STUDIES ON KININS AND PROSTAGLANDINS

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C O N T E N T S

KININS

1. The isolation of bradykinin, a plasma kinin from ox blood.

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2. The structure of bradykinin.

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4. Actions of pure bradykinin.

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The Isolation of Bradykinin, a Plasma Kinin from Ox Blood

By D. F. ELLIOTT, E. W. HORTON and G. P. LEWIS

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The Isolation of Bradykinin, a Plasma Kinin from Ox Blood

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Bradykinin belongs to a group of peptides which stimulate smooth muscle and have been given the generic name of plasma kinin; this name has been adopted by general consent of most workers in the field (see Lewis, 1958). The formation of bradykinin from ox-plasma proteins by the action of trypsin or the venom of the snake, *Bothrops jararaca*, was first observed by Rocha e Silva, Beraldo & Rosenfeld (1949). Blood and many other tissues are known to contain enzymes which can be activated under certain conditions and will then cause liberation of a plasma kinin from the plasma proteins (Frey, Kraut & Werle, 1950). Bradykinin is pharmacologically indistinguishable from other plasma kinins (Lewis, 1958; Gaddum & Horton, 1959; Werle, 1953; Holdstock, Mathias & Schachter, 1957; Mathias & Schachter, 1958; Hilton & Lewis, 1956).

The experiments described in this paper have led to the isolation of bradykinin, which is the first member of the plasma-kinin group to be obtained in the pure state.

EXPERIMENTAL

Materials. $(NH_4)_2SO_4$ was a commercial grade. Ammonium acetate was A.R. grade. Ammonium acetate buffer, pH 5, was prepared from stock solutions of 10M-

ammonium acetate and 10M-acetic acid. The solutions were diluted to the required molarity and then the acetic acid solution was added to the ammonium acetate solution until pH 5 was reached (glass electrode). Approximately 160 ml. of acetic acid soln./500 ml. of ammonium acetate soln. was required.

Carboxymethyl(CM)-cellulose was prepared from Solka Floc cellulose powder by a slight modification of the method of Peterson & Sober (1956). Before its conversion into the carboxymethyl derivative, fine particles were removed from the cellulose by sedimentation. The cellulose powder was stirred with about 6 vol. of water and the mixture was allowed to settle for several hours. Particles which had not settled by this time were rejected by decantation. The process was repeated several times. The sludge was filtered, washed with methanol and dried in air. After the reaction of 60 g. of cellulose with chloroacetic acid and addition of 500 ml. of 10% acetic acid to the reaction mixture, as described by Peterson & Sober (1956), a further addition of acetic acid was made, with stirring, until a pH of 5–6 was obtained. Fine particles were removed by sedimentation as before. It was then transferred to a glass tube 7 cm. × 50 cm., fitted with a filtration plate at the bottom and washed with 10% (v/v) acetic acid until the flame test in the eluate was negative. It was washed with 2 l. of water, transferred to a filter funnel, washed with ethanol and dried in air. The yield was approx. 20 g.

Except in two-dimensional chromatography butan-1-ol was redistilled.

Trichloroacetic acid was A.R. grade, redistilled at 65°/15 mm. Hg.

Ether (d 0·720) was allowed to stand for several days over NaOH flakes and redistilled.

Phenol–water for chromatography was prepared by adding 5 vol. of liquid phenol to 2 vol. of water and dissolving in the mixture 0·0003% of ethylenediaminetetraacetic acid (EDTA).

Butanol–acetic acid for chromatography was prepared by shaking together butan-1-ol, water and acetic acid (63:27:10, by vol.) and keeping for several days before using the upper layer.

Bradykinin at P4 stage and subsequent stages was handled in glassware which had been treated with a solution of dimethyldichlorosilane in carbon tetrachloride and washed with warm water.

Fractionation of ox blood. Fresh ox blood was collected at the slaughterhouse and defibrinated immediately. The red cells were separated by centrifuging in a De Laval Industrial Separator, type 1229G, at room temperature with a gravity disk of 37 mm. internal diam. The rate of feed was 90 l./hr. and the yield of serum was about 44 l. To this amount of serum was added, with mechanical stirring, 22 l. of $(NH_4)_2SO_4$ soln. saturated at room temperature. The ppt. was removed as completely as possible by centrifuging once in a Sharples no. 5 Centrifuge with a 5 mm. diam. jet and the supernatant (60 l.) was treated with 13 l. of saturated $(NH_4)_2SO_4$. Except that a 2 mm. diam. jet was used, the ppt. was collected by centrifuging as before, dissolved in 5 l. of water and dialysed in Visking dialysis tubing against deionized tap water. The latter was prepared with the aid of a Permutit Portable Deminrolit Plant MK4. Dialysis was continued until SO_4^{2-} ion had been removed. The final volume was 9 l.

Preparation of crude bradykinin. The substrate solution, prepared as described above, was treated with 90 ml. of 10N-HCl and then heated at 37° for 30 min. with gentle stirring (cf. Gaddum & Horton, 1959). The pH of the solution was adjusted to 7·5 with 4N-NaOH and incubated for 6 hr. at 37° with crystallized salt-free trypsin (Armour Pharmaceutical Co. Ltd., Eastbourne). The trypsin was added in portions of 75, 37·5 and 37·5 mg. respectively at zero time, 2 hr. and 4 hr. from the commencement of the incubation. The solution was then poured rapidly into 30 l. of boiling ethanol and when the mixture had cooled it was centrifuged and the ppt. was discarded. The supernatant was evaporated under reduced pressure at a temperature not exceeding 40° to a volume of approximately 300 ml. Redistilled butanol was added in small portions to this solution, with gentle shaking, until no more would dissolve. Trichloroacetic acid solution (50%, w/v) was then added to bring the concentration of trichloroacetic acid in the solution to 0·3%. The resulting solution formed the first lower phase of a countercurrent-distribution process carried out in five bottles. The solvent system was prepared by equilibration of equal volumes of butanol and aqueous 1% (w/v) trichloroacetic acid solution. The volumes of upper and lower phases were equal to each other and to the volume of the crude bradykinin soln. forming the lower layer in the first bottle. Emulsions which formed were separated by centrifuging. The process was continued until all bottles were filled with both layers, the upper layer forming the moving phase. The upper phase in the fifth bottle was removed and equilibrations were continued, with addition of a fresh upper phase to the first bottle, until all bottles were again filled. The lower phases in the first two bottles were rejected. The upper phase that had been removed from the fifth bottle was mixed with twice its volume of peroxide-free ether and equal volumes of water and the mixture were equilibrated. The resulting upper layer was rejected and the aqueous phase was combined with the remaining upper and lower phases. The two-phase mixture was evaporated under reduced pressure to a volume of about 200 ml. at a temperature not exceeding 35°. The mixture was fed continuously into a cyclic evaporator, it being so arranged that the upper (butanol) layer formed the major portion of the liquid entering the evaporator until it had all been removed. The aqueous solution was treated with 3N-HCl until pH 1 (glass electrode) was reached and was then extracted nine times with equal volumes of peroxide-free ether, and the ether extracts were rejected. After the fifth extraction the pH of the aqueous layer was readjusted to 1. The aqueous solution was freed of ether by heating it for about 10 min. under atmospheric pressure in a steam bath and then heating it for a few minutes under reduced pressure at about 35°. It was then freeze-dried, yielding a pale-yellow powder. The amount obtained varied considerably, but on average was about 5 g. It was stored in a vacuum desiccator at −2° over NaOH pellets. The crude material (P2) was stable for long periods under these conditions.

Biological assay of bradykinin. The biological activity of samples was estimated by assay on the isolated guinea-pig intestine against a standard sample of P2 which had been set aside at the beginning of this investigation. Results were expressed in terms of the weight of P2 to which the test sample was equivalent in biological activity. The terminal portion of the guinea-pig ileum was suspended in 15 ml. of oxygenated Tyrode solution and maintained

at 34°. Contractions of the tissue were recorded by means of a frontal lever writing on a smoked drum of a kymograph.

Chromatography of bradykinin on carboxymethylcellulose. Columns were run at 20°; they were packed without application of external pressure, but during equilibration the head of liquid used generally caused further settlement. Buffer solutions were saturated with toluene to inhibit growth of micro-organisms. Chromatography of P2 was carried out on 32 g. of CM-cellulose in a column of 3·2cm. diam. through which 0·01 M-ammonium acetate had been passed at approx. 50 ml./hr. until the pH values of the influent and effluent fluids were identical. The pH of the solution was not accurately controlled and varied with different batches of the A.R.-grade ammonium acetate used; it was generally pH 6·4–6·5. A portion (4 g.) of P2 was dissolved in 100 ml. of 0·01 M-ammonium acetate and allowed to run into the column. The column was then washed with 0·01 M-ammonium acetate soln. at the rate of 50 ml./hr. until the extinction of the effluent at 280 mμ was less than 0·02. Gradient elution was then commenced with 0·2 M-ammonium acetate in the reservoir and with a mixing vessel of 250 ml. capacity. In this and other gradient experiments the mixing vessel had only two outlets, one to the column and the other to the reservoir; it had no outlet to the atmosphere. The upper reservoir was open to the air. The effluent was collected in fractions of 30 ml. Fractions containing biological activity were combined, boiled for 2 min., cooled and evaporated to dryness under reduced pressure at a temperature of approx. 35°. The evaporation was completed in a rotary evaporator and the syrupy residue heated at 50°/0·01 mm. Hg until ammonium acetate had been removed. This product was described as P4.

Chromatography of P4 was carried out on 2 g. of CM-cellulose in a column of 9 mm. diam. which had been equilibrated with 0·01 M-ammonium acetate buffer, pH 5, in the manner already described. A portion (50 mg.) of P4 was dissolved in 0·1 ml. of 0·01 M-ammonium acetate buffer and, after allowing liquid to drain from the top of the column, the solution was added to the column and allowed to soak in. Two successive portions of 0·1 ml. of the buffer were then added in a similar way. The column was then washed with 0·01 M-ammonium acetate buffer, pH 5, which was also 1 mM with respect to EDTA. The hydrostatic head was adjusted to give a flow rate of 5–6 ml./hr and washing was continued until at least 80 ml. of effluent had been collected. The washing fluid was then changed to 0·01 M-ammonium acetate, pH 5; 40 ml. of effluent was collected. Gradient elution was then commenced with a mixing vessel of 100 ml. capacity and 0·5 M-ammonium acetate in the reservoir. Under these conditions the gradient was approximately linear. Tubes containing biologically active material were placed in a vacuum desiccator over NaOH and H_2SO_4, until a solid residue remained. They were then placed in a wide glass tube closed at one end and open at the other and fitted with a side arm near the open end. The position of the side arm was above the open ends of the tubes containing the solid. The tube was stoppered, evacuated to approx. 0·01 mm. Hg and immersed as far as the side arm in a bath of water at 50°. Heating under these conditions was continued for 1 hr. The combined solid was described as P5.

Chromatography of P5 was carried out on 4 g. of CM-cellulose in a column of 9 mm. diam. It was carried out exactly as for P4 with a mixing vessel of identical capacity.

Electrophoresis of P5. This was carried out on Whatman no. 1 filter paper which had been washed by descending solvent flow with 2 N-acetic acid for 3 days and then with water for 1 day. P5 at a concentration of 10 μg./μl. was applied in the form of a band 2–3 mm. wide and 2 cm. less in length than the total width of the paper. The loading was generally about 100 μg./cm. of paper width; occasionally double this amount was applied with successful results. The buffer used was 2 N-acetic acid and the voltage gradient was 25 v/cm. Electrophoresis was continued for 1 hr. in an apparatus similar to that of Durrum (1950). At the conclusion of the experiment the strip was dried in air at room temperature and a marker strip 0·3 cm. wide was cut lengthwise from the centre of the paper. It was stained with ninhydrin and with Sakaguchi reagent. The section of paper containing the bradykinin was cut out and eluted with water by descending capillary flow, water being allowed to drip from the bottom end of the strip. Elution was generally continued for 16 hr. and the eluate was evaporated to dryness in a vacuum desiccator. The recovery of biological activity was about 70% of that applied to the paper.

Paper chromatography. Two-dimensional chromatograms were carried out with butanol–acetic acid in the first dimension and phenol–water–NH_3 in the second dimension on Whatman no. 1 paper. For one-dimensional chromatography butanol–acetic acid or pyridine–water (80:20, v/v) was used. Chromatograms were dried at 40°. Quantitative amino acid analysis was carried out by the method of Mandelstam & Rogers (1959), a slight modification being made to permit estimation of proline. The chromatogram was developed in butanol–acetic acid on Whatman no. 3 paper, and, after formation of the spots with ninhydrin, all those except the yellow spot of proline were cut out. The remainder of the sheet was moistened by steaming and heated at 110° for 15 min. The yellow spots due to proline became purple and were then cut out. Reproducible results were obtained in the range 0·01–0·1 μM.

Chromatography of dinitrophenyl amino acids (DNP amino acids) was carried out on Whatman no. 1 paper with the *tert.*-pentanol (2-methylbutan-2-ol) system of Blackburn & Lowther (1951) and 1·5 M-phosphate buffer (Levy, 1954), used either singly or for two-dimensional chromatograms. For quantitative work, sections of paper of known area surrounding the spots were cut out, shredded and allowed to stand with 4·5 ml. of 1% $NaHCO_3$ soln. at room temperature for 10 min. Extinction of the extract was measured at 350 mμ, except with DNP-proline, where measurement was made at 370 mμ. Blank estimations were made on pieces of paper cut from the same sheet.

Use of fluoro-2:4-dinitrobenzene for end-group determination and dinitrophenylation of amino acid mixtures. To the peptide or mixture of amino acids (containing between 0·1 and 1 μequiv. of free amino groups), dissolved in a mixture of 1 ml. of 2% (w/v) $NaHCO_3$ and 1 ml. of ethanol, was added 5 μl. of fluoro-2:4-dinitrobenzene. The mixture was shaken by hand for a few minutes, when a homogeneous solution resulted; this was allowed to stand in the dark at room temperature for 3 hr. The solution was then extracted three times with an equal volume of ether. In the determination of end groups the aqueous layer was made

acid (pH 1), evaporated to dryness and hydrolysed with 6N-HCl at 115° for 16 hr. DNP amino acids with the exception of DNP-arginine were extracted from acid solution (pH 1 or less) with ether. If much dinitrophenol was present in the ether extract, this was removed by the vacuum-sublimation technique of Mills (1952). DNP-arginine was extracted with butanol.

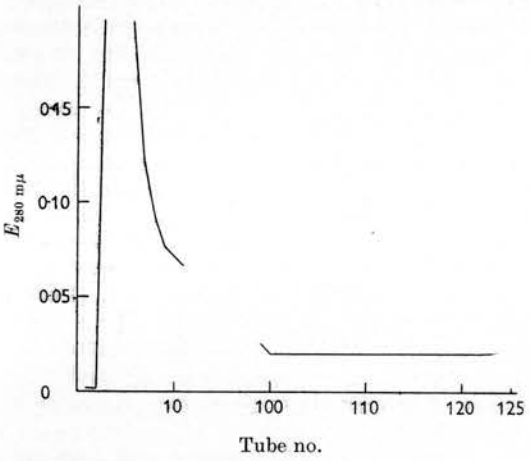


Fig. 1. Results of chromatography of 4 g. of P2 on 32 g. of CM-cellulose at pH 6·4. The fraction volume was 30 ml. The gradient from 0·01 to 0·2M-ammonium acetate was commenced at tube no. 100. Smooth-muscle-stimulating activity was found in tubes nos. 102–125.

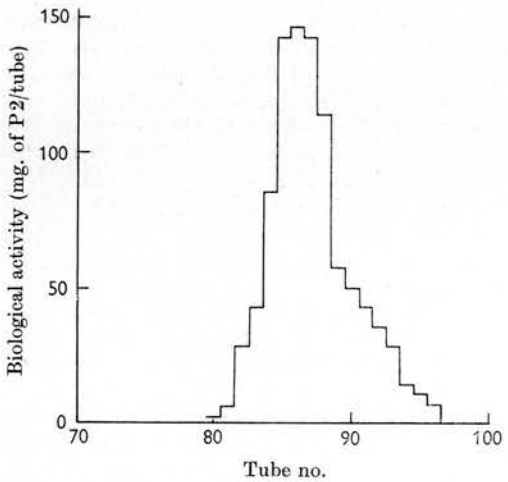


Fig. 2. Results of chromatography of 32 mg. of P4 on 2 g. of CM-cellulose at pH 5. The fraction volume was 1·4 ml. After washing with EDTA (see text) the gradient from 0·01 to 0·5M-ammonium acetate was commenced at tube no. 1. Results are expressed in terms of the wt. of standard P2 to which the tube contents were equivalent in biological activity.

RESULTS

Chromatography of bradykinin on carboxymethylcellulose

Fig. 1 illustrates the results obtained on chromatography of 4 g. of crude bradykinin (P2) on CM-cellulose. It will be seen that a large amount of inactive peptide that absorbs light at 280 mμ emerges in the first 300 ml. of effluent. Further washing with 0·01M-ammonium acetate decreased the extinction of the effluent to about 0·02 at 280 mμ and thereafter it remained almost constant. Tubes nos. 102–125 each contained smooth-muscle-stimulating activity equivalent to more than 5 mg. of P2 and the contents of these were combined. The yield of P4 obtained in this experiment and similar ones was 60–70 mg., which was equivalent in biological activity to 2·4–2·8 g. of P2. The recovery was therefore 60–70 %. Inferior results were obtained when chromatography was carried out at 2°.

In Fig. 2 are shown the results of chromatography of P4 on CM-cellulose. The contents of tubes containing biological activity equivalent to more than 10 mg. of P2 were combined. The yield of P5 was 7·8 mg., which was equivalent in biological activity to 925 mg. of P2. An amount of P4 equivalent to 1·4 g. of P2 was used in this experiment and therefore the recovery was 66 %.

The results shown in Fig. 3 were obtained on chromatography of 15 mg. of P5 on CM-cellulose. In this experiment there was false indication of incipient separation into two peaks. The contents of tubes nos. 105–110 had the properties of pure bradykinin. The yield was 5·3 mg. It was concluded that the low yield of biological activity in tube 108 was due to a defect in the experimental procedure.

Electrophoresis and paper chromatography of P5

When P5 was submitted to chromatography on Whatman no. 1 paper with butanol–acetic acid as solvent, separation of bradykinin from the inactive peptides was achieved after the chromatogram had been allowed to develop for several days. These results were not sufficiently reproducible to warrant their continued use. At times no separation was achieved and the bradykinin did not leave the origin. It was considered possible that this difficulty was due to contamination with metal ions. Flame-spectrographic analysis (by Johnson, Matthey and Co. Ltd., Wembley, Middx.) revealed that P5 contained 1 % by wt. of magnesium. A number of other metallic elements were present, but these were in much smaller quantities than magnesium.

In subsequent experiments paper electrophoresis was used in preference to paper chromatography,

as it was more easily applicable to preparative work. In order to achieve the isolation of pure bradykinin it was necessary to include in the chromatography of P 5 a stage of washing with EDTA. Plate 1 shows the results obtained on electrophoresis of P 5 prepared with and without the aid of EDTA. It would clearly be impossible to achieve the isolation of pure material from an electrophoresis experiment such as that illustrated in strip no. 1. Strips nos. 2 and 3 show that bradykinin stains weakly with ninhydrin and strongly with the Sakaguchi reagent. By the use of the dipping technique of Jepson & Smith (1953) it was possible to stain the same strip with both ninhydrin and Sakaguchi reagents. The substances stained strongly by ninhydrin on strips nos. 2 and 3 in Plate 1 were biologically inactive.

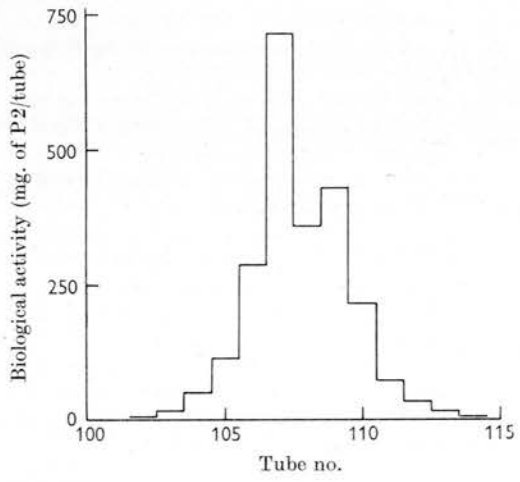


Fig. 3. Results of chromatography of 15 mg. of P 5 on 4 g. of CM-cellulose at pH 5. Conditions were identical with those given in Fig. 2.

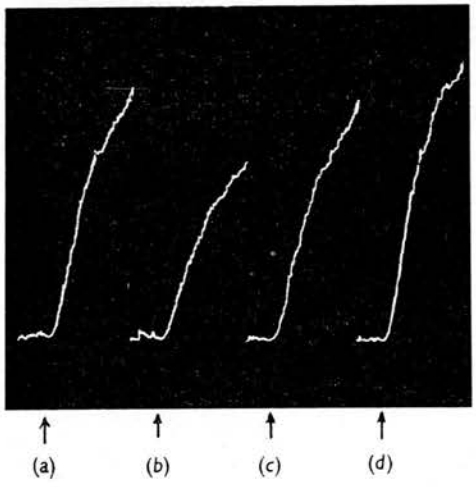


Fig. 4. Responses of the guinea-pig ileum suspended in 15 ml. of Tyrode solution to pure bradykinin (P 6), 40 μmg. and 50 μmg. at (*b*) and (*d*) respectively, and to P 2, 22·5 μg. and 20 μg. at (*a*) and (*c*) respectively.

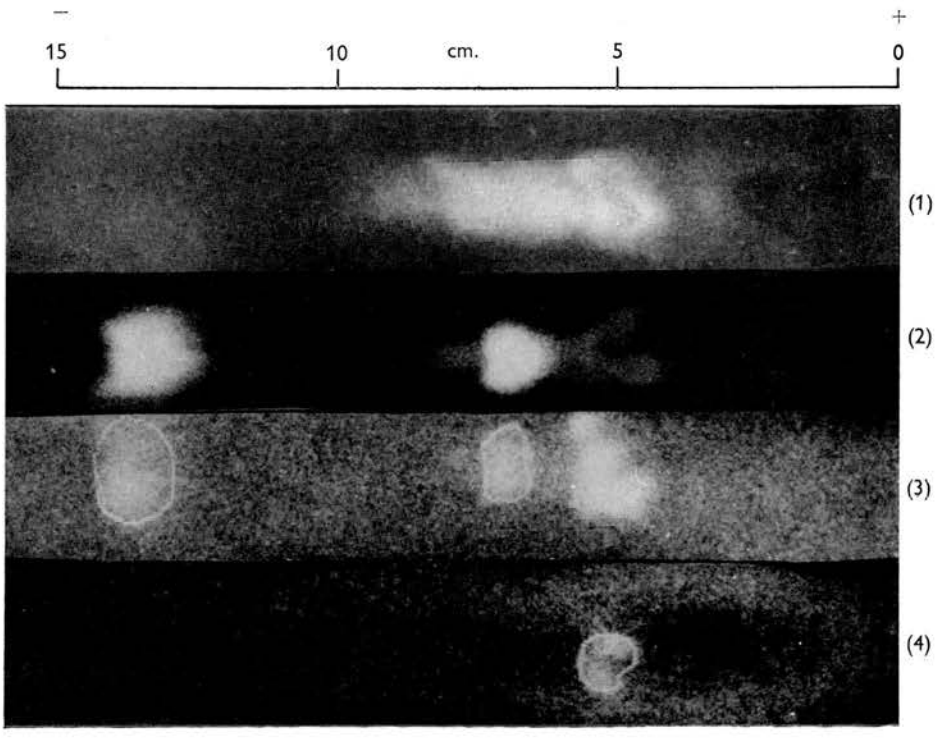
Properties of pure bradykinin

The substance prepared by electrophoresis of P 5 as shown in Plate 1 or by further CM-cellulose chromatography of P 5 as shown in Fig. 3 was found to contain a single *N*-terminal residue, that of arginine. Hydrolysis followed by two-dimensional paper chromatography revealed the presence of five amino acids, serine, glycine, proline, arginine and phenylalanine. The molar proportions of each amino acid, found by quantitative paper chromatography of the amino acids themselves or of their DNP derivatives, were serine 1, glycine 1, proline 2, arginine 2 and phenylalanine 2. [Degradative work recently carried out by the authors (Elliott, Lewis & Horton, 1960) has revealed that the result obtained for proline is incorrect. The molecule of bradykinin contains three residues of proline.]

Bradykinin was found to possess very high biological potency. For example, the threshold dose for contraction of the isolated guinea-pig ileum was approx. 1 μmg./ml. Fig. 4 shows the results obtained when the biological activity of pure bradykinin (P 6) was compared with that of P 2. The slow contraction is a characteristic feature of the response of guinea-pig ileum to bradykinin. P 6 at a concentration of 3 μmg./ml. produced a marked contraction of the tissue. From these results it was concluded that P 6 was 444 times as active as P 2. The biological activity of bradykinin was destroyed by chymotrypsin, but not by trypsin. Bradykinin resisted attack by 0·1 N-HCl at 100° for at least 1 hr. and by 0·1 N-NaOH at room temperature for at least 2 hr.

DISCUSSION

The purification of bradykinin on columns of IRC-50 resin was described by Andrade & Rocha e Silva (1956). These authors used phosphate buffers for elution and were unable to remove inorganic ions from the eluates. In the present investigation it was decided that if a volatile buffer could be used this would be much more convenient than a non-volatile buffer. It was decided to explore the potentialities of CM-cellulose in the chromatography of bradykinin. In the course of another investigation, one of us (E.W.H., unpublished work) found that urinary kinin, a peptide closely related to, if not identical with, bradykinin, was irreversibly adsorbed on to IRC-50 in the presence



Results of four separate electrophoresis experiments performed under identical conditions (see text). (1) Electrophoresis of P5 which had been prepared on a CM-cellulose column without EDTA treatment. The strip was stained with ninhydrin. (2) The same technique applied to P5 from a CM-cellulose column with EDTA treatment. (3) Identical with 2, except that the strip was stained with Sakaguchi reagent after ninhydrin. The spots marked by a (pencil) line were positive only to ninhydrin. (4) Electrophoresis of pure bradykinin. The strip was stained with ninhydrin and Sakaguchi reagents.

of ammonium acetate. Losses of activity occurred in all the CM-cellulose columns used and these could have been due to irreversible adsorption.

It was surprising to find that bradykinin contained magnesium after passage twice through CM-cellulose. Accurate data are not available for the magnesium content of pure bradykinin obtained from EDTA-treated columns, owing to the relatively large amount needed for flame-spectrographic analysis. Qualitative results show that it is still present in the purest material, although in decreased amount compared with P5 prepared without the aid of EDTA.

Recent work has shown (Elliott, Horton & Lewis, 1960) that bradykinin exerts all the actions which have been attributed to one or another of the plasma kinins (smooth-muscle stimulation, vasodilatation, increased capillary permeability, pain production), but the question whether or not the plasma kinins constitute several biologically active peptides or a single peptide (bradykinin) must remain open until other plasma kinins have been isolated and their structures determined.

SUMMARY

1. Bradykinin was formed by trypsin digestion of the fraction of ox-serum proteins which precipitated between 33 and 45% of saturation with ammonium sulphate.

2. Bradykinin was isolated in the pure state by application of the techniques of countercurrent distribution, chromatography on carboxymethylcellulose and paper electrophoresis.

3. Bradykinin contained the five amino acids glycine, serine, proline, phenylalanine and arginine in the molar proportions 1:1:3:2:2 and one of the arginine residues was *N*-terminal.

The authors are indebted to Mr A. W. Hemmings, who devised techniques for the large-scale fractionation of blood, and to Mr R. Blakemore, Miss M. Kinsella, Mr E. Stevens and Mr I. Croker for valuable experimental assistance.

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The Structure of Bradykinin. By D. F. ELLIOTT, G. P. LEWIS and E. W. HORTON. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Bradykinin, isolated from ox blood, was found to have the amino acid composition Ser(1):Gly(1):Pro(2):Phe(2):Arg(2) and a single *N*-terminal residue of arginine (Elliott, Lewis & Horton, 1960). The structure has now been elucidated by chemical and enzymic degradation. Chymotrypsin hydrolysed two peptide bonds in the molecule, one bond being more rapidly split than the other. The main hydrolysis products were arginine and a heptapeptide; the latter was shown to possess a *C*-terminal phenylalanine residue by the use of carboxypeptidase. At a higher concentration of chymotrypsin, appreciable hydrolysis of the second phenylalanyl bond took place yielding, as one of the products, a pentapeptide containing arginine, proline, glycine and phenylalanine and as another a peptide yielding serine and phenylalanine on hydrolysis. Stepwise degradation of bradykinin by the Edman method indicated the sequence Arg.Pro.Pro.Gly.Phe for the first five residues. Therefore the structure of bradykinin is Arg.Pro.Pro.Gly.Phe.Ser.Phe.Arg.

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THE STRUCTURE OF BRADYKININ - A PLASMA KININ FROM OX BLOOD

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Received July 5, 1960

In a previous communication it was stated that bradykinin yielded on acid hydrolysis the amino acids serine, glycine, proline, phenylalanine and arginine in the molar proportions 1:1:2:2:2 and contained a single N - terminal residue of arginine (Elliott, Lewis and Horton, 1960a). In a later communication the sequence Arg.Pro.Pro.Gly.Phe.Ser.Phe.Arg. was proposed for bradykinin (Elliott, Lewis and Horton, 1960b). In the light of synthetic work carried out by Dr. Boissonnas and his collaborators (Boissonnas, Guttmann, Jaquenoud, Konzett and Stürmer, 1960) and communicated to the authors before publication, the above structure for bradykinin was clearly incorrect. Discussions between Dr. Boissonnas and ourselves led to the view that bradykinin contained three residues of proline and that its structure was Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg. Further degradative work has confirmed that this structure is the correct one.

Edman degradation of bradykinin.

Bradykinin (0.5 mg.) was degraded by the Edman method using the technique adopted for hypertensin (Elliott and Peart, 1957) whereby the phenylthiohydantoins obtained at each stage were converted into amino acids by reductive hydrolysis with hydriodic acid. Under these conditions a synthetic sample of

the PTH-derivative of serine was converted into alanine. The amino acids were identified by paper chromatography in pyridine:water (4:1). As expected, the first stage of the degradation gave no amino acid because arginine gives a PTH-derivative which cannot be extracted from aqueous solution. The sequence of the next four amino acids was found to be Pro.Pro.Gly.Phe., consequently the sequence of the first five residues of bradykinin was Arg.Pro.Pro.Gly.Phe. It was decided not to continue beyond this point owing to the accumulation of artefacts which made chromatographic identification progressively more difficult.

Action of chymotrypsin on bradykinin.

Bradykinin (0.5 mg.) was dissolved in 2 ml. of 0.05M ammonium bicarbonate:ammonium acetate buffer, pH 7.5, the solution was mixed with 0.15 ml. of a solution of chymotrypsin in the same buffer containing 2 mg. of the enzyme per ml. and was incubated for 18 hr. at 37° After addition of 0.1 ml. of glacial acetic acid the solution was evaporated to dryness in a vacuum desiccator at room temperature. The residue had no action upon isolated smooth muscle preparations. Electrophoresis, on Whatman No. 1 paper, of a small portion of the residue in 2M-acetic acid using a voltage gradient of 25V/cm., gave the results shown in Fig. 1. Bands 2, 3 and 4, could be

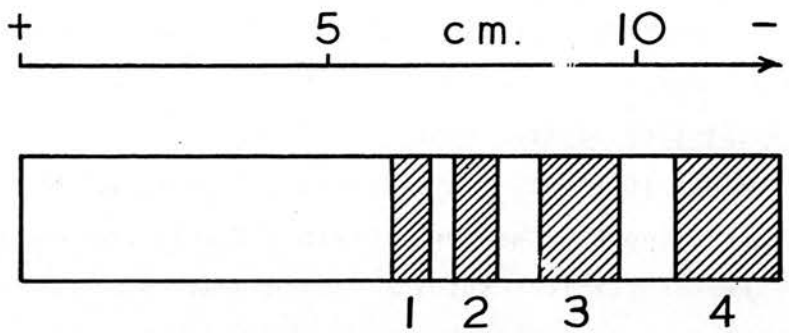

Fig. 1. Electrophoresis of a chymotryptic digest of bradykinin.

revealed by the Sakaguchi reagent (Jepson and Smith, 1953), or by ninhydrin, whereas band 1 was stained only by ninhydrin. The enzyme:substrate molar ratio in this experiment was 1:40; at a ratio of 1:100 bands 2 and 4 formed the main products and bands 1 and 3 were scarcely visible. This showed that one bond was hydrolysed more rapidly than the other by the enzyme. The main bulk of the chymotryptic digest was then submitted to electrophoresis as before and the products isolated by a technique previously described (Elliott and Peart, 1957). It was deduced that band 4 was arginine on the basis of the chromatographic behaviour of the substance itself and of its dinitrophenyl derivative; band 2 yielded on hydrolysis all five amino acids present in bradykinin. These two substances were clearly the products of fission of the first bond in bradykinin by chymotrypsin. When band 2 was submitted to the action of carboxypeptidase at 37^{o} and pH 8 phenylalanine was liberated, thus showing that the C - terminal sequence of bradykinin was Phe.Arg. Hydrolysis of band 3 yielded arginine, proline, glycine and phenylalanine which were estimated as their dinitrophenyl derivatives by the method of Elliott, Lewis and Horton (1960b) and found to be in the molar proportions 1:2:1:1. Band 3 was evidently one of the products of fission of the second bond in bradykinin by chymotrypsin and its amino acid composition was consistent with the sequence Arg.Pro.Pro.Gly.Phe. deduced from the results of the Edman degradation on bradykinin. The N - terminal residue of band 1 was found to be serine by the fluorodinitrobenzene technique. Hydrolysis of band 1 and chromatography of the hydrolysate in butanol/acetic acid revealed the presence of serine, phenylalanine and a smaller amount of proline, but in view of the fact that two proline residues had already been placed it

was assumed that the presence of proline was due to contamination of band 1 by self-digestion products of the enzyme. Band 1 was therefore assigned the structure Ser.Phe. (Elliott, Lewis and Horton, 1960b), but as a result of the information given to us by Dr. Boissonnas it has now been examined more closely. After separation from a chymotryptic digest of 0.5 mg. of bradykinin by electrophoresis as already described, band 1 was further purified by chromatography on Whatman No. 1 filter paper in n-butanol:acetic acid:water (63:27:10).

Two equal portions of the product were hydrolysed with 6N-HCl for 16 hr. and 48 hr. respectively and the products subjected to chromatography in butanol/acetic acid. The yields of the amino acids in the hydrolysates were roughly estimated by comparison of the size and intensity of the spots with those of standard amounts of serine, proline and phenylalanine on the same chromatogram. The yield of proline from both the 16 hr. and the 48 hr. hydrolysates of band 1 was about 50% of the theoretical amount. Evidently destruction of proline took place at some stage before complete hydrolysis, but these results leave no doubt that band 1 has the structure Ser.Pro.Phe and not Ser.Phe as originally thought. The presence of proline in this position is also consistent with the fact that carboxypeptidase had no further action on the octapeptide (band 2) or its dinitrophenyl derivative after it had liberated phenylalanine. This was the case even at an enzyme:substrate molar ratio of 1:10.

Conclusions

Bradykinin has the structure Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg and not Arg.Pro.Pro.Gly.Phe.Ser.Phe.Arg as originally proposed (Elliott, Lewis and Horton, 1960b). Destruction during acid hydrolysis probably accounts for the incorrect result obtained

for the proline content of bradykinin.

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ACTIONS OF PURE BRADYKININ

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(*Received* 9 *May* 1960)

Bradykinin, first described by Rocha e Silva, Beraldo & Rosenfeld (1949), is one of a group of polypeptides called plasma kinins. Plasma kinins are known to stimulate certain types of smooth muscle, to cause vasodilatation, to increase capillary permeability and to produce pain when brought into contact with pain fibres. It has not been clear hitherto whether bradykinin is responsible for all these actions or whether several peptides are involved, as in all experiments crude extracts have been employed. However, recently bradykinin has been isolated (Elliott, Lewis & Horton, 1960*a*) and its structure determined (Elliott, Lewis & Horton, 1960*b*,*c*). It is therefore now possible to examine the actions of the pure peptide.

The present investigation was carried out to answer the question, does the crude preparation of bradykinin contain one peptide having all the actions reported for the crude mixture, or does it contain a group of related peptides each of which is responsible for one or more of the biological actions? The results show that pure bradykinin possesses all the actions of the crude mixture, that is, smooth-muscle stimulation, vasodilatation, increased capillary permeability and pain production.

METHODS

Where comparisons have been made on a molar basis the molecular weight of bradykinin has been taken as 1131, which is the molecular weight of bradykinin dihydrochloride.

Actions on isolated smooth-muscle preparations

Guinea-pig ileum. A 3–4 cm segment of terminal ileum from animals weighing 250–400 g was suspended in a 15 ml. bath containing oxygenated Tyrode solution at 34° C. Tests were made every 4 min, and the contact time was 45 sec.

Rat uterus. Virgin rats weighing 120–200 g were injected subcutaneously with stilboestrol (10 μg/100 g) 16–18 hr before use. A 2 cm segment of uterine horn was suspended in a 10 ml. bath containing de Jalon's uterus solution at 30° C. Tests were made every 4 min and the time of contact was 45 sec.

Rat duodenum. The proximal 3 cm of duodenum from rats weighing 120–200 g was suspended in a 10 ml. bath containing de Jalon's solution at 30° C. Tests were made every 3 min, and the contact time was 30 sec.

Rabbit duodenum. The proximal 4–5 cm of duodenum from rabbits was suspended in a 15 ml. bath containing Locke's solution at 37° C. Tests were made every 3 min, and the contact time was 1 min.

Blood pressure experiments

Cats were anaesthetized with pentobarbitone sodium 40 mg/kg. The trachea was cannulated and blood pressure was recorded from the femoral artery. Intravenous injections were made into a cannula tied into the femoral vein.

Rats (300–400 g) were anaesthetized with urethane 175 mg/100 g, and were prepared by the method of Crawford & Outschoorn (1951). Blood pressure was recorded from the carotid artery with a Condon capillary manometer.

Vasodilatation was examined in the skin of the hind limb supplied by the saphenous artery. Cats were anaesthetized with chloralose 60 mg/kg. The preparation was the same as that described previously (Lewis, 1958).

Capillary permeability was examined by the method of Miles & Miles (1952). Guinea-pigs and rabbits were injected intravenously with Pontamine sky blue 60 mg/kg. A few minutes after injection of the dye pure or crude bradykinin or histamine in 0·1 ml. saline was injected intradermally into the abdominal skin. One hour later the area of blueing was measured.

Pain production was examined by the method of Armstrong, Dry, Keele & Markham (1953). Blisters were raised on the flexor surface of the forearms of human subjects by the application of cantharidin plasters, 2 × 2 cm. The plasters were applied in the evening before the experiment and allowed to act for 6 hr. The area was covered with a sterile dressing and a blister allowed to form during the night. The skin of the blister was cut away and the blister base was washed with a warm Ringer's solution containing (g/l.) NaCl 9·2, KCl 0·4, $CaCl_2$ 0·24 and $NaHCO_3$ 0·15. Drugs to be tested were dissolved in physiological saline and kept at 37° C during the experiment.

The test was carried out as follows. The solution to be tested was applied to the blister area with a Pasteur pipette until the area was filled. Each dose was allowed to act until the pain reached a plateau, or began to subside, up to a maximum of 2 min. The area was then thoroughly washed with the Ringer's solution. Doses were given at intervals of 10 or 20 or 30 min or 1 hr, and before each application the area was washed periodically with Ringer's solution. The subject was not told the nature of the applied solution; he assessed the pain intensity of each solution subjectively, grading it from 0 to + + +.

Guinea-pig bronchoconstriction

The method described by Konzett & Rössler (1940) was used. Injections were made intravenously through a cannula in the superior vena cava.

RESULTS

A comparison was made of the actions of pure bradykinin (P_6) and of the crude mixture (P_2) produced at the first stage of purification in the procedure of Elliott *et al.* (1960*a*). It was found that the increase in activity from P_2 to P_6 was of the same order when estimated on various isolated smooth-muscle preparations as well as when estimated for bronchoconstriction in guinea-pigs, for vasodilatation on the skin vessels of the cat hind limb, for depressor activity in cats and rats, for increase in capillary permeability in guinea-pigs and rabbits and for pain production in man.

Table 1 shows the relative potencies P_6:P_2 on these biological preparations. The variations shown in the Table are probably within experimental variations of the tests and suggest that all the actions of crude mixture are inherent properties of the pure peptide.

Actions on smooth muscle

Several isolated smooth-muscle preparations respond to low concentrations of pure bradykinin. The guinea-pig ileum responds with a slow contraction to bradykinin 10^{-9} and the rat uterus to 10^{-10}. It has been shown previously that crude bradykinin has an inhibitory effect on certain types of smooth muscle, particularly those showing rhythmical activity and a high resting tone. The pure peptide also has this inhibitory action. On the isolated rat duodenum bradykinin 8×10^{-9} caused only relaxation (Fig. 1), whereas with the rabbit duodenum and the rat colon the response was biphasic, an initial inhibitory action followed by a contraction.

TABLE 1. Ratios of the relative potency of pure (P_6) and crude (P_2) bradykinins in different tests

Vasodilatation	Cat skin	365
Depressor action	Cat B.P.	480
	Rat B.P.	265
Increased capillary permeability	Guinea-pig skin	390
	Rabbit skin	270
Smooth-muscle contraction	Guinea-pig ileum	450
	Rat uterus	405
Smooth-muscle relaxation	Rat duodenum	225
Bronchoconstriction	Guinea-pig	400
Pain production	Human blister base	180

Collier, Holgate, Schachter & Shorley (1959) have shown that crude kinins cause bronchoconstriction in anaesthetized guinea-pigs. In the present experiments pure bradykinin 0·5 μg injected intravenously into guinea-pigs caused a bronchoconstriction equal to that caused by the same dose of histamine. On a molar basis, therefore, bradykinin is about 10 times more active than histamine when tested in this way. When injections of bradykinin were made at 5 or 10 min intervals the preparation quickly lost its sensitivity to bradykinin. An interval of about 15–20 min between injections of bradykinin was required in order to avoid loss of sensitivity in this preparation.

Vasodilatation

The vasodilator action of crude plasma kinin has been studied in investigations on skin (Lewis, 1958), skeletal muscle and salivary glands (Hilton & Lewis, 1955). In the present experiments a dose as low as 100 ng of pure bradykinin caused vasodilatation in the skin of the cat hind limb compared with 10–20 ng acetylcholine. Figure 2 illustrates the vasodilatation produced by the pure peptide (P_6) compared with that produced by much larger doses of crude (P_2) bradykinin. Therefore, when calculated on a molar basis, bradykinin is approximately as active as acetylcholine on this preparation. When compared with histamine on a molar basis bradykinin is about 10 times more active as a vasodilator.

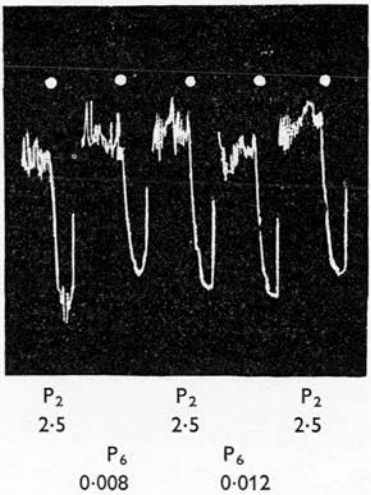

Fig. 1. Isolated rat duodenum suspended in a 10 ml. bath containing oxygenated de Jalon's solution at 30° C. Responses to crude (P_2) and pure (P_6) bradykinin; doses in μg.

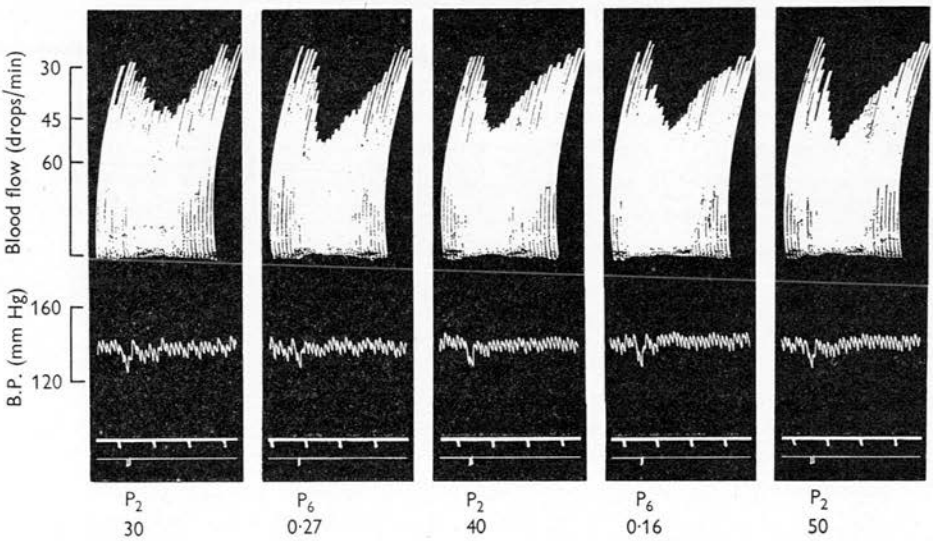

Fig. 2. Venous outflow from skin of hind limb (upper tracing) and arterial blood pressure (lower tracing). Cat, 2·5 kg, sodium pentobarbitone anaesthesia (40 mg/kg.). Intra-arterial injections of P_2 and P_6; doses in μg. Time marker, 30 sec.

Depressor action

Crude plasma kinin has been shown to lower the arterial blood pressure on intravenous injection (Rocha e Silva *et al.* 1949). Pure bradykinin injected intravenously into both anaesthetized cats and rats also caused a fall of arterial blood pressure. The threshold dose in the cat was 400 ng/kg. On a molar basis bradykinin was 10 times less active than acetylcholine and as active as histamine. The threshold dose in the rat was 150 ng/g.

Increased capillary permeability

The action of increasing capillary permeability was demonstrated for crude kinins by Holdstock, Mathias & Schachter (1957) and Herxheimer & Schachter (1959). In the present experiments intradermal injection of the pure peptide into guinea-pigs and rabbits, previously treated with Pontamine sky blue, caused an intense local blueing, indicative of increased capillary permeability (Fig. 3). In both species pure bradykinin increased capillary permeability in concentrations of 10^{-9} to 10^{-8} and therefore on a molar basis bradykinin was approximately 15 times more active in increasing permeability than histamine. Accurate comparison was difficult owing to a qualitative difference between the responses to bradykinin and those to histamine. This difference was particularly pronounced in the rabbit, where histamine produced a ring of blueing surrounding a central area which had been only slightly blued. Bradykinin on the other hand gave rise to an area of intense blueing. Furthermore, with higher concentrations of histamine (10^{-4}) the area of the response was much greater than that produced by the highest concentrations (10^{-5}) of bradykinin tested.

Stimulation of pain fibres

Armstrong, Jepson, Keele & Stewart (1957) showed that crude plasma kinin caused pain when applied to the exposed base of a cantharidin blister. In addition they claimed that large doses caused not only pain but also itching, as well as a flare, and a wheal (Armstrong, Keele, Jepson & Stewart, 1954). In the present experiments with pure bradykinin pain was produced when concentrations of 10^{-7} to 10^{-6} were applied to a blister base, but there was no itching, no flare and no wheal. On a molar basis pure bradykinin was somewhat less active than 5-hydroxytryptamine, which was the most potent pain-producing substance in the experiments of Armstrong *et al.* (1953; 1957). Histamine was usually active at a concentration of 10^{-6} but the sensation produced by histamine was different from that produced by bradykinin or 5-hydroxytryptamine. Histamine caused itching or pricking, whereas bradykinin and 5-hydroxytryptamine caused a burning sensation. Even when histamine 10^{-4} was

applied to the blister base no painful burning sensation resulted. With concentrations of bradykinin 10^{-7} to 10^{-6} the pain was transient; it sometimes disappeared in less than 1 min. With concentrations of 10^{-6} to 10^{-5} pain lasted for 2–3 min.

Repeated administration of bradykinin resulted in a gradual decrease in the sensitivity of the preparation, as is shown by the following experiment. Two exposed blister bases in the same subject were each tested at

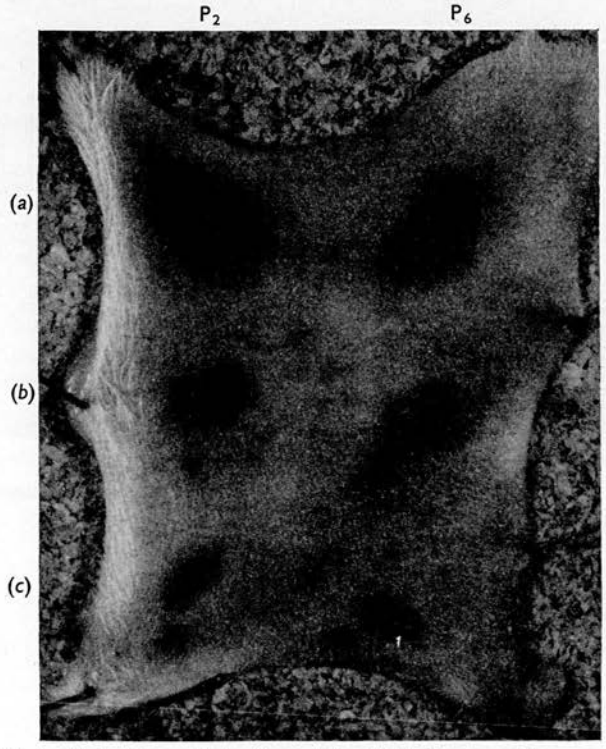

Fig. 3. Effect of intradermal injections of bradykinin on capillary permeability in a guinea-pig injected intravenously with Pontamine sky blue (60 mg/kg). The dark areas represent the areas of blueing 1 hr after the injections. Crude bradykinin (P_2) was injected in doses of (*a*) 1 mg. (*b*) 100 μg and (*c*) 10 μg. Pure bradykinin (P_6) was injected in concentrations of (*a*) 5 μg/ml., (*b*) 0·5 μg/ml. and (*c*) 0·05 μg/ml.

20 min intervals, one with bradykinin 0·1, 1·0, 1·0, 1·0 and 10·0 μg/ml. and the other with histamine 1·0 μg/ml. The application of bradykinin 0·1 μg/ml. produced a painful burning sensation but the effect of 10 times this concentration 20 min later was no greater and no longer-lasting. Subsequent applications of 1 μg, 1 μg and 10 μg/ml. produced no sensations. On the other hand, the five histamine applications to the other blister produced effects without any loss of sensitivity. After a further

20 min the blister base treated with bradykinin failed to respond even to 500 μg/ml. bradykinin, whereas the blister base which had only received histamine responded to 0·1 μg/ml. bradykinin with the usual burning sensation. Finally, both blisters, the one sensitive and the other insensitive to bradykinin, responded with a strong sensation of pain on application of 5-hydroxytryptamine creatinine sulphate 0·1 μg/ml.

DISCUSSION

All the pharmacological actions attributed to the crude plasma kinins were found to be inherent properties of the pure peptide, bradykinin. As a vasodilator substance bradykinin was found to be as active as acetylcholine, the most potent naturally occurring vasodilator substance. In increasing capillary permeability bradykinin was found to be more active than the most active substance, histamine. In producing superficial pain bradykinin was almost as active as 5-hydroxytryptamine.

Isolated smooth muscle preparations are the most sensitive tissues to bradykinin and are therefore the most commonly used in its detection. The rat uterus contracts in the presence of 0·1 ng/ml. of bradykinin, which is therefore about as active as the polypeptide oxytocin on this preparation. The rat duodenum is relaxed by bradykinin; the concentration necessary is less than the concentration of adrenaline which is required to relax it.

The action of bradykinin on the blood vessels is to dilate them and to increase their permeability—this is perhaps its most important action. This pronounced vasodilator action is consistent with the view that bradykinin plays an important role in functional vasodilatation (Hilton & Lewis, 1957). The finding that bradykinin was less active than acetylcholine in lowering the blood pressure, in spite of the fact that acetylcholine is destroyed in the blood much more rapidly, suggests that it acts on a part of the vascular tree different from that on which acetylcholine acts, and which is involved mainly in local vascular effects.

The differences we observed between bradykinin and histamine in their effects on capillary permeability were similar to those described by Menkin (1936) between histamine and a mixture of peptides called 'leukotaxine', and between histamine and crude plasma kinin described by Bhoola & Schachter (1959).

Thus bradykinin in low concentration produces two of the essential reactions of the inflammatory response—vasodilatation and increased capillary permeability. In addition it produces the typical burning sensation which is experienced in conditions of local tissue injury. Thus bradykinin may play the role of chemical mediator of the inflammatory response.

SUMMARY

1. The properties of pure bradykinin have been examined and compared quantitatively with those of a crude preparation of bradykinin.

2. The pure peptide was found to have the following actions: vasodilatation in the skin of the cat's hind limb; depressor action in cats and rats; increased capillary permeability in guinea-pigs and rabbits; contraction of isolated guinea-pig ileum and rat uterus; relaxation of rat duodenum; bronchoconstriction in guinea-pigs; and pain production when applied to the exposed base of a cantharidin blister in man.

3. It therefore seems likely that all the actions so far attributed to the crude plasma kinins can be accounted for by this single peptide, bradykinin.

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Visceral receptors, pain, bradykinin, and analgesic agents

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Pericapillary non-myelinated fibres and branching varicose terminals course alongside, but do not end upon, the capillary wall (methylene blue and silver methods in rat and cat). These pericapillary terminals appear similar to the free-branching 'pain' receptors in cutaneous epithelium (Weddell & Pallie, 1954; Lim, 1960).

The perivascular network in larger vessels containing one or more layers of muscle consists largely of non-myelinated sympathetic efferents, which disappear after sympathectomy. The remaining fibres are derived from myelinated axons and are sensory fibres arising from spinal ganglia. Some of these terminate as pericapillary receptors of the *vasa vasorum* (and *vasa nervorum*).

Intra-arterial injection of KCl (0·5 ml., 18·75 mg), bradykinin* (0·5 ml. = 1·2 μg pure bradykinin) or acetylcholine (0·5 ml., 0·5 mg) in the chloralose dog (80 mg/kg I.V.) evokes a response consisting of vocalization, hyperpnoea and vasomotor change, which disappears on section of the nerves to the organ or part supplied by the injected vessels. On the other hand, intravenous and caval injections are without effect. Intra-arterial injections into the leg after removal of the skin, or into the splenic, gastric, intestinal or internal carotid arteries, or portal vein, all give the response.

Excluding KCl as a general depolarizing agent which may excite axons, and assuming that the other agents excite receptors (Diamond, 1959), it is evident that they are capable of exciting both the cutaneous and the visceral (pericapillary) receptors, which evoke a common response. In view of the fact that bradykinin is the most effective agent and produces pain in man (Armstrong, Keele, Jepson & Stewart, 1954), it may well be one of the agents responsible for the causation of visceral pain under different conditions of inflammation or internal nociception. Rocha e Silva & Antonio (1960) report that thermal injury to the rat's paw liberates bradykinin into the warmed tissues. These observations support the idea that bradykinin acts as a mediator of inflammatory changes (Elliott, Horton & Lewis, 1960).

Preliminary tests have shown that the intravenous administration of sodium acetylsalicylate (ASA) 25–50 mg/kg and *N*-acetyl-*p*-aminophenol

* Crude bradykinin used was from a sample kindly provided by Drs D. F. Elliott and G. P. Lewis and from other batches prepared in this laboratory.

[P.T.O.

(APAP) 10–20 mg/kg may block the response to bradykinin, etc., completely (ASA 4/11, APAP 2/8 dogs), or partially (ASA 5/11, APAP 1/8 dogs), and that morphine sulphate 1·0 mg/kg is the most powerful blocking agent (6/7 dogs). At these dose levels, the response begins to recover in 45–60 min.

These observations suggest that if the features of the response, particularly vocalization, are indicators of visceral pain, the latter may be produced by the action of bradykinin on visceral (pericapillary) receptors and reduced by the action of non-narcotic and narcotic analgesics.

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THE ROLE OF BRADYKININ IN THE PERIPHERAL NERVOUS SYSTEM

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THE ROLE OF BRADYKININ IN THE PERIPHERAL NERVOUS SYSTEM

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In 1957, Keele and his co-workers showed that both plasma and inflammatory exudates after contact with glass cause pain when applied to an exposed blister base on the human forearm. They compared this "pain-producing substance" with various pharmacological agents and concluded that it might be identical with bradykinin but was certainly different from all other known substances (Armstrong, Jepson, Keele and Stewart, 1957).

When bradykinin, prepared from ox plasma, was isolated (Elliott, Horton and Lewis, 1960b), the pure peptide was shown to have potent pain-producing activity, being active in a threshold concentration of 0.1 μg/ml on the blister base, the only known substance of greater activity on this preparation being 5-hydroxytryptamine (Elliott, Horton and Lewis, 1960a). One striking feature we noticed was the marked tachyphylaxis which developed to pure bradykinin.

Intra-arterial Injections of Braydkinin

Additional evidence for the action of bradykinin on the peripheral nervous system came from work on animals. Lim and his colleagues showed that intra-arterial injection of 1 microgram of bradykinin into lightly anaesthetized dogs results in vocalization, increased respiration and a transient rise in blood pressure (Braun, Guzman, Horton, Lim and Potter, 1961; Guzman, Braun, Lim, Horton and Potter, 1961). These effects are reminiscent of the pseudaffective response described by Woodworth and Sherrington in the decerebrate preparation in response to what would in the intact animal have been a painful stimulus (Woodworth and Sherrington, 1904). This similarity of response was strongly suggestive that intra-arterial injections of bradykinin were causing pain. Other known algesic agents such as potassium chloride and acetylcholine also produced this response but were far less active than bradykinin.

Section of the sensory nerve to the limb or organ under test or ipsilateral dorsal root ganglionectomy abolished the response whereas sympathectomy was without effect. The response has been obtained from injections into the femoral, internal carotid, coronary, splenic and mesenteric arteries and into the portal vein. Systemic intravenous injection failed to evoke the response (Guzman, Braun and Lim, 1962).

Histologically, it has been shown that some capillaries in all areas investigated are accompanied by paravascular nerves which terminate in the adjacent connective tissue and free branching unmyelinated receptors (Lim, Liu, Guzman and Braun, 1962). It is postulated that algesic agents such as bradykinin pass through the capillary wall or are formed in the interstitial spaces where they act upon these receptors, afferent impulses passing via the appropriate sensory nerve and dorsal roots.

This pain-producing action of bradykinin is blocked by non-narcotic analgesics such as sodium acetylsalicylate and N-acetyl-p-aminophenol when injected intravenously in the equivalent of therapeutic dosage.

Guinea-pig Bronchoconstriction

Collier and his co-workers have shown that bradykinin-induced bronchoconstriction in guinea-pigs is also antagonized by the same group of analgesics (Collier, Holgate, Schachter and Shorley, 1960; Collier and Shorley, 1960). Two significant features relate these experiments to work on sensory nerve endings. On both the blister base in man and bronchoconstriction in the guinea-pig, the response to bradykinin is markedly tachyphylactic. Secondly, both bronchoconstriction in guinea-pigs and the pain response in dogs are antagonized by the salicylate group of analgesics (FIGURE 1).

	Human blister base	Guinea-pig bronchioles	Dog, visceral pain receptors
Tachyphylaxis	+	+	−
Acetyl salicylate	−	+	+
Denervation	+	−	+

FIGURE 1. Methods of reducing or abolishing bradykinin response on the human blister base, the guinea-pig bronchioles and the dog visceral pain receptors.
+ = Effective method of reducing response

This raised the important question—is the guinea-pig bronchoconstriction a reflex response to bradykinin acting upon afferent nerve endings, the pathway being tachyphylactic like the sensory receptors on the blister base and blocked by aspirin like the vocalization in dogs? Collier has shown that bradykinin also produces bronchoconstriction in the isolated lungs of the guinea-pig and we have shown that the spinal, vagotomized guinea-pig also responds to bradykinin by bronchoconstriction. Thus a pathway involving the central nervous system seems to be ruled out; however, there is the possibility that bronchoconstriction is mediated by the equivalent of an axon reflex within the lungs. There is, however, no evidence for the existence of such a pathway.

Intra-epidermal Injections in Man

Intra-epidermal injections of crude bradykinin in man produce pain and a flare (Harpman and Allen, 1959). Both the flare and the pain were absent when bradykinin was injected into an area of denervated skin, indicating that the flare was the result of an axon reflex. The occurrence of a flare has been confirmed using synthetic bradykinin. 5 micrograms produced a flare about 5 cm. in diameter and lasting for 5 to 10 minutes.

Possible Role of Bradykinin

These experiments in man and animals provide clear evidence that bradykinin is a potent algesic agent, but they do not tell us anything about the role of bradykinin under physiological or pathological conditions. It has been postulated that bradykinin might be a mediator of the inflammatory response, accounting for the vasodilatation, increased capillary permeability and pain. Although its algesic action is undoubted, the ability of bradykinin to account for inflammatory pain, at least in man, is less certain. One finding which raised doubts in my mind was the extremely marked tachyphylaxis which Elliott, Lewis and I observed with pure ox bradykinin. I therefore decided to re-investigate the actions of bradykinin on the human blister base.

Action of Bradykinin on the Blister Base

The marked tachyphylaxis with synthetic bradykinin was confirmed. The threshold concentration was 0.1 μg/ml and doses were applied at 20 minute intervals (FIGURE 2). It has been shown that increasing the dose interval to 60 minutes does not reduce the speed of onset of tachyphylaxis. Sometimes, however, when concentrations which produced only a mild response were

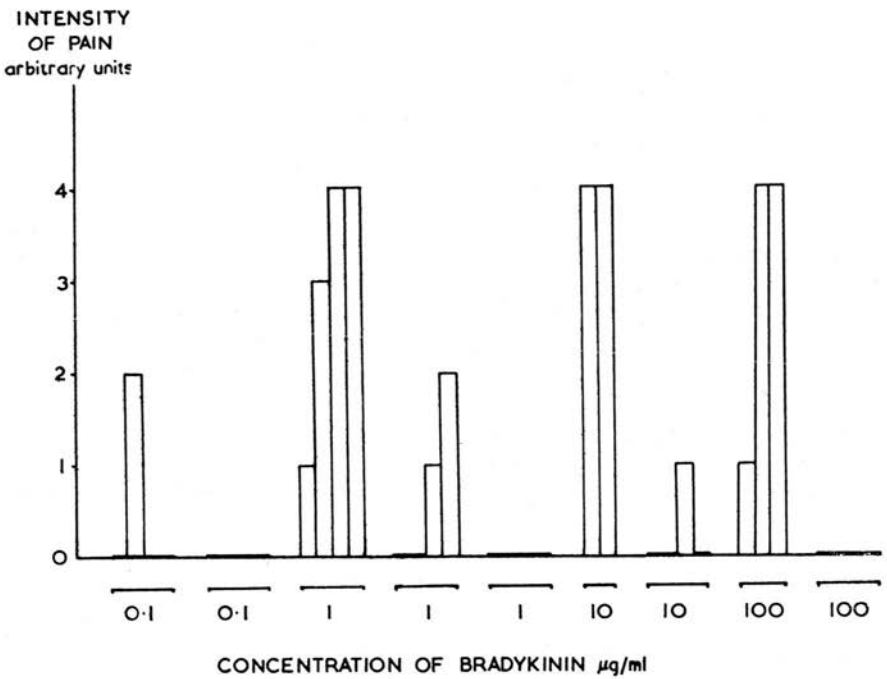

FIGURE 2. Tachyphylaxis to bradykinin on the blister base. Ordinate: Intensity of pain in arbitrary units. Abscissa: Concentration of bradykinin (μg/ml) applied at 20 minute intervals. Pain was assessed every 30 seconds during the period of bradykinin application.

applied repeatedly tachyphylaxis to bradykinin did not occur so readily, but any dose which gave rise to severe pain was always followed by tachyphylaxis. It would seem that doses which cause any appreciable pain are so unphysiological that tachyphylaxis rapidly ensues. However, with the dilutions required to avoid tachyphylaxis the response to bradykinin is so feeble that it is difficult to believe that it can exert a physiological role as an algesic agent.

Could this tachyphylaxis be due to an accumulation of inactivated bradykinin on the receptor sites? It seems likely as we have heard that bradykinin is inactivated by a carboxypeptidase and this would result in the same octapeptide as brief incubation with chymotrypsin. Chymotrypsin attacks first the Phe Arg linkage and less readily the Phe Ser linkage (FIGURE 3) (Elliott, Horton and Lewis, 1960b). Bradykinin solution (10 μg/ml) was incubated with chymotrypsin (0.1 mg/ml) for 5 minutes and then the reaction was stopped by boiling. Control samples without chymotrypsin and without bradykinin were also incubated. The control containing bradykinin produced intense pain and the usual rapid tachyphylaxis ensued. However the chymotryptic digest had no action on the blister base, nor did it affect the development of tachyphylaxis (FIGURE 4).

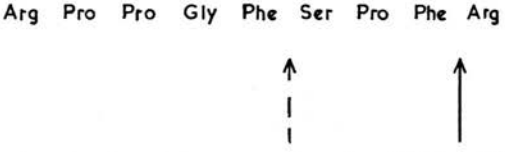

FIGURE 3. The sites of action of chymotrypsin on bradykinin. Primary site of action indicated by solid arrow, secondary site of action by interrupted arrow.

Comparison of Synthetic Bradykinin and Pain-producing Substance

In contrast to our experience with pure bradykinin, Keele and his co-workers, in their paper on pain-producing substance made no mention of the occurrence of tachyphylaxis. I therefore compared the action of glass-activated human plasma with bradykinin on the blister base. The pain producing substance was prepared as follows using siliconed glassware throughout. The plasma was separated from fresh heparinized human blood by centrifugation. It was acidified with $\frac{1}{10}$ volumes of *N*-hydrochloric acid and incubated at 37° for 10 minutes then neutralized with *N*-sodium bicarbonate solution. This acid-treated plasma was divided into two equal portions—one of which was shaken with unsiliconed glass beads—the other was shaken without beads, at 37° C. The appearance of biological activity was monitored by frequent testing of both samples on the isolated rat duodenum. Kinin formation in the tube containing the beads occurred after about 15 minutes at which time no kinin could be detected in the control tube. Without the beads maximum kinin concentration was reached after about 2 hours. The glass-activated kinin solution was heated to 95° C. and was used in the pain experiments. In the concentrations used, the initial application produced marked pain with both pain producing substance and brady-

kinin. Tachyphylaxis developed rapidly with bradykinin but only slowly with glass-activated kinin even though marked pain was felt during the first three applications (FIGURE 5). It was concluded that pain-producing substance must differ in some way from bradykinin, although parallel assays have previously revealed no difference between these substances (Gaddum and Horton, 1959). Is this a species difference, a difference in the mode of

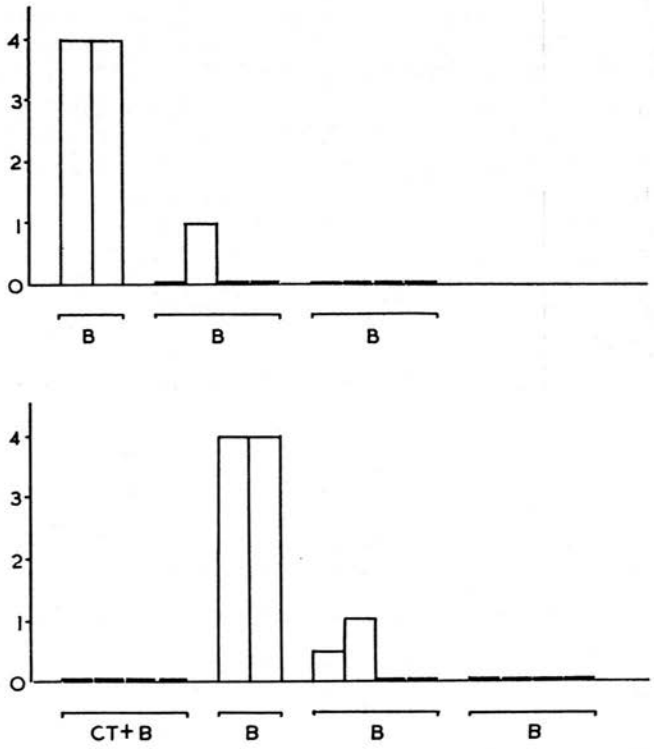

FIGURE 4. Effect of chymotryptic digest of bradykinin on tachyphylaxis. Ordinate: Intensity of pain in arbitrary units. Abscissa: Applications of bradykinin 10 μg/ml (B) and chymotryptic digest (CT + B). Interval between applications 15 minutes. Upper and lower records represent experiments on two different blister bases. Pain was assessed every 30 seconds during the period of application.

formation or due to the presence of interfering substances? A sample of human bradykinin was prepared from human plasma by the same method as that used to prepare glass-activated kinin but adding trypsin (1 mg/ml final concentration) instead of glass beads. The trypsin was finally inactivated by heating to 95° C. This human bradykinin showed the same marked tachyphylaxis as synthetic bradykinin, indicating that the presence of the constituents of plasma does not modify the rate of onset of tachyphylaxis. The incidence of rapid tachyphylaxis appears to depend upon the mode of kinin formation.

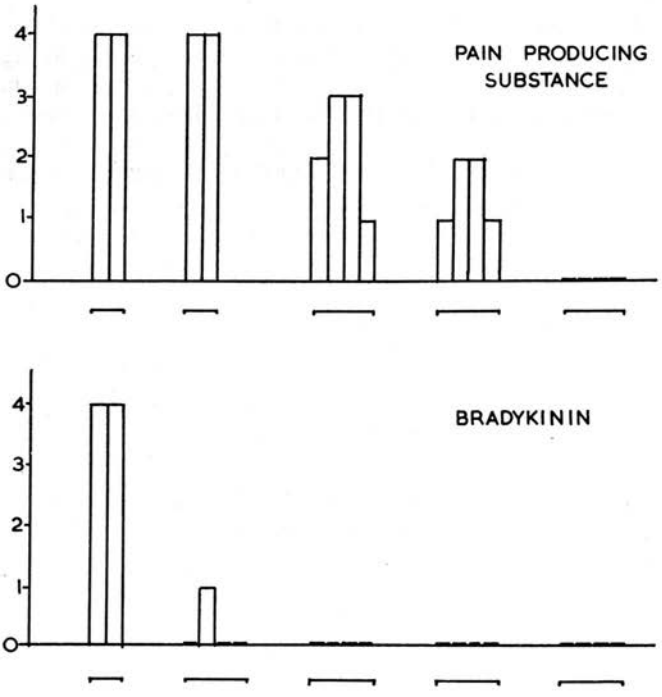

FIGURE 5. Tachyphylaxis to pain producing substance and synthetic bradykinin on two blister bases. Ordinate: Intensity of pain in arbitrary units. Abscissa: Applications of (a) pain producing substance and (b) bradykinin at 15 minute intervals on two blister bases. Pain was assessed every 30 seconds during the period of application.

Conclusion

If the nerve endings subserving pain in inflamed tissues behave like those in the human blister base, release of bradykinin could not account for continued pain since the concentration required to produce this would produce tachyphylaxis. It is possible that the kinin mediator in human inflammation may be a slightly different peptide from the synthetic nonapeptide—showing less tachyphylaxis and thus allowing continued severe pain. However, tachyphylaxis undoubtedly would tend to occur. It is possible that receptors subserving pain in inflamed tissues differ from those subserving cutaneous pain, the visceral receptors described by Lim and his colleagues probably belong to this group. However an alternative explanation is that the mediator of inflammation does not itself cause the pain but lowers the threshold for firing of afferent impulses and so renders the area hyperalgesic to otherwise subthreshold stimuli. It is this action of bradykinin which I believe deserves further study.

Acknowledgment

I am grateful to Dr. E. D. Nicolaides of Parke, Davis and Company, Ann Arbor for the synthetic bradykinin used in these experiments.

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CHAPTER 15

Bradykinin and the Inflammatory Response

*E. W. HORTON, M. B., Ph.D.**

The purpose of this paper is to examine the evidence in support of the working hypothesis that bradykinin is a mediator of the inflammatory response, and to outline what further evidence is required.

Quantitative Aspects

If the changes of the inflammatory response are due to a chemical mediator produced as a direct consequence of the cell damage, then there should be some quantitative relationship between the number of cells damaged, the amount of mediator formed and the extent of the inflammatory changes. The relationship may not be a simple one, but it must have a quantitative element which can be measured.

According to the hypothesis under consideration the following sequence will occur: cell damage; release or activation of plasma kinin forming enzyme; kinin formation; inflammatory response. If the hypothesis is true, it should be possible to relate the amount of plasma kinin forming enzyme released to the amount of cell damage. The evidence so far available is not quantitative enough. It has been shown that heating the skin to above body temperature results in increased bradykinin in the interstitial fluid (1, 2), but it would be more convincing if the amount of bradykinin released were shown to be in direct proportion to the amount of tissue subjected to heat treatment. Lewis in describing his interesting experiments on injury to the dog hind limb has said that the concentration of plasma kinin forming activity is increased 2 to 8.3 times (3). At present these techniques are not sufficiently refined to enable us to obtain a quantitative

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relationship between the amount of enzyme formed and the amount of cell damage.

The second quantitative consideration concerns the ability of the proposed mediator to reproduce the signs of inflammation when injected or infused. Elliott *et al.* (4) showed that pure bradykinin produces vasodilation, increased capillary permeability and pain and that it is one of the most active substances known in these respects. These are the effects seen in the inflammatory response. However, it is not certain that bradykinin can reproduce the changes of the inflammatory response in the concentrations in which it occurs in the interstitial fluid during inflammation.

Stores of Mediator

There is no evidence that bradykinin itself is stored in the body. It is formed from a protein, known as bradykininogen, present in the alpha-2 globulin fraction of blood and interstitial fluid. The evidence so far indicates that bradykininogen levels in the blood are maintained at a relatively constant level. It is possible that, if the hypothesis under discussion is true, a fall in bradykininogen level might be detectable during extensive and prolonged inflammation unless this is accompanied by an increased synthesis of bradykininogen. Conversely if some means could be found to deplete the body of bradykininogen, possibly by blocking its synthesis, it might prove possible to prevent the inflammatory response to injury. Such experiments could provide additional evidence in support of the hypothesis.

Inactivating Enzymes

Inflammation in response to local injury is normally localized to the injured area and its immediate surroundings. Consequently if bradykinin is a chemical mediator of this response, it is likely that its effects will be localized by inactivation before it can reach the systemic circulation. We know that such an enzyme is present in blood and interstitial fluid. Furthermore an inactivator of kallikrein, the plasma kinin forming enzyme, has been found in lymph nodes (5). This is interesting in view of Lewis's finding of increased plasma kinin forming activity in the lymph from injured areas. One might postulate that the kinins are inactivated by kininase very soon after their formation, hence the failure to detect kinins in the lymph as mentioned by Lewis, but that the enzyme on the other hand, is not inactivated until it reaches a lymph node. In both cases access of the active substance to the general circulation would be prevented.

Antagonists

Certain anti-inflammatory agents, notably the salicylates and phenylbutazone have been reported to antagonize some of the pharmacologic

actions of bradykinin. The evidence that the bronchoconstrictor action (6, 7) of bradykinin is blocked by these drugs is convincing, but it is difficult to relate this antagonism to the anti-inflammatory actions of these drugs. What is required is a drug which will block all the pharmacologic actions of bradykinin specifically. This would be a useful research tool, and a substance of potential therapeutic value, which could be used to test the hypothesis that bradykinin is a mediator of the inflammatory response. Support for the hypothesis would be obtained if the antagonist were equally effective in blocking inflammatory responses and the actions of injected bradykinin.

Release of Mediator

A means of inhibiting the plasma kinin forming enzyme, kallikrein, or inhibiting its activation or release could provide further evidence for or against the hypothesis. It has been reported that aspirin and phenylbutazone will antagonize the action of kallikrein both *in vivo* and *in vitro* (8). These observations could not be confirmed by Lewis (9) or in our laboratory. However, if drug inhibition of the enzyme does prove possible the hypothesis would require that the inflammatory response should also be inhibited by the drug in similar concentrations.

This summary of the evidence available and evidence required to substantiate the claim that bradykinin is a mediator of the inflammatory response clearly indicates that more experiments, particularly more quantitative experiments, are needed before a verdict can be given.

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AN INCREASE IN BUTANOL-EXTRACTABLE 5-HYDROXY-TRYPTAMINE IN VENOUS BLOOD DURING REACTIVE HYPERAEMIA

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Reactive hyperaemia which follows temporary occlusion of the vessel supplying a tissue has been attributed to the accumulation of metabolic products during the period of circulatory arrest (Lewis & Grant, 1926). Either carbon dioxide, lactic acid or one of the adenosine compounds could be the vasodilator substance concerned.

Frey, Kraut & Werle (1950) suggested that the slight drop in blood pH which occurs during the period of occlusion might be sufficient to activate the kallikrein system, and thus lead to the formation of plasma kinins which are potent vasodilators. The present investigation was undertaken to test this hypothesis.

By means of a method of extraction designed to recover kinins quantitatively from whole blood, and by assaying the extracts on the isolated rat uterus, an attempt was made to estimate the kinin content of blood collected before and immediately following vascular occlusion of dogs' hind limbs. In none of the experiments could kinins be detected. On the other hand, an apparent increase in 5-hydroxytryptamine (5-HT) was invariably found in extracts of blood from hyperaemic limbs.

METHODS

Extraction of blood. Samples (usually 30 ml.) were collected from the femoral vein via polythene tubing. The blood ran directly into an equal volume of 0·2 N–HCl saturated with sodium chloride. The collecting vessel was placed on a two-pan balance and counterpoised with tares. Weights corresponding to the amount of blood to be collected were added to the opposite pan. Blood was collected until the balance needle just reached the zero mark. The volume was calculated by assuming a specific gravity of 1·060 (see below). Sodium chloride (1 g/3 ml. blood) was added to ensure complete saturation. After the addition of an equal volume of *n*-butanol the mixture was shaken gently by repeated inversion of the bottle. After centrifugation at 2500 rev/min (1000 *g*) for 20 min, the butanol phase was separated by pipetting and stored overnight at −4° C. Little of the hydrochloric acid or sodium chloride pass into the butanol phase. The butanol was extracted with 0·2 vol. distilled water. The aqueous phase was separated and extracted with 5 vol.

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chloroform to remove traces of butanol. The final aqueous solution, which was approximately pH 2, was heated on a boiling water-bath for 10 min to remove traces of chloroform. It was then neutralized with sodium bicarbonate before testing biologically. Routinely, the isolated rat uterus was used, but specificity of the method for kinins was from time to time confirmed by parallel assay on the isolated rat duodenum which relaxes in the presence of kinins (Horton, 1959).

Recovery experiments. In control experiments bradykinin was added to blood which was immediately extracted by the method described above. Recoveries, as estimated on the isolated rat uterus, varied between 83 and 97 % for bradykinin concentrations of 250 ng/ml. and above. Within the range 10–125 ng/ml. recoveries were lower, varying between 17 and 43 %. The minimum concentration of bradykinin which could be detected was 1 ng/ml. whole blood. These recoveries were low, but as they were reproducible it was considered justifiable to use this method in an attempt to detect an increase in kinin concentration in blood.

Estimation of specific gravity of blood. In two experiments the specific gravity of the blood samples was estimated at the time of collection, by the copper sulphate method (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945). The specific gravity was always between 1·058 and 1·062. The error in assuming a specific gravity of 1·060 was less than 0·5 %.

Preparation of the dog hind limb. Dogs of either sex weighing 11–27 kg were used. They were anaesthetized with pentobarbitone sodium 45–75 mg/kg injected intravenously. The trachea was cannulated. All the tissues of one hind limb, except the femoral artery and femoral vein, were divided at the mid-thigh level, diathermy being used for the soft tissues. All muscles were doubly ligated before section and the exposed ends of the femur were filled with plasticine. The femoral vein was cannulated proximally and distally, the two cannulae being connected by a glass T-piece. To produce reactive hyperaemia both the femoral artery and femoral vein were occluded with bull-dog clamps. Carotid blood pressure was recorded with a mercury manometer. The blood pressure tended to fall as the experiment proceeded because of the removal of the relatively large volume of blood as samples. Experiments were discontinued if the mean pressure fell below 85 mm Hg.

Measurement of hind-limb blood flow. Blood flow was measured by timing the collection of the samples with a stop clock.

Isolated rat uterus. Female rats weighing 120–200 g were injected with stilboestrol (10 μg/100 g) 18 hr before use. One uterine horn was suspended in a 5 ml. bath containing de Jalon's solution at 30° C. Doses were added at intervals of 5 min and left in contact with the tissue for 45–60 sec.

Chymotrypsin inactivation. Chymotrypsin (twice crystalline) was dissolved in de Jalon's solution and then added to solutions of the extracts, previously adjusted to pH 7·5. The final concentration of chymotrypsin was 100 μg/ml. The mixture was incubated at 37° C for 10 min and then placed in the boiling water-bath for 5 min.

RESULTS

Oxytocic activity of butanol extracts of whole blood following vascular occlusion

Samples of femoral venous blood were collected before and immediately after vascular occlusion of dogs' hind limbs. The period of occlusion varied in different experiments from 5 to 45 min, but was usually 10 min. Butanol extracts of all the blood samples contained a substance which caused contraction of the isolated rat uterus. In sixteen samples collected

immediately after vascular occlusion the concentration of this oxytocic substance was on average 3·4 times greater than in the control samples. An experiment in which this characteristic increase after vascular occlusion occurred is illustrated in Fig. 1. The results of all the experiments are plotted in Fig. 2. Concentrations of oxytocic substance were expressed in terms of 5-hydroxytryptamine. The mean value ($\pm$ S.E.) for samples collected after vascular occlusion was $16{\cdot}1 \pm 13{\cdot}6$ ng/ml., whereas for the controls it was $4{\cdot}7 \pm 2{\cdot}5$ ng/ml. This difference is statistically significant ($P < 0{\cdot}001$).

The butanol extracts caused a contraction not only of the isolated rat uterus but also of the isolated guinea-pig ileum and the isolated rat duodenum. Unfortunately the sensitivity of these tissues was too low to

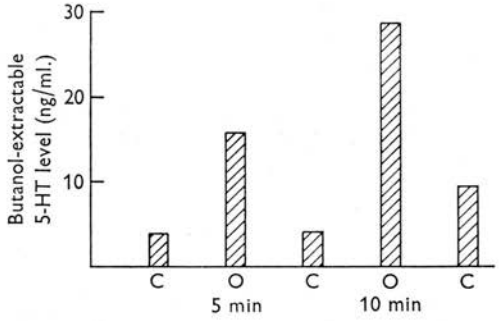

Fig. 1. Concentrations (ng/ml.) of butanol-extractable 5-HT in venous blood collected from the isolated hind limb of a dog (No. 4) before and immediately following vascular occlusion. C = control samples, O = samples collected after 5 and 10 min occlusion. There was a 10-min interval between collection of samples.

allow parallel assays to be carried out. The oxytocic action of the samples was not affected by the presence of atropine sulphate (0·1 μg/ml.) or by incubation with chymotrypsin (100 μg/ml.). On the other hand, their activity like that of 5-hydroxytryptamine, was completely inhibited by lysergic acid diethylamide (0·1 μg/ml.). These results strongly suggested that the substance being estimated was 5-hydroxytryptamine, and consequently all estimates of oxytocic activity have been expressed as ng 5-hydroxytryptamine base/ml. whole blood. On all the tests applied the active principle in the extracts prepared from control blood was qualitatively identical with that in the extracts from blood collected after vascular occlusion.

Recovery experiments. The butanol extraction procedure was developed for the efficient recovery of kinins and was specifically designed to exclude other naturally-occurring pharmacologically active substances such as 5-HT. It was, therefore, unlikely to be a good method for the estimation

of 5-HT. This was confirmed experimentally. Recovery experiments in which 5-HT was added to blood before butanol extraction and was estimated after butanol extraction on the isolated rat uterus showed that this method always extracted less than 1 % of the added 5-HT. In view of this poor recovery and of the probability that the substance being estimated was 5-HT other methods of extraction were investigated.

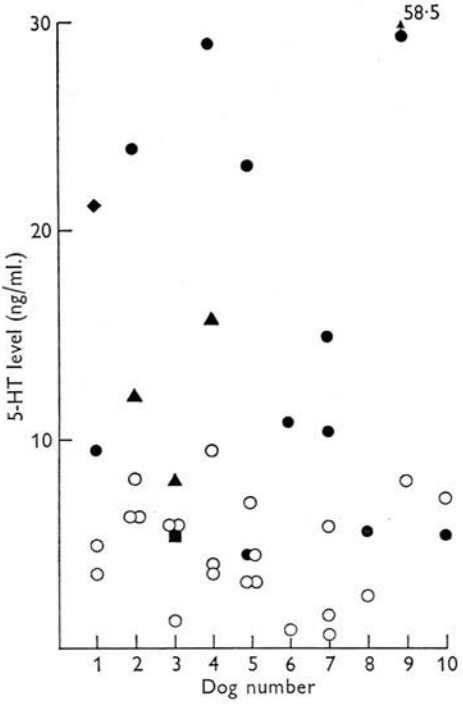

Fig. 2. Scatter diagram of the thirty-eight estimates of butanol-extractable 5-HT in ten dogs. ▲, ●, ■ and ♦ represent samples collected after 5, 10, 20 and 45 min vascular occlusion of the hind limb respectively. ○ indicates control samples collected under resting conditions.

Acetone extraction of whole blood or of plasma, to which 5-HT had been added, gave variable results, but recoveries were always less than 10 %.

A simpler method was tried. Blood samples were allowed to clot and their 5-HT content was estimated on the isolated rat uterus by adding the unextracted serum directly to the organ bath. With this method recoveries of 70–80 % of added 5-HT were obtained. The estimated blood 5-HT levels fell within the range 135–315 ng/ml. (after correction for 75 % recovery, 180–421 ng/ml., or 1·8–4·2 μg/10^9 platelets). Since these values agreed with those found by other workers (Humphrey & Toh, 1954), the

method was considered to be reliable for estimations of 5-HT. Antagonism by lysergic acid diethylamide confirmed that more than 95 % of the activity being estimated was in fact due to 5-HT.

Blood levels of 5-hydroxytryptamine following vascular occlusion

With this direct method of estimating 5-HT in serum, the apparent increase in 5-HT content of blood following vascular occlusion was re-investigated. Blood samples collected before and immediately following a period of vascular occlusion were allowed to clot. The oxytocic activity of the serum was then estimated on the isolated rat uterus. The mean activity expressed as 5-HT of eight samples collected after vascular occlusion was 177 ($\pm$ 70) ng/ml., whereas that of the twelve control samples

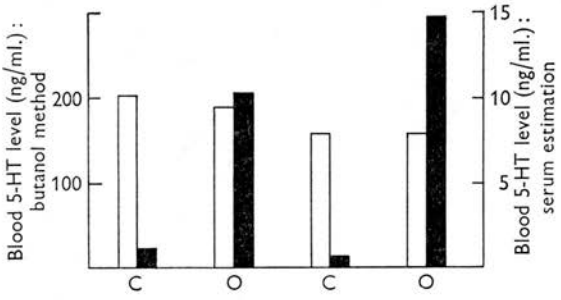

Fig. 3. Concentrations (ng/ml.) of serum 5-HT (□, left-hand ordinate) and butanol-extractable 5-HT (■, right-hand ordinate) in venous blood from the isolated hind limb of dog No. 7 before (C) and immediately following (O) two 10-min periods of vascular occlusion.

was 179 ($\pm$ 80) ng/ml. Thus, when a method was used which recovers most of the 5-HT from blood, no significant difference ($P > 0{\cdot}9$) could be found in its concentration following vascular occlusion. These results were in marked contrast to those found when the butanol extraction procedure was used. A direct comparison was therefore made.

Parallel estimations by the two methods on the same samples. In each of these experiments two blood samples were collected, one before and one immediately after vascular occlusion. Both samples were divided into two portions, one portion being allowed to clot and the other being extracted by the butanol procedure. The two sera and the two butanol extracts were assayed on the isolated rat uterus. In this way parallel estimates by the two methods were made on one and the same blood sample. Previous results were confirmed. Estimated by adding crude serum to the organ bath, the 5-HT content of blood from the occluded limb was no greater than that of the control blood. On the other hand, the 5-HT content was,

as usual, considerably greater in the butanol extract of blood from the occluded limb (Fig. 3). These experiments confirm the conclusion that only the extractable 5-HT and not the total blood 5-HT is increased in reactive hyperaemia. On the average butanol-extractable 5-HT in blood from a non-hyperaemic limb represented only 2·5 % of the total blood 5-HT (compare the two ordinate scales in Fig. 3).

Relation between 5-hydroxytryptamine and rate of hind-limb blood flow

In most experiments the hind-limb blood flow was measured during collection of the samples, and Table 1 shows how the increase in flow rate

TABLE 1. Rates of hind-limb blood flow and blood concentrations of butanol-extractable 5-hydroxytryptamine before and after vascular occlusion

Dog no.	Weight (kg.)	Duration of occlusion (min)	Rate of blood flow (ml./min) (*a*) Before occlusion	(*b*) After occlusion	Increase in flow (*a*)/(*b*)	Blood 5-HT level (ng/ml.) (*c*) Before occlusion	(*d*) After occlusion	Increase in 5-HT (*c*)/(*d*)
1	11	5	60	144	2·40	3·6	9·4	2·60
2 (*a*)	18	5	38	52	1·37	6·3	12·2	1·93
(*b*)		10	24	67	2·79	8·1	24·9	2·94
3 (*a*)	14	5	63	71	1·13	5·6	8·1	1·44
(*b*)		20	49	41	0·84	5·7	5·4	0·95
4 (*a*)	20	5	71	120	1·69	4·0	15·8	3·98
(*b*)		10	45	123	2·73	4·1	28·8	6·96
5 (*a*)	18	10	73	88	1·21	7·0	23·1	3·33
(*b*)		10	41	57	1·39	3·2	4·5	1·39
8	17	10	75	78	1·04	2·5	5·6	2·27
9	16	10	41	69	1·68	8·0	58·5	7·34
10	22	10	86	78	0·91	7·2	5·4	0·75

following vascular occlusion compared with the increase in butanol-extractable 5-HT. In general an increase in flow was associated with a proportionate increase in 5-HT (the coefficient of correlation, r, $= +0{\cdot}63$). In two experiments (dogs 3 (*b*) and 10), where vascular occlusion was followed not by reactive hyperaemia but by a slight diminution in blood flow, the butanol-extractable 5-HT did not increase. These results indicate that the increase in butanol-extractable 5-HT is related to the hyperaemia and not merely to the vascular occlusion.

On the other hand, there appeared to be no correlation between absolute blood-flow rates and amounts of butanol-extractable 5-HT. For example, the resting blood-flow rates in dog 2 (*b*) and 10 were 24 and 86 ml./min. whereas the butanol-extractable 5-HT concentrations were 8·1 and 7·2

ng/ml. respectively. Thus a $3\frac{1}{2}$ times difference in blood flow was associated with little difference in 5-HT concentration.

Since there appeared to be an association between 5-HT and reactive hyperaemia, the effect of 5-HT on blood flow was investigated. Retrograde injections were made via a cannula in the saphenous artery of a hind limb prepared in the usual way. In doses up to 225 μg (base), injected at different rates in different experiments, 5-HT had no effect on the rate of venous outflow from the dog's hind limb. These large doses would certainly have doubled the 5-HT concentration of the blood. The absence of any vascular effect is consistent with the finding that reactive hyperaemia is associated, not with changes in total 5-HT concentration, but with changes in a particular fraction which can be extracted with butanol.

DISCUSSION

Plasma kinins and reactive hyperaemia

Kinins are formed very readily when blood comes into contact with a foreign surface, particularly glass (Armstrong, Jepson, Keele & Stewart, 1957). Furthermore, kinins, whether they are formed endogenously in the blood or they are added to it, are rapidly inactivated by a peptidase (Erdös, Renfrew, Sloane & Wohler, 1963). In order to obtain an estimate of the kinin content of blood *in vivo* at a given instant, it is clearly essential that all enzyme reactions should be stopped at the moment of collecting the blood sample. Not only must the enzyme reactions be stopped, which might be achieved by rapid cooling, but they must also be prevented from recurring during the interval before the biological assay. This can most conveniently be achieved by enzymic denaturation. In the present investigation this was achieved by precipitation with hydrochloric acid.

It is known that when urine is acidified and saturated with sodium chloride, the kinins can be extracted with butanol (Gomes, 1955; Gaddum & Horton, 1959). The method has now proved successful with blood. Recovering by this method averaged 25 % when concentrations of bradykinin as low as 10 ng/ml. were estimated in control experiments. Since the presence of kinins was not detected in samples from hyperaemic limbs, it may be concluded that concentrations of these substances in dogs' blood during reactive hyperaemia are certainly less than 10 ng/ml. and probably less than 1 ng/ml. The failure to demonstrate their presence does not exclude the possibility that kinins are involved in reactive hyperaemia. Kinins are potent vasodilator substances and concentrations of less than 1 ng/ml. may be sufficient to produce the increases in blood flow observed after vascular occlusion. It is possible, therefore, that an increase does occur but that the method used was not sufficiently sensitive to detect it.

5-Hydroxytryptamine and reactive hyperaemia

The identification of the oxytocic substance in the butanol extracts as 5-HT was based upon its biological activity and the effect of specific antagonists. Although such evidence is not conclusive, all other known pharmacologically active substances of natural occurrence have been excluded, with the exception of tryptamine, which is far less active biologically (Gaddum, 1953).

It is apparent from the results of this investigation that only a small fraction (about 2·5 %) of total 5-HT can be extracted from whole blood with butanol, and that it is this fraction, and not the total 5-HT which is increased in reactive hyperaemia. Humphrey & Toh (1954), after taking precautions to avoid platelet damage, found the 5-HT concentration of fresh dog plasma to lie between 2 and 6 ng/ml. In the present investigation a very similar range (2·5 and 8·1 ng/ml.) has been found for the butanol-extractable 5-HT concentration of whole blood.

The positive correlation observed between increase in blood flow and increase in butanol-extractable 5-HT is suggestive that the two changes are not independent, though the relation does not necessarily imply cause and effect. Since intra-arterial injections of large doses of 5-HT did not affect hind-limb blood flow, it seems improbable that 5-HT is the mediator of the vascular changes observed in reactive hyperaemia.

An alternative role is suggested by the experiments of Kwaan & McFadzean (1956). They observed that following arterial occlusion in man the fibrinolytic activity of blood in the occluded limb is increased. Moreover, they showed that intravenous or paravenous injection of 5-HT also increases the fibrinolytic activity (Kwaan, Lo & McFadzean, 1957). This suggests the possibility that vascular occlusion results in the release of (butanol-extractable) 5-HT from the platelets, and that this 5-HT activates the fibrinolytic system and so counteracts any tendency of the blood in the occluded vessels to clot.

SUMMARY

1. Blood collected from dogs' hind limbs during reactive hyperaemia contained no detectable plasma kinins.

2. An oxytocic substance, closely resembling 5-hydroxytryptamine, was found in butanol extracts of whole blood. The concentration of this substance was found to have increased up to seven-fold during reactive hyperaemia.

3. There was no increase in the total blood 5-hydroxytryptamine of which the butanol-extractable 5-hydroxytryptamine represents about 2·5 %.

4. There was a positive correlation between increase in butanol-extractable 5-hydroxytryptamine and increase in blood flow following vascular occlusion. Intra-arterial injection of up to 225 μg 5-hydroxytryptamine (base), however, did not affect hind-limb blood flow.

5. It is postulated as a tentative working hypothesis that 5-hydroxytryptamine is released from platelets during vascular occlusion and that this may lead to increased fibrinolytic activity of the blood, thus counteracting the tendency for coagulation to occur in the occluded vessel.

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A COMPARISON OF THE PLASMA KININ FORMING ENZYMES IN URINE; KALLIKREIN AND UROKINASE

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The experiments of the present paper show that urine contains two enzymes capable of forming plasma kinin; one of these enzymes acts quickly and is probably identical with kallikrein, the other acts more slowly and is probably identical with urokinase.

Kallikrein is the name given by Frey & Kraut (1928) to a non-dialysable depressor substance obtained from urine. This was later shown to be an enzyme capable of acting on plasma proteins to form a vasodilator and smooth-muscle-stimulating polypeptide; this plasma kinin was originally called substance DK (Werle, 1937) and later kallidin (Werle & Berek, 1950).

Urokinase is the name given by Sobel, Mohler, Jones, Dowdy & Guest (1952) to an activator of plasminogen, the precursor of the proteolytic enzyme plasmin (Astrup & Sterndorff, 1952). Subsequent work by Lewis & Work (1957) and Lewis (1958, 1959) has shown that plasmin and its activators form a plasma kinin when incubated with the pseudoglobulin fraction of plasma. Urokinase should therefore form plasma kinin by first activating plasminogen. The question thus arose whether urinary kallikrein and urokinase were identical, both forming kinins by first activating plasminogen. The results show first a distinct difference in the rate of plasma kinin formation with the two enzymes. Secondly, the urokinase was weight-for-weight 300 times more potent in activating plasminogen, whereas the kallikrein was 40–50 times more potent in producing vasodilatation and about seven times more potent in contracting the dog's ileum.

METHODS

Preparation of kallikrein. Pooled male human urine was added to 4 vol. acetone at 4° C. The precipitate was collected by filtration, dissolved in a small volume of water and dialysed against running tap water for 48 hr. The dialysed solution was then freeze-dried and stored at 4° C.

Urokinase was kindly supplied by Dr J. Ploug, of Leo Pharmaceuticals, Copenhagen, and was prepared by the method of Ploug & Kjeldgaard (1957). The essential steps of this procedure are adsorption on silica gel, precipitation at pH 1·5, dialysis, and chromatography on IRC-50.

The kallikrein and urokinase extracts were compared in the following tests:

Plasma kinin formation. The enzymes were incubated with a pseudoglobulin solution at 34° C, and samples from the incubated mixture were tested at intervals on the isolated guinea-pig's ileum suspended in 15 ml. of Mg-free Tyrode solution at 34° C. The pseudoglobulin was prepared from dog plasma by $(NH_4)_2SO_4$ fractionation: the fraction which precipitated between 33 and 46 % saturation was dissolved in water, dialysed for 48 hr against running tap water and freeze-dried. For use the powder was dissolved in 0·9 % NaCl solution in a volume equal to the original volume of plasma.

Perfusion of dog's skin. Dogs were anaesthetized with pentobarbitone sodium 40 mg/kg. The two enzyme preparations were injected via the saphenous artery into a patch of skin prepared as has already been described for cats (Lewis, 1958). The outflow from the saphenous vein was recorded with a Gaddum drop recorder.

Isolated dog's ileum. The smooth-muscle-stimulating activity of each enzyme preparation was tested on a 4 cm segment of terminal ileum suspended in a 25 ml. bath of Tyrode solution at 34° C, containing atropine 10^{-6}.

Activation of plasminogen. When plasminogen is activated plasmin is formed, which is able to digest casein with the production of free tyrosine, giving rise to absorption in u.-v. ight at 275 mμ. The rate of plasminogen activation can therefore be estimated by measuring the increase in optical density at 275 mμ.

Plasminogen was prepared from citrated human plasma by the method of Kline (1953); and goat casein by the method of Müllertz (1955). The incubation mixture contained: casein (3 %, w/v), 3·5 ml., 0·1 M phosphate buffer, 3·5 ml.; plasminogen 14 mg, and varying amounts of either kallikrein or urokinase. The mixture was incubated at 34° C, and 2 ml. samples were transferred at 0, 10 and 20 min to tubes containing 3 ml. of 1·7 M perchloric acid. The precipitated proteins were separated by centrifugation and the increase in optical density of the supernatant at 275 mμ was measured in a Hilger Uvispek absorption spectrophotometer.

Antiplasmin (antifibrinolysin, 6 u./mg) was a gift from Dr E. C. Loomis of Parke, Davis and Company.

RESULTS

Rate of formation of plasma kinin. When the two purified enzyme preparations were incubated with pseudoglobulin it was found that urokinase was a slow-acting enzyme and that kallikrein was a quick-acting enzyme. Thus, in the experiment of Fig. 1, on incubation of the kallikrein preparation with pseudoglobulin maximum formation of plasma kinin occurred after 1·5 min (Fig. 1*A*), but when the urokinase was incubated with pseudoglobulin, maximum formation occurred after 10 min, as is shown in Fig. 1*B*.

To distinguish two enzymes by their rate of action is open to the criticism that the difference is due only to their concentrations. This criticism does not apply to these results, as is shown by the following experiment. Various concentrations of the kallikrein (25, 50, 100 and 200 μg/ml.) and of the urokinase preparation (250, 500 and 1000 μg/ml.) were incubated with a standard concentration of pseudoglobulin and the mixture was tested at various intervals of time. Although the time of maximum plasma kinin formation occurred later as the concentration of the kallikrein decreased, the maximum formation took not more than

10 min (Fig. 2). With the lowest concentration of kallikrein (Fig. 2 *D*) there was little or no plasma kinin formation. On the other hand, when urokinase was similarly incubated with pseudoglobulin in various concentrations, increasing the urokinase concentration occasionally decreased the time of maximal formation, although this did not occur before 30 min (Fig. 3).

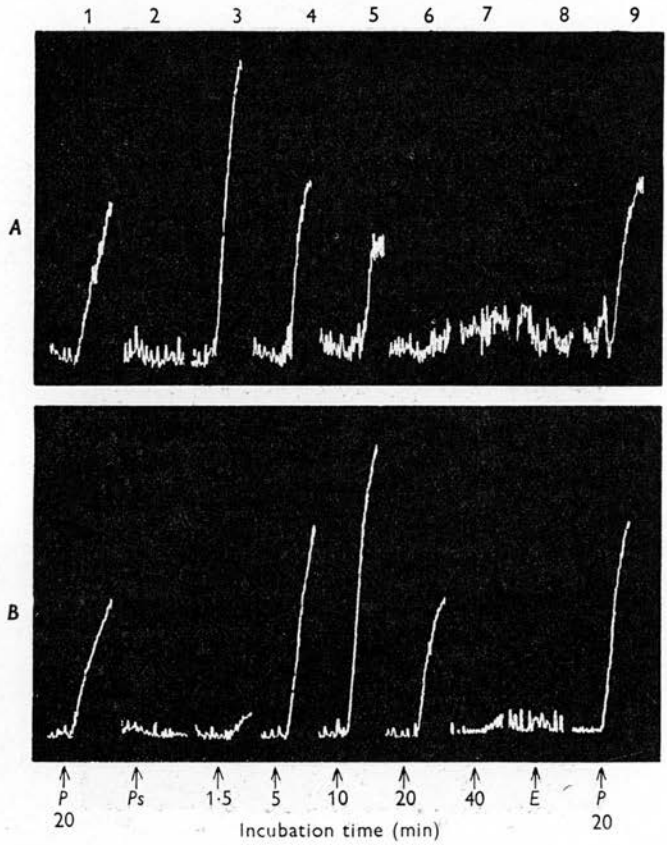

Fig. 1. Guinea-pig ileum preparation suspended in 15 ml. of Tyrode solution. At *P* response to 20 μg of standard plasma kinin, at *Ps* 0·1 ml. pseudoglobulin solution. Contractions 3–7 produced by successive 0·1 ml. samples taken from an incubated mixture of 2 ml. pseudoglobulin solution with 2 mg kallikrein (*A*), and of 2 ml. of pseudoglobulin solution with 1 mg of urokinase (*B*). At *E*, kallikrein 200 μg (*A*) and urokinase 100 μg (*B*).

It is not possible to make an accurate comparison of the potencies of urokinase and the urinary kallikrein in forming plasma kinins because of the differences in the time of formation. But weight-for-weight the kallikrein preparation was usually more active than the urokinase preparation.

The plasma kinin forming activity of human urine can be readily explained in terms of these two plasma kinin forming enzymes. When freshly collected human urine (dialysed against running tap water for 4–8 hr) was incubated with pseudoglobulin solution, the mixture developed smooth-muscle-stimulating activity within 1–5 min, reaching a maximum

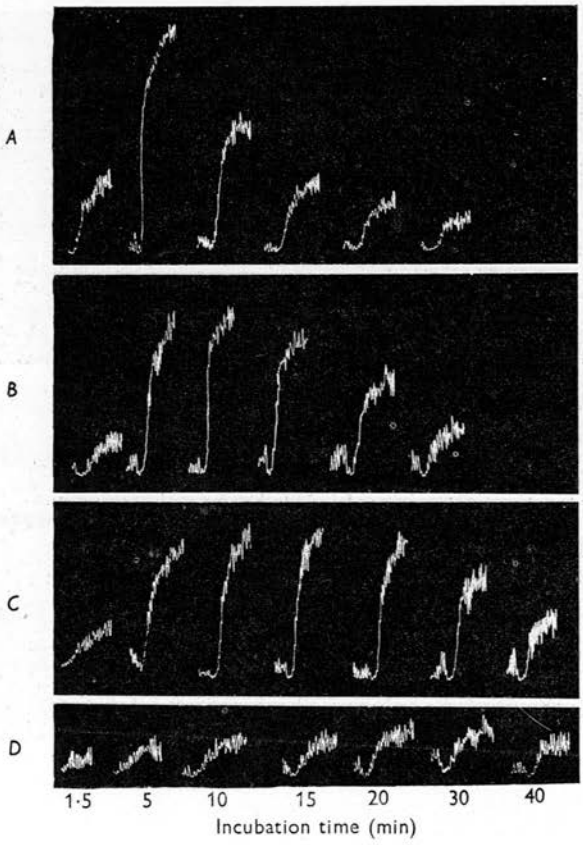

Fig. 2. Contractions of the isolated guinea-pig ileum produced by successive samples containing pseudoglobulin solution 0·1 ml. and kallikrein *A* 200 μg, *B* 100 μg, *C* 50 μg and *D* 25 μg, incubated for times shown.

at 5 min. The amount of kinin in the incubation mixture was undiminished after 30 min, despite the presence in pseudoglobulin of an enzyme which is known to inactivate the kinin (Fig. 4*A*). However, when the experiment was repeated in the presence of the plasmin inhibitor antiplasmin (3 mg/ml.), the maximum activity was reached in 5 min, but subsequently the amount of kinin decreased and after incubation for 30 min had fallen considerably (Fig. 4*B*). These results can be explained by the

presence in dialysed urine of two enzymes: one a slow-acting plasma kinin forming enzyme (urokinase) which was inhibited by antiplasmin, and the other a quick-acting enzyme (kallikrein) which was resistant to antiplasmin.

Perfusion of the dog's skin. Arterial injection of both the kallikrein and the urokinase preparations caused vasodilatation in the dog's skin. In the experiment illustrated in Fig. 5, dilatation of about the same order was obtained with 5 mg of the urokinase preparation and with 125 μg of the kallikrein. Usually the kallikrein was 40–50 times more active.

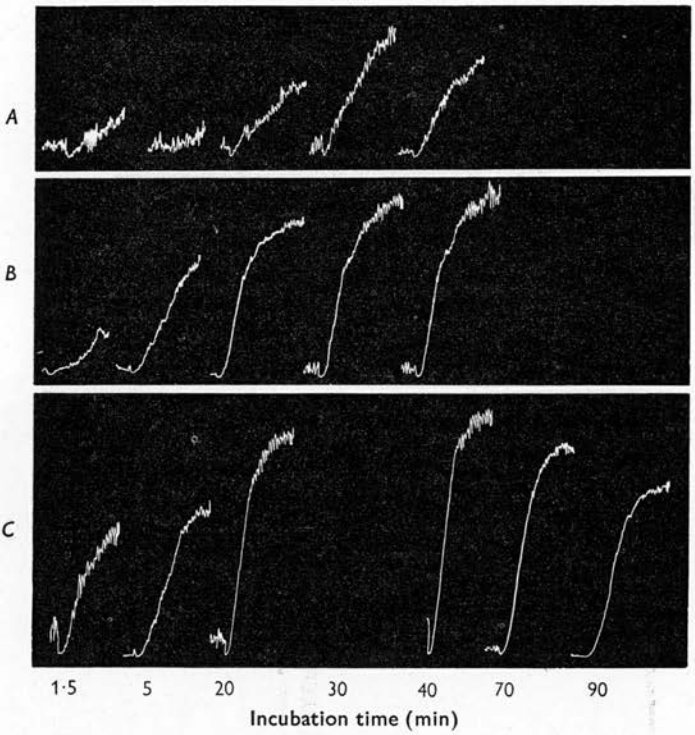

Fig. 3. Contractions of the isolated guinea-pig ileum to a mixture of pseudoglobulin solution 0·1 ml. and urokinase, *A* 250 μg, *B* 500 μg, and *C* 1000 μg; incubated for times shown. The mixtures in *A* and *B* were not tested at 70 min and 90 min, and the mixture in *C* was not tested at 30 min.

Isolated dog's intestine. Both the kallikrein and the urokinase preparation produced contractions of the isolated dog's ileum. When the two preparations were compared the kallikrein was not fifty, but only seven, times as active as the urokinase preparation. There was, in addition, a qualitative difference in that the contraction due to urokinase was sometimes steeper than that caused by the kallikrein (Fig. 6). It was observed

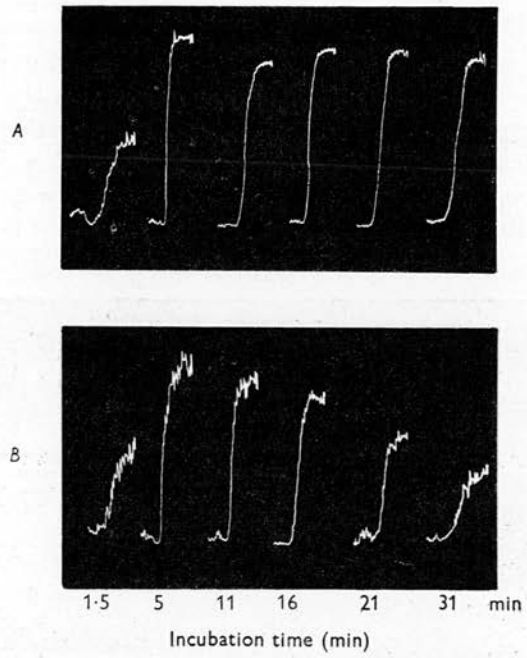

Fig. 4. Contractions of the isolated guinea-pig ileum produced by successive samples of 0·4 ml. taken from a mixture of dialysed human urine 3 ml. and pseudoglobulin solution 1 ml. (*A*) and from the same mixture with antiplasmin 3 mg/ml. (*B*), incubated for times shown.

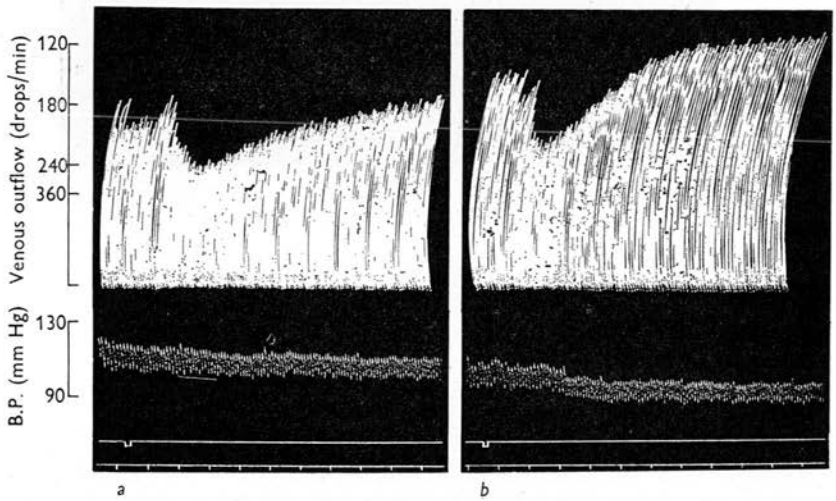

Fig. 5. Venous outflow from skin of hind limb (upper tracing) and arterial blood pressure (lower tracing). Dog, 14 kg, sodium pentobarbitone anaesthesia (40 mg/kg). At (*a*) 5 mg urokinase and at (*b*) 125 μg kallikrein injected arterially. Time, 30 sec.

that this tissue responded slowly even to very large doses of kallikrein (5–10 mg).

Plasminogen activation. The results shown in Fig. 7 confirm the fact that urokinase is a potent activator of plasminogen; on the other hand, kallikrein was about 300 times less active in this respect. The low activity was not due to the presence in the kallikrein extract of an inhibitory substance, since it did not inhibit the action of small doses of the urokinase preparation when mixed with it. In the absence of plasminogen neither the urokinase preparation nor the kallikrein caused hydrolysis of casein, as measured by the optical density at 275 mμ.

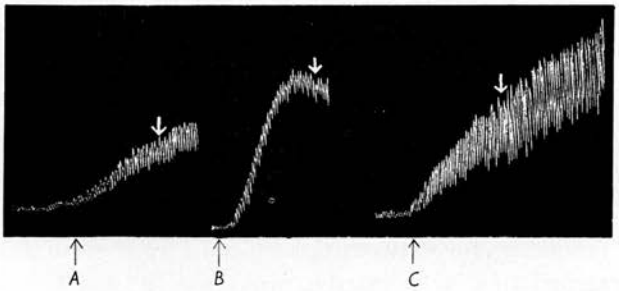

Fig. 6. Isolated dog's ileum suspended in 25 ml. of atropinized (10^{-6}) Tyrode solution. At (*A*) kallikrein 250 μg and at *C* 500 μg; at *B* urokinase 5 mg. The drugs were in contact with the tissue for 2 min and injections were repeated at 15 min intervals.

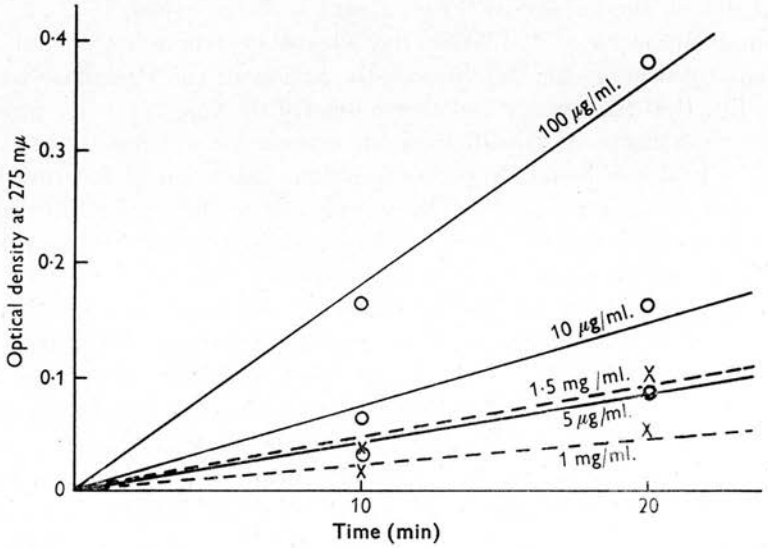

Fig. 7. Spectrophotometric measurements of the activation of plasminogen to plasmin by urokinase, ○—○, in concentrations shown; and by kallikrein, × – – – ×.

DISCUSSION

The present experiments show that human urine contains two enzymes which form plasma kinins on incubation with pseudoglobulin. They differ in their rate of kinin formation. One is a relatively quick-acting, the other a relatively slow-acting enzyme. In the kallikrein preparation from urine the main activity is due to the quick-acting enzyme, whereas the action of the urokinase preparation is due to the action of the slow-acting enzyme. In these experiments only partially purified urinary extracts have been used, and the presence of a small amount of the slow-acting enzyme in the kallikrein, and vice versa, is not excluded.

It is clear from the assays of the two principles on various preparations that their effects cannot be due to the same active substance. As a vasodilator kallikrein was 45 times more active than urokinase, but for plasma kinin formation the activity ratio of kallikrein to urokinase was less than 7. As an activator of plasminogen kallikrein had only 1/300th of the activity of urokinase, but in stimulating the dog's isolated intestine kallikrein was about seven times more active.

Since the procedure for extracting kallikrein from urine did not include any step designed to inactivate the urokinase, it was possible that the plasminogen-activating action of the kallikrein extract is due to the presence in it of traces of urokinase. On the other hand, the preparation of the urokinase involved precipitation at low pH, which would inactivate most if not all the kallikrein (Frey, Kraut & Werle, 1950).

If small amounts of kallikrein did escape destruction they might be sufficient to account for the vasodilator action of the urokinase extract. The finding that the latency and time course of the vasodilatation produced by the two extracts do not differ might be regarded as evidence in support of this. Urokinase has a slower action than kallikrein in forming kinins *in vitro*; if it is assumed that the vasodilatation following injections of these substances is due to the production of kinins *in vivo*, then urokinase would be expected to have a slower vasodilator action.

Plasmin, or plasminogen with an activator, will form plasma kinins when incubated with pseudoglobulin. The formation proceeds slowly and can be inhibited by antiplasmin (Lewis, 1958). Since urokinase also forms kinins slowly and since it is a potent activator of plasminogen, it seems likely that it forms kinins by first activating plasminogen to plasmin. In contrast, kallikrein forms kinins more quickly and since it has been shown to have negligible, if any, plasminogen-activating action and is not antagonized by antiplasmin, it probably acts independently of the plasminogen system. It is concluded that the two urinary fractions, urokinase and kallikrein, are not identical but are capable of forming plasma kinins by different mechanisms.

SUMMARY

1. Partially purified extracts of urokinase and kallikrein have been compared by parallel assays. Urokinase was 300 times more potent as a plasminogen activator, whereas kallikrein was 40–50 times more potent as a vasodilator and 7 times more potent as a smooth-muscle stimulator. It is concluded that the two active principles are different.

2. Urokinase forms a plasma kinin when incubated with pseudoglobulin. This reaction proceeds slowly, is inhibited by antiplasmin, and is probably due to the activation of plasminogen to plasmin.

3. Kallikrein forms plasma kinins more quickly and is thought to act independently of the plasminogen system.

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10

ACTION OF PROSTAGLANDIN E_1 ON TISSUES WHICH RESPOND TO BRADYKININ

By

Dr. E. W. HORTON

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Action of Prostaglandin E_1 on Tissues which respond to Bradykinin

PROSTAGLANDIN E_1 (9-keto, 11α, 15, dihydroxy-prost-13-enoic acid, PGE_1) was the first of several prostaglandins to be isolated by Bergström *et al.*[1,2]. It is a member of a chemically new group of vasodilator substances. Other naturally occurring vasodilators such as bradykinin, acetylcholine and histamine have effects on several types of tissue in addition to smooth muscle. For example, bradykinin increases capillary permeability, produces pain on application to a blister base and releases adrenaline from the adrenal medulla[3,4]. The actions of PGE_1 on these and other biological preparations have been investigated using bradykinin as a standard for comparison. The results are summarized in Table 1.

The vasodilator activity of PGE_1 was confirmed on the flow of blood in the hind-limb of a cat. Like bradykinin, it increased capillary permeability as shown by intradermal injection into guinea pigs pre-treated with Pontamine sky blue. A dose of 1 μg (in 0·1 ml.) produced a maximal effect. The intensity of bluing with PGE_1 was less for a given area of lesion than with bradykinin and the very intense bluing produced by bradykinin was never observed with PGE_1. Estimates of relative activity were therefore difficult to make but PGE_1 was at least 10–100 times less active than bradykinin.

PGE_1, unlike bradykinin (0·1 μg/ml.), did not cause pain on application to human blister bases in concentrations from 0·1 to 100 μg/ml. Retrograde injections of bradykinin (2 μg) into the cœliac artery of the 'Nembutal' anæsthetized cat released the equivalent of 1 μg of adrenaline from the adrenal medulla as detected by the denervated nictitating membrane. PGE_1 did not cause any detectable adrenaline release ($< 0{\cdot}2$ μg) in doses up to 20 μg.

Table 1. THRESHOLD DOSES OR CONCENTRATIONS OF PROSTAGLANDIN E_1 AND BRADYKININ REQUIRED TO PRODUCE EFFECTS ON THE BIOLOGICAL PREPARATIONS USED IN THIS INVESTIGATION

Response	Preparation	PGE_1	Bradykinin
Vasodilatation	Cat hind limb	200 ng	20–100 ng
Increased capillary permeability	Guinea pig skin	100 ng	1–10 ng
Pain production	Human blister base	No effect (> 100 μg/ml.)	0·1 μg/ml.
Adrenaline release	Cat adrenal medulla	No effect ($> 7{\cdot}5$ μg/kg)	0·15 μg/kg
Reduced air entry	Guinea pig lungs	No effect (> 50 μg/kg)	4–10 μg/kg
Contraction of isolated smooth muscle	Rat uterus	60 ng/ml.	0·5 ng/ml.
	Guinea pig ileum	8 ng/ml. (Contraction)	10 ng/ml. (Relaxation)
	Rat duodenum	250 ng/ml.	5 ng/ml.

PGE_1 also differed from bradykinin in that it did not reduce air entry into the lungs of the guinea pig as measured by the Konzett and Rössler method. Like bradykinin, however, it caused contraction of the isolated rat uterus and the isolated guinea pig ileum; it further contracted the isolated rat duodenum, which is relaxed by bradykinin.

I thank Prof. S. Bergström of the Karolinska Institute, Stockholm, for the prostaglandin E_1, and Dr. E. D. Nicolaides, of Parke, Davis and Co., Ann Arbor, for the synthetic bradykinin used in these experiments.

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A COMPARISON OF THE BIOLOGICAL ACTIVITIES OF FOUR PROSTAGLANDINS

BY

E. W. HORTON and I. H. M. MAIN

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A COMPARISON OF THE BIOLOGICAL ACTIVITIES OF FOUR PROSTAGLANDINS

BY

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From the Miles-Ames Research Laboratories, Stoke Poges, Bucks

(*Received April* 25, 1963)

The biological activities of prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$ have been compared. Prostaglandins E_1, E_2 and E_3 were qualitatively similar; E_1 and E_2 were about equiactive, but E_3 was less active on all preparations. Prostaglandin $F_{1\alpha}$ was a less potent vasodilator than E_1 on the cat gastrocnemius muscle blood flow and skin blood flow and a less potent depressor drug on rabbit blood pressure. On the rabbit isolated jejunum $F_{1\alpha}$ was twice as active as E_1 but on the guinea-pig isolated ileum E_1 was about forty times more active than $F_{1\alpha}$. One qualitative difference between these prostaglandins was observed; on the rabbit fallopian tube *in vivo* prostaglandins of the E series decreased both the tone and the peristalsis of the tube whereas prostaglandin $F_{1\alpha}$ increased tubal tone.

Goldblatt (1933, 1935) and Euler (1934, 1935a) independently described the presence of a substance which contracted smooth muscle and lowered blood pressure and occurred in semen and extracts of prostate glands. The active principle was given the name " prostaglandin " (Euler, 1935b).

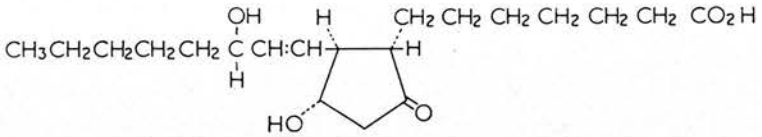
More recently Bergström and his colleagues have isolated several different prostaglandins from sheep prostate gland (Bergström & Sjövall, 1960a, b; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962), sheep semen (Bergström, Krabisch & Sjövall, 1960), sheep and pig lung (Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962) and human semen (Bergström & Samuelsson, 1962).

Six naturally-occurring prostaglandins have been described. Their structures have been elucidated by Bergström and his co-workers (Bergström & Samuelsson, 1962; Bergström *et al.*, 1962a, b). The three prostaglandin E's differ only in the number of double bonds. Reduction of the keto-group in a prostaglandin E can give two isomeric alcohols (Bergström *et al.*, 1962b), those from prostaglandin E_1, for example, being referred to as $F_{1\alpha}$ and $F_{1\beta}$ (Bergström, Ryhage, Samuelsson & Sjövall, 1963).

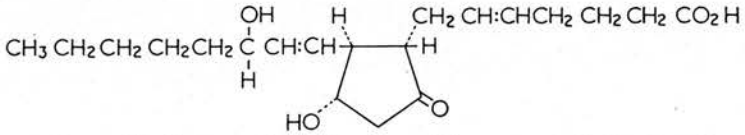
In the present investigation the actions of four prostaglandins (E_1, E_2, E_3 and $F_{1\alpha}$) (Fig. 1) have been compared on various biological preparations. Some of these comparisons have been made previously (Bergström, Eliasson, Euler & Sjövall, 1959; Euler & Bergström, unpublished).

METHODS

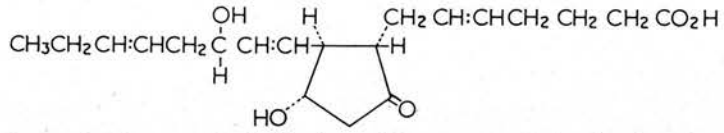
Isolated smooth muscle preparations. Segments of various organs were suspended in a 4 ml. organ-bath. Longitudinal contractions were recorded isotonically with a frontal-writing lever on



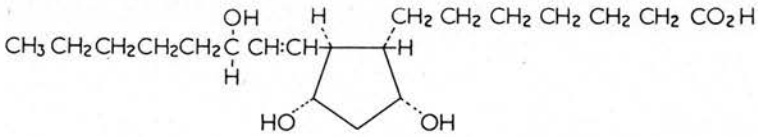
Prostaglandin E_1. 11α,15-dihydroxy-9-keto-prost-13-enoic acid



Prostaglandin E_2. 11α,15-dihydroxy-9-keto-prost-5,13-dienoic acid



Prostaglandin E_3. 11α,15-dihydroxy-9-keto-prost-5,13,17-trienoic acid



Prostaglandin $F_{1\alpha}$. 9α,11α,15-trihydroxy-prost-13-enoic acid

Fig. 1. Formulae of the four naturally-occurring prostaglandins studied in this investigation.

a smoked drum, or isometrically with a force-displacement transducer (Grass FT.03) and an ink-writing polygraph. A dose-cycle of 4 to 6 min was used with 45 to 90 sec contact time.

Guinea-pig ileum. Terminal ileum, from guinea-pigs weighing 200 to 400 g, was suspended in Tyrode solution at 37° C, gassed with air.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 1.5 to 2.5 kg, was suspended in Tyrode solution at 35 to 36° C, gassed with air.

Hamster colon. The ascending colon, from hamsters weighing 100 to 200 g, was suspended in de Jalon solution at 35 to 36° C, gassed with air.

Rat uterus. A segment of uterine horn, from rats weighing 150 to 250 g which had been injected subcutaneously, 18 hr previously, with stilboestrol (100 μg), was suspended in de Jalon solution at 32° C, gassed with air.

Pithed rat blood pressure. Rats, weighing 200 to 350 g, were anaesthetized with ether and pithed by passing a strong wire through the orbit and down the cerebrospinal axis. Artificial ventilation was maintained by a Palmer small-animal respiration pump. Blood pressure was recorded from a carotid artery via a cannula connected to a Statham P23A transducer. Injections were made into a jugular or femoral vein.

Cat gastrocnemius muscle blood flow. Cats, weighing 2.5 to 5 kg, were anaesthetized with sodium pentobarbitone (40 mg/kg) injected intraperitoneally. The trachea, a carotid artery and a jugular vein were cannulated. Blood pressure was recorded by a mercury manometer. The femoral vessels were ligated beyond the branches to the gastrocnemius muscle. All other branches and tributaries proximal to this level were ligated, and the artery supplying the gracilis muscle was

cannulated with fine polyethylene tubing connected to a three-way tap for retrograde intra-arterial injections. Blood flow was maintained by stimulation of the sciatic nerve (3 to 4 V, 0.2 msec duration, 6 shocks/min). Heparin (1,000 U/kg) was injected intravenously and further doses of 500 U/kg were given every 2 hr. Venous outflow was recorded by passing blood from the femoral vein through a Palmer drop-chamber connected to a Gaddum drop-recorder or Thorp impulse-counter, or through a Grass drop-chamber connected to a Grass polygraph, the blood being returned into the jugular vein.

Cat hind-limb skin blood flow. The preparation was identical to that used for recording gastrocnemius muscle blood flow, except that the femoral artery and vein were ligated immediately distal to the saphenous vessels, which were left intact.

Fallopian tubal tone and peristalsis. Rabbits, weighing 1.5 to 3 kg, were anaesthetized with intraperitoneal urethane (175 mg/kg). The trachea was cannulated. A jugular vein was cannulated for intravenous injections. Blood pressure was recorded from a carotid artery with a Statham transducer. The abdomen was opened with a mid-line incision, the alimentary viscera were displaced to one side and a fallopian tube was identified. A polyethylene cannula was inserted into the uterine end of the tube through an incision in the uterine horn and tied in position, taking care not to occlude tubal blood supply. The cannula was attached to a Statham transducer P23AC, the side-limb of which was connected via polyethylene tubing to a 20 ml. syringe placed in a Palmer slow-injection apparatus.

The perfusion system from syringe to cannula contained Tyrode solution. An inflow rate of 27 μl./min was usually adequate to stimulate peristalsis, which was recorded with a suitable pen-writer.

RESULTS

Prostaglandins E_2, E_3 and $F_{1\alpha}$ were assayed by bracketing against prostaglandin E_1 on eight different biological preparations. The activities expressed relative to prostaglandin E_1 (=1) are shown in Table 1. The threshold doses of prostaglandin E_1 are shown in Table 2.

Isolated smooth muscle preparations. Each of the four prostaglandins contracted smooth muscle. In general, prostaglandin E_2 was slightly more active, and prostaglandin E_3 rather less active, than E_1. Prostaglandin $F_{1\alpha}$ was twice as active as E_1 on the rabbit jejunum (Fig. 2) but about forty times less active on the guinea-pig ileum (Fig. 3). The guinea-pig ileum, rabbit jejunum and hamster colon were

TABLE 1

BIOLOGICAL ACTIVITY OF PROSTAGLANDINS E_2, E_3 AND $F_{1\alpha}$ RELATIVE TO PROSTAGLANDIN E_1

Figures represent mean activity (±standard errors) relative to prostaglandin E_1 (=1). Numbers of assays are shown in parentheses. * In four of five preparations prostaglandin $F_{1\alpha}$ increased the tone of the fallopian tubes, in contrast to the prostaglandin E's which caused relaxation

Preparation	Biological activity of E_2	E_3	$F_{1\alpha}$
Guinea-pig ileum	1·56±0·78 (6)	0·23±0·19 (3)	0·023±0·013 (4)
Hamster colon	2·75±1·50 (4)	0·19±0·14 (4)	0·26 ±0·16 (4)
Rabbit jejunum	1·50±0·50 (3)	0·99±0·90 (3)	2·22 ±1·72 (2)
Rat uterus	1·09±0·37 (3)	0·31±0·03 (2)	0·94 ±0·75 (4)
Cat gastrocnemius muscle blood flow	0·76±0·31 (4)	0·53±0·46 (3)	0·22 (1)
Cat hind limb skin blood flow	0·91 (1)	0·23 (1)	0·22 (1)
Rabbit blood pressure	1·00±0·00 (4)	0·34±0·03 (2)	0·075±0·04 (2)
Rabbit fallopian tube	0·93±0·08 (4)	0·43±0·16 (4)	*

TABLE 2

THRESHOLD DOSES OF PROSTAGLANDIN E_1

Figures indicate concentrations (in ng/ml.) for the isolated tissues and doses (in μg/kg) for the *in vivo* experiments

Guinea-pig isolated ileum	Contraction	8
Hamster isolated colon	Contraction	12
Rabbit isolated jejunum	Contraction	12
Rat isolated uterus	Contraction	60
Cat gastrocnemius muscle blood flow	Vasodilatation	0·5
Cat skin blood flow	Vasodilatation	0·1
Rabbit blood pressure	Depression	0·6
Rabbit fallopian tube	Inhibition	0·8

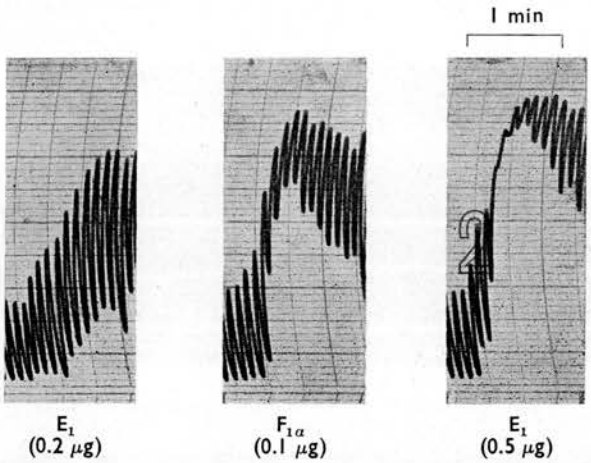


Fig. 2. Contractions of rabbit isolated jejunum, suspended in a 4 ml. organ-bath containing Tyrode solution. Contractions were recorded isometrically with a Grass force-displacement transducer (model FT.03) and recorded on a Grass polygraph. E_1=prostaglandin E_1; $F_{1\alpha}$=prostaglandin $F_{1\alpha}$.

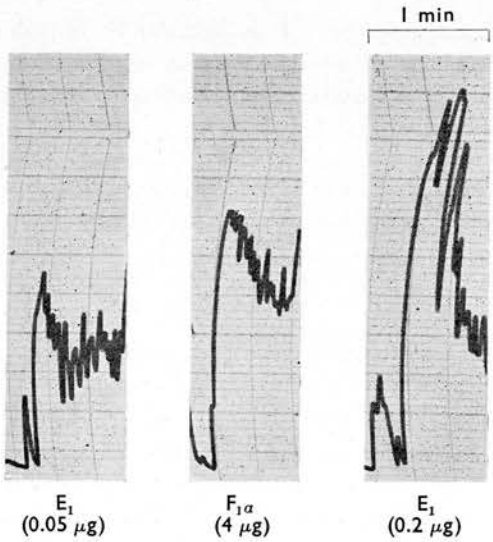


Fig. 3. Contractions of guinea-pig isolated ileum. Records as for Fig. 2.

about equally sensitive to prostaglandin E_1, responding to a concentration of 8 to 12 ng/ml.

Blood pressure and blood flow measurements. Prostaglandins E_1 and E_2 were equiactive in lowering the rabbit blood pressure, and produced a threshold effect in concentrations of 600 ng/kg, but prostaglandin E_3 was less active. Prostaglandin $F_{1\alpha}$ was fifteen to twenty times less potent than E_1 on the blood pressure. Similarly, on the cat gastrocnemius muscle blood flow and the cat skin blood flow prostaglandins E_1 and E_2 were equiactive, but E_3 and $F_{1\alpha}$ were less active (Fig. 4).

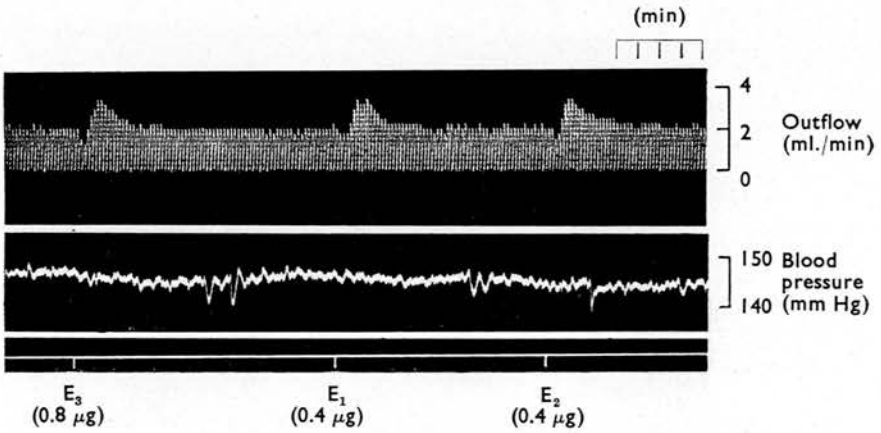


Fig. 4. Cat 3.7 kg, anaesthetized with sodium pentobarbitone (40 mg/kg). Uppermost trace, venous outflow from gastrocnemius muscle recorded with a Thorp impulse counter; middle trace, arterial blood pressure; lowest trace, event marker. E_1, E_2 and E_3=prostaglandins E_1, E_2 and E_3 respectively.

Fallopian tubal tone and peristalsis. Prostaglandin E_1, in doses as low as 800 ng/kg. reduced the tone of the rabbit fallopian tube *in vivo* and diminished its peristalsis (Horton, Main & Thompson, 1963). Similar effects were seen with prostaglandins E_2 and E_3, E_2 being equiactive with E_1 and E_3 being about half as active. In contrast, prostaglandin $F_{1\alpha}$ in doses of 5 μg/kg increased the tone of the fallopian tubes in four of the five rabbits tested (Fig. 5). In the remaining animal 5 μg/kg of $F_{1\alpha}$ caused a transient reduction in tone and in peristalsis.

DISCUSSION

Prostaglandins E_1, E_2 and E_3 differ chemically only in their degree of unsaturation, having one, two and three double bonds respectively (Fig. 1). Biologically, they were qualitatively similar and the ratios of activity were very similar on different preparations. In general prostaglandin E_2 was either equiactive to or slightly more active than E_1, whereas prostaglandin E_3, which has a double bond in the terminal pentyl group (Bergström *et al.*, 1962b), was less active than E_1 and E_2 on all preparations, On the rabbit jejunum and guinea-pig ileum the ratios are in fairly good agreement

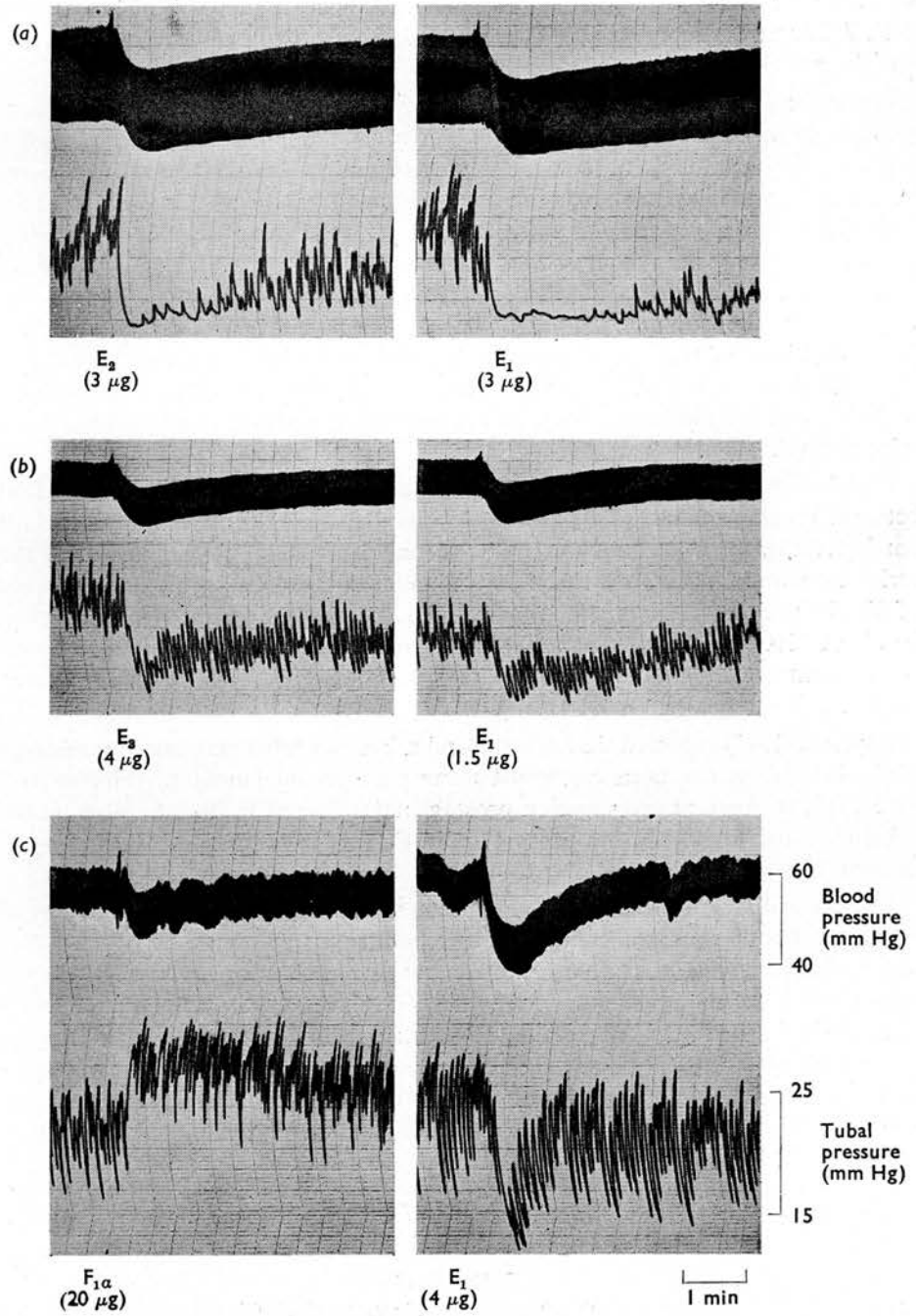


Fig. 5. Records of rabbit blood pressure (upper traces) and intraluminal pressure in a fallopian tube (lower traces). (*a*), (*b*) and (*c*) represent three different experiments. E_1, E_2, E_3 and $F_1{}^{\alpha}$= prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$ respectively.

with those of Euler & Bergström (unpublished), but on the rabbit blood pressure Euler & Bergström reported that E_2 was definitely less active than E_1, whereas in our experiments there was no difference.

Prostaglandin $F_{1\alpha}$ has a hydroxyl substituent in the cyclopentane ring instead of the keto-group of prostaglandin E_1. This structural difference has a more profound effect on biological activity than the degree of unsaturation. The ratio of activity of $F_{1\alpha}$ to E_1 varied greatly from one preparation to another. For example, on the rabbit jejunum $F_{1\alpha}$ was twice as active as E_1, but on the guinea-pig ileum it was forty times less active. Bergström *et al.* (1959) found similar ratios for E_1 and $F_{1\alpha}$ on these tissues. They also reported that $F_{1\alpha}$ had no depressor activity in doses up to 10 μg in the rabbit, in contrast to E_1 which was a potent depressor substance. We found that each of these prostaglandins lowered rabbit blood pressure although the threshold dose for $F_{1\alpha}$ (5 μg/kg) was approximately ten times that for E_1. It seems probable that the maximum doses of $F_{1\alpha}$ used by Bergström *et al.* (1959) were just subthreshold.

It is clear that none of the preparations we have tested are suitable for distinguishing between prostaglandins E_1, E_2 and E_3 by parallel biological assay. On the other hand, prostaglandin $F_{1\alpha}$ might easily be distinguished from prostaglandins of the E series by parallel assays on the rabbit jejunum and guinea-pig ileum. The index of discrimination (Gaddum, 1955) between E_1 and $F_{1\alpha}$ using these two tissues would be about 100. Similarly, such a combination of tissues might be used to estimate the amounts of E_1 and $F_{1\alpha}$ in a mixture using Euler's (1948) method, as already suggested by Bergström *et al.* (1959).

Asplund (1947) reported that prostaglandin inhibits tubal peristalsis in the rabbit. This observation has been confirmed using pure prostaglandin E_1 (Horton *et al.*, 1963). In the present investigation prostaglandins E_2 and E_3, like E_1, also inhibited tubal tone and peristalsis, but prostaglandin $F_{1\alpha}$ had the opposite effect, an increase in tubal tone being observed.

Prostaglandins are present in high concentrations in human semen. It has been suggested that they may aid conception by relaxing the smooth muscle of the fallopian tubes, thus allowing easier access of sperm to an ovum (Asplund, 1947). The relaxant effect of the prostaglandin E's on the rabbit fallopian tubes agrees with this hypothesis, but the stimulant action of prostaglandin $F_{1\alpha}$ does not. It is possible that under physiological conditions prostaglandin $F_{1\alpha}$ does not reach the tubes, or at least not in sufficiently high concentration to affect their tone, and that the inhibitory effect of prostaglandins E_1 and E_2, which are more potent, is the predominant physiological response.

We are very grateful to Professor S. Bergström for gifts of the pure prostaglandins.

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A COMPARISON OF THE ACTIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_1 ON SMOOTH MUSCLE

BY

E. W. HORTON and I. H. M. MAIN

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A COMPARISON OF THE ACTIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_1 ON SMOOTH MUSCLE

BY

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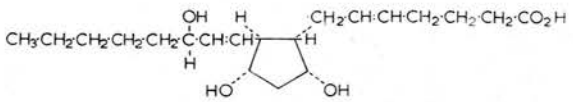
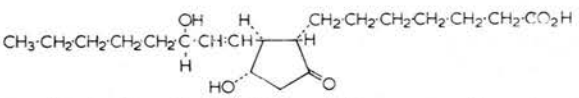
(*Received August* 11, 1964)

In a previous investigation we compared the biological activities of four prostaglandins, E_1, E_2, E_3 and $F_{1\alpha}$ (Horton & Main, 1963). Prostaglandins of the E series were qualitatively very similar in their effects and their ratios of activity were approximately constant on all the biological preparations tested. In contrast, the biological activity of prostaglandin $F_{1\alpha}$ relative to E_1 varied widely on different preparations, and on one, the rabbit fallopian tube intraluminal pressure, the two prostaglandins produced opposite effects.



Prostaglandin $F_{2\alpha}$: 9α,11α,15-trihydroxy-prost-5,13-dienoic acid



Prostaglandin E_1: 11α,15-dihydroxy-9-oxo-prost-13-enoic acid

Fig. 1. Formulae of prostaglandin $F_{2\alpha}$ and prostaglandin E_1

In this investigation we have compared a fifth prostaglandin, $F_{2\alpha}$ (Fig. 1), directly with prostaglandin E_1 on eight smooth muscle preparations selected for their known sensitivity to E_1. We have found that, on preparations which are inhibited by prostaglandins, $F_{2\alpha}$ is always less active than E_1, but that, on preparations which are contracted, $F_{2\alpha}$ is usually more active than E_1. These findings are of interest since $F_{2\alpha}$ is the prostaglandin which predominates in several tissues (Bergström, 1964).

METHODS

Smooth muscle preparations in vitro

Segments of various organs were suspended in a 4- or 10-ml. organ-bath. Longitudinal contractions were recorded either isotonically with a frontal-writing lever on a smoked drum, or isometrically with a

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force-displacement transducer and an ink-writing polygraph. A dose cycle of 4 to 6 min with a contact time of 45 to 90 sec was used for all preparations except the cat trachea.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 2 to 3 kg, was suspended in Tyrode solution at 35 to 36° C, gassed with air.

Guinea-pig ileum. Terminal ileum, from guinea-pigs weighing 200 to 450 g, was suspended in Tyrode solution at 37° C, gassed with air.

Hamster colon. The ascending colon, from hamsters weighing 100 to 200 g, was suspended in de Jalon solution at 35 to 36° C, gassed with air.

Cat trachea. Tracheas were obtained from cats which had been anaesthetized with sodium pentobarbitone, and which had been used for other experiments. Two or three rings of trachea were tied together with the muscle in alignment; the cartilage was removed. The preparations were suspended in Krebs-Henseleit solution at 37° C, gassed with 95% oxygen and 5% carbon dioxide.

Rat uterus. Segments of uterus from rats weighing 150 to 250 g, which had been injected subcutaneously with stilboestrol (100 μg) 18 hr previously, were suspended in de Jalon at 32° C, gassed with air.

Smooth muscle preparations in vivo

Rabbit blood pressure and fallopian tube intraluminal pressure. Rabbits weighing 2 to 3 kg were anaesthetized with urethane (1.75 g/kg) injected intraperitoneally. Arterial blood pressure and fallopian tube intraluminal pressure were recorded with pressure transducers as described previously (Horton & Main, 1963).

Cat gastrocnemius muscle blood flow. In one cat weighing 3.4 kg gastrocnemius muscle blood flow was recorded as described previously (Horton & Main, 1963). Prostaglandins were injected intra-arterially.

RESULTS

In all experiments the relative activity of the two prostaglandins was estimated by bracketing. Threshold doses quoted for prostaglandin $F_{2\alpha}$ are approximate. The results are summarized in Table 1.

TABLE 1

APPROXIMATE THRESHOLD DOSES OF PROSTAGLANDIN $F_{2\alpha}$ AND ITS BIOLOGICAL ACTIVITY RELATIVE TO PROSTAGLANDIN E_1

In three out of four experiments prostaglandin $F_{2\alpha}$ increased the tone of the fallopian tubes, in contrast to prostaglandin E_1 which caused relaxation. Relative activities are means, with ranges in parentheses

Preparation	Response	Threshold dose (ng/ml. *in vitro*) (μg/kg *in vivo*)	Relative activity, $F_{2\alpha}$ to E_1	No. of experiments: Qualitative difference between $F_{2\alpha}$ and E_1	No. of experiments: Total
Rabbit jejunum	Contraction	0·25–1	26 (5–100)	5	7
Guinea-pig ileum	Contraction	10–50	0·55 (0·015–3)	3	7
Hamster colon	Contraction	1–5	3·5 (1–8)	0	5
Cat trachea	Relaxation	50–1,000	0·03 (0·0025–0·05)	0	4
Rat uterus	Contraction	25–50	8 (5–15)	0	4
Rabbit fallopian tube	Contraction	1·3–5	—	3	4
Rabbit blood pressure	Depression	0·7–2·5	0·11 (0·08–0·2)	3	4
Cat gastrocnemius muscle blood flow	Vasodilatation		<0·05	0	1

Intestinal smooth muscle

The rabbit isolated jejunum contracted in response to prostaglandin $F_{2\alpha}$ in concentrations of 0.25 ng/ml. In five out of seven experiments there was a qualitative difference in the responses to prostaglandin $F_{2\alpha}$ and prostaglandin E_1. The contraction due to prostaglandin $F_{2\alpha}$ was slower in onset and reached a maximum more slowly than the contraction to prostaglandin E_1 (Fig. 2). This qualitative difference made meaningful quantitative com-

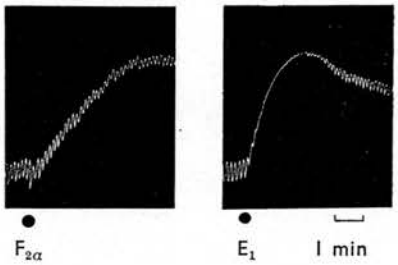

Fig. 2. Isotonic responses of isolated rabbit jejunum preparation suspended in 4-ml. organ-bath containing Tyrode solution. $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 4 ng/ml.; E_1=prostaglandin E_1, 20 ng/ml.

parisons difficult. Prostaglandin $F_{2\alpha}$ was always more active than prostaglandin E_1, the amount varying from 5- to 100-times with different intestines. The biggest differences were found in preparations which were less sensitive than usual to prostaglandin E_1 although the sensitivity to prostaglandin $F_{2\alpha}$ was normal. The reason for this discrepancy is unexplained. It could not be accounted for by deterioration in the prostaglandin solutions because freshly prepared solutions were also less active on these less sensitive preparations.

A similar qualitative difference between the prostaglandins was sometimes observed (three out of seven experiments) on the guinea-pig isolated ileum. On this preparation the ratio of activity of E_1 to $F_{2\alpha}$ was even more variable than on the rabbit jejunum. On average, prostaglandin E_1 was slightly more active but the relative activities varied from 0.015 to 3.0 on different preparations.

No qualitative differences in the stimulant action of the two prostaglandins were observed on the hamster isolated colon. In four out of five experiments prostaglandin $F_{2\alpha}$ was more active than E_1, in the remaining experiment they were equiactive.

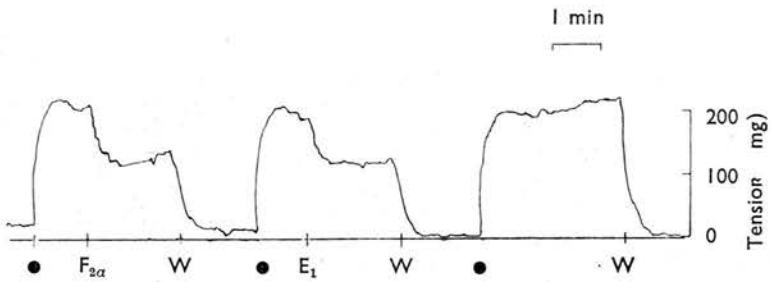

Fig. 3. Isometric responses of cat isolated trachea, suspended in 4-ml. organ-bath containing Krebs-Henseleit solution. At the dots acetylcholine (12.5 ng/ml.) was added. $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 0.75 μg/ml.; E_1=prostaglandin E_1, 1.9 ng/ml.; W=wash.

Respiratory smooth muscle

The cat isolated tracheal chain has little or no inherent tone; inhibitory responses are therefore difficult to detect. If a contraction of the preparation is produced by acetylcholine, prostaglandins can be shown to have inhibitory effects (Main, 1964). Using this technique, we have shown that, like prostaglandin E_1, $F_{2\alpha}$ also inhibits this preparation. The activity of prostaglandin $F_{2\alpha}$ was, however, only about one-thirtieth of that of E_1 (Fig. 3). In view of the small amounts of prostaglandin $F_{2\alpha}$ available, comparisons were not made on other preparations of respiratory smooth muscle which are affected by prostaglandin E_1 (Main, 1964).

Reproductive smooth muscle

Both prostaglandins contracted the isolated uterus of the rat, prostaglandin $F_{2\alpha}$ being more active than E_1. The threshold concentration of prostaglandin $F_{2\alpha}$ was about 25 ng/ml. The rabbit fallopian tube *in vivo*, which is relaxed by small doses of prostaglandin E_1, was

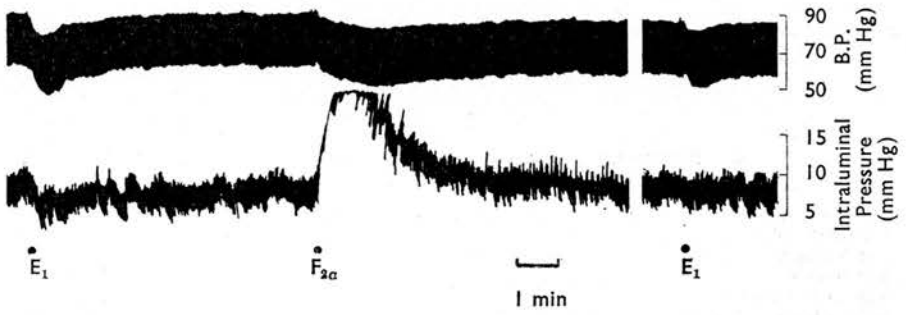

Fig. 4. Record of rabbit (2.8 kg) blood pressure (B.P., upper trace) and fallopian tube intraluminal pressure (lower trace). $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 10 μg; E_1=prostaglandin E_1, 2 and 1 μg at first and second injections respectively.

contracted by prostaglandin $F_{2\alpha}$ in three out of four experiments (Fig. 4). In the fourth experiment prostaglandin $F_{2\alpha}$ produced a short-lasting relaxation. Prostaglandin $F_{2\alpha}$ was much less active than E_1 on this preparation, the threshold dose being about 5 μg/kg.

Vascular smooth muscle

On intravenous injection into the anaesthetized rabbit both prostaglandins caused a fall in arterial blood pressure. Prostaglandin $F_{2\alpha}$ was about one-tenth as active as prostaglandin E_1. In three out of four experiments prostaglandin $F_{2\alpha}$ produced a more gradual and more prolonged fall of pressure than occurred with prostaglandin E_1 (Fig. 4). In one experiment on the cat gastrocnemius muscle blood flow, prostaglandin $F_{2\alpha}$ was shown to have vasodilator activity, but it was less active than prostaglandin E_1. In view of this low activity and of the need to conserve supplies of prostaglandin $F_{2\alpha}$ no further blood flow experiments were carried out.

DISCUSSION

Prostaglandins E_1, E_2 and E_3 differ chemically only in their degree of unsaturation and have very similar biological activities; E_3 is usually the least potent (Horton & Main, 1963; Bergström & Euler, 1963; Horton, 1964; Main, 1964). On the other hand, prostaglandin $F_{1\alpha}$, which can be formed by the reduction of the oxo substituent in the cyclopentane ring of prostaglandin E_1, differs more significantly from prostaglandins of the E series (Horton & Main, 1963). It could be predicted that the introduction of a second double bond in the prostaglandin $F_{1\alpha}$ molecule would have a similar effect upon its biological activity as a change from prostaglandin E_1 to prostaglandin E_2, and that, in view of the similarity of E_1 and E_2, the two prostaglandin Fs would also have very similar activity. How far is this prediction substantiated by the experimental findings? In our previous investigation we estimated the relative activities of E_2 to E_1 and of $F_{1\alpha}$ to E_1; the expected relative activities of $F_{2\alpha}$ to E_1 can therefore be calculated. The general trend of the predictions is supported

TABLE 2

BIOLOGICAL ACTIVITY OF PROSTAGLANDIN $F_{2\alpha}$ RELATIVE TO PROSTAGLANDIN E_1

Results predicted from previous studies are compared with observed results. * Prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$ increased the tone of the fallopian tubes in contrast to prostaglandins of the E series which caused relaxation

	Relative biological activity				
Preparation	Observed E_2/E_1	Observed $F_{1\alpha}/E_1$	Predicted $F_{2\alpha}/E_1$	Observed $F_{2\alpha}/E_1$	Calculated $F_{2\alpha}/F_{1\alpha}$
Rabbit jejunum	1·5	2·2	3·3	26	12
Guinea-pig ileum	1·6	0·02	0·03	0·55	27
Hamster colon	2·8	0·26	0·73	3·5	13
Cat trachea	1·0	0·002	0·002	0·03	15
Rat uterus	1·1	0·9	1·0	8	9
Rabbit fallopian tube	0·9	*	*	*	*
Rabbit blood pressure	1·0	0·08	0·08	0·1	1·25
Cat gastrocnemius muscle blood flow	0·8	0·2	0·16	<0·05	0·3

by the experimental results (Table 2). Thus $F_{2\alpha}$ has weak inhibitory actions on cat isolated trachea and on vascular smooth muscle, while on the rabbit fallopian tube prostaglandin $F_{2\alpha}$, like $F_{1\alpha}$ but unlike E_1, increases intraluminal pressure. However, on all tissues (except vascular smooth muscle) the activity of $F_{2\alpha}$ was some ten times greater than predicted. The reason for this apparent discrepancy cannot be explained. Anggård & Bergström (1963) also found that $F_{2\alpha}$ is more potent than $F_{1\alpha}$ but they made no direct comparison. We have been able to confirm other observations of Anggård & Bergström (1963), namely that the rabbit isolated duodenum or jejunum is very sensitive to $F_{2\alpha}$ and that $F_{2\alpha}$ is not a very potent depressor substance but that like other prostaglandins it does increase blood flow through skeletal muscle. We were unable to confirm that the rat isolated uterus will respond to $F_{2\alpha}$ in concentrations as low as 1 ng/ml. although it is more active than other prostaglandins on this preparation. It is possible that the exact stage in the oestrous cycle is critical for such high sensitivity.

One important reason for comparing the activities of different prostaglandins is to establish which tissue is most suitable for the assay of a particular prostaglandin and

which pair of tissues might be used to distinguish between two prostaglandins or to estimate their concentrations in a mixture. Biological methods of estimation are more sensitive than any known chemical methods and are the only means of assaying the minute quantities found in small amounts of tissue. We have previously concluded that no combination of biological preparations yet known will distinguish between the three prostaglandin Es but that $F_{1\alpha}$ and E_1 could be distinguished by parallel assays on rabbit jejunum and guinea-pig ileum (Horton & Main, 1963) or rabbit jejunum and cat trachea (Main, 1964). The rabbit jejunum and cat trachea could also be used to distinguish between the prostaglandin Es and prostaglandin $F_{2\alpha}$ (index of discrimination=800). It is doubtful whether any pair of the tissues tested in this investigation would enable us to distinguish between prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$. A combination of, say, guinea-pig ileum and rabbit blood pressure might provide a high enough index of discrimination. However, the rabbit blood pressure is too insensitive for use as a routine assay method where only nanograms of prostaglandins may be present in the sample to be assayed.

In addition to a qualitative difference in the response of the rabbit fallopian tube, $F_{2\alpha}$ and E_1 differed in the type of response on the rabbit blood pressure and certain types of isolated intestinal smooth muscle. On both rabbit blood pressure and rabbit isolated jejunum the responses to $F_{2\alpha}$ were usually slower in onset and took longer to reach a maximum than the responses to E_1. Similar differences were not observed between prostaglandin $F_{1\alpha}$ and prostaglandin E_1 in our previous investigation, but Bergström, Eliasson, Euler & Sjövall (1959) did report that the response to $F_{1\alpha}$ was slower than that to E_1. It is unlikely that the slower response is due to conversion of prostaglandin $F_{2\alpha}$ to E_2 since $F_{2\alpha}$ is active at lower concentrations than E_2. Possibly it is related to the ease with which this compound is transported to the active site.

One of the most interesting features which has emerged from this investigation is the contrast in potency of prostaglandin $F_{2\alpha}$ as an inhibitor and as a stimulator of smooth muscle. It had already become apparent from our previous work (Horton & Main, 1963; Main, 1964) that $F_{1\alpha}$ is less potent than E_1 on smooth muscle which is inhibited and the present results suggest that this is a general property of prostaglandin Fs. In some respects the difference is similar to that existing between adrenaline and noradrenaline. Prostaglandin E_1, like adrenaline, is potent on tissues which are inhibited, whereas both catechol amines and both prostaglandins are potent as stimulators. It is perhaps premature to speculate about two kinds of prostaglandin receptor.

Information about the distribution of the different prostaglandins in different tissues is still incomplete. Prostaglandin $F_{2\alpha}$ appears to be more widely distributed than the others; it has been isolated from lung, brain, iris, semen and menstrual fluid. In view of its presence in lung and brain it is of interest that it is considerably less active than the corresponding prostaglandin E in relaxing respiratory smooth muscle and in causing stupor and sedation (Horton & Main, 1965). If prostaglandins are concerned in the control of smooth muscle tone in the lungs (Main, 1964), it would seem likely that prostaglandin E_2 rather than prostaglandin $F_{2\alpha}$ would be of more immediate physiological importance. Possibly prostaglandin $F_{2\alpha}$ in lung and brain represents a tissue store of prostaglandin which is either a relatively inactive metabolite of prostaglandin E_2 or a precursor which exerts physiological effects only after conversion to prostaglandin E_2.

SUMMARY

1. The biological activities of four prostaglandins have previously been reported; in the present paper the activity of a fifth prostaglandin, $F_{2\alpha}$, is compared with prostaglandin E_1 on smooth muscle preparations.

2. Both prostaglandins contract intestinal smooth muscle of the rabbit, hamster and guinea-pig *in vitro*. Responses of the rabbit jejunum to prostaglandin $F_{2\alpha}$ are usually slower in onset and more prolonged than those to prostaglandin E_1.

3. Both prostaglandins inhibit acetylcholine-induced contractions of the cat isolated tracheal chain, but $F_{2\alpha}$ has only one-thirtieth of the activity of E_1.

4. The rat isolated uterus is contracted by both prostaglandins, but is more sensitive to $F_{2\alpha}$. On the other hand, the rabbit fallopian tube *in vivo*, which is relaxed by small doses of prostaglandin E_1, is contracted by $F_{2\alpha}$.

5. Both prostaglandins increase blood flow through skeletal muscle in the cat and lower arterial blood pressure; $F_{2\alpha}$ is less potent in this respect than E_1.

6. It is concluded that prostaglandin $F_{2\alpha}$ is a less potent inhibitor of smooth muscle than prostaglandin E_1, but that, on smooth muscle preparations which are contracted, $F_{2\alpha}$ is often more active than E_1.

We are indebted to Professor Sune Bergström of the Karolinska Institute for pure samples of the prostaglandins.

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THE RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURE OF PROSTAGLANDINS AND THEIR BIOLOGICAL ACTIVITY

By E. W. HORTON and I. H. M. MAIN

INTRODUCTION

The term 'prostaglandin' was introduced by Euler in 1935 to describe a lipid-soluble substance present in human seminal plasma, which contracts isolated smooth muscle and lowers arterial blood pressure and which differs from all other known naturally occurring substances. In 1962, Bergström and his colleagues at the Karolinska Institute reported the isolation of six prostaglandins from various animal tissues (see Horton, 1965, for references). In view of this it became important to know whether and how the prostaglandins differ from each other in their biological properties.

We were very fortunate in obtaining samples of five prostaglandins from Professor Bergström and Dr Samuelsson and we are greatly indebted to them for supplying us with them at a time when only very small quantities had been isolated. One of the most significant recent advances in this field was the observation made independently by two groups of workers that the prostaglandin E's are biosynthesized from the essential fatty acids (Bergström, Danielsson & Samuelsson, 1964; Dorp, Beerthuis, Nugteren & Vonkeman, 1964). Furthermore, the same tissue enzyme systems can form 'unnatural' prostaglandins when incubated with analogues of the essential fatty acids. We have studied one of these, *nor*-prostaglandin E_1, very kindly supplied by Dr van Dorp, and also the precursors of prostaglandins E_1 and E_2, *bis*-homo-γ-linolenic acid and arachidonic acid respectively.

METHODS

The methods used in this investigation have been described fully in our previous papers (Horton & Main, 1963; Horton, 1964; Main, 1964; Horton & Main, 1965*a–c*).

RESULTS

Effects on biological activity of increasing the degree of unsaturation of the prostaglandin E molecule

The prostaglandin E's differ from each other only in their degree of unsaturation having one, two and three double bonds (Fig. 1). We have

compared them on eleven biological preparations, and on all of these the responses to the three prostaglandins were qualitatively very similar. They all produced a contraction of the rat uterus, guinea-pig ileum, rabbit jejunum and hamster colon. They all inhibited cat tracheal smooth muscle,

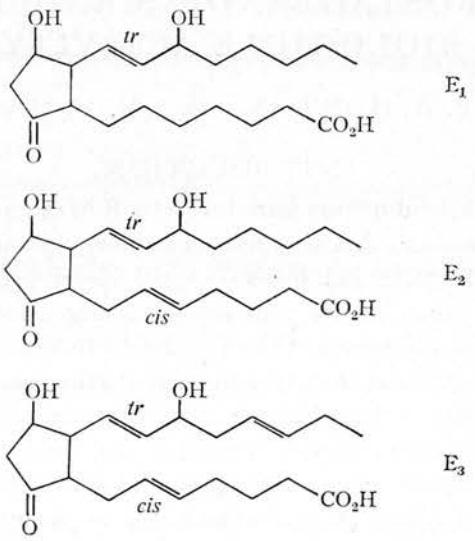

Fig. 1. Formulae of prostaglandins E_1, E_2 and E_3.

cat vascular smooth muscle and rabbit oviduct smooth muscle. They all lowered the arterial blood pressure of the anaesthetized rabbit and had a sedative-like action in young chicks. On injection into the cerebral ventricles of the unanaesthetized cat, E_2 and E_3 (like E_1) produced a state of catatonic stupor but there were insufficient experiments for an estimate of their relative potencies to be made.

The activities of E_2 and E_3 relative to E_1 did not vary greatly from one preparation to another (Table 1). E_2 was a little more active than E_1 on the

Table 1. *Biological activity of prostaglandins E_2 and E_3 relative to prostaglandin E_1 (= 1)*

Preparation	Response	E_2/E_1	E_3/E_1
Rat uterus	Contraction	1·1	0·3
Guinea-pig ileum	Contraction	1·6	0·2
Rabbit jejunum	Contraction	1·5	1·0
Hamster colon	Contraction	2·8	0·2
Cat trachea	Relaxation	1·0	0·2
Rabbit oviduct *in vivo*	Inhibition	0·9	0·4
Rabbit blood pressure	Depression	1·0	0·3
Cat muscle blood flow	Vasodilatation	0·8	0·5
Cat skin blood flow	Vasodilatation	0·9	0·2
Chick	Sedation	0·7	0·3

guinea-pig ileum, rabbit jejunum and hamster colon and rather less active in producing sedation in the chick and as a vasodilator in the cat. Allowing for variation in the response of tissues, it is unlikely that the biological activities of E_2 and E_1 differ very significantly. On the other hand E_3 had only one fifth to one half of the activity of E_1 except on the isolated rabbit jejunum on which they were equi-active.

Effects on biological activity of reduction of the 9 oxo substituent to a cis-hydroxyl

Prostaglandin F's differ from the corresponding E's by the presence of a hydroxyl substituent in the 9 position instead of an oxo (Fig. 2). In the naturally occurring F's the two hydroxyls on the ring are in the *cis* configuration.

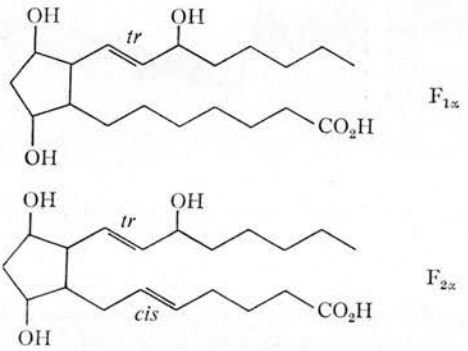

Fig. 2. Formulae of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$.

On comparing E's and F's biologically several qualitative differences were observed. Whereas E's inhibit smooth muscle of the rabbit oviduct, $F_{1\alpha}$ and $F_{2\alpha}$ both increase oviduct smooth muscle tone. The difference between $F_{2\alpha}$ and E_1 is illustrated in Fig. 3. The threshold dose of F for producing an increase in oviduct tone is five to ten times greater than the threshold dose for E for producing a decrease in tone.

Another qualitative difference in the biological actions of F's and E's is seen on intravenous injection in the unanaesthetized chick. The E's produce a condition resembling sedation, but $F_{1\alpha}$ and $F_{2\alpha}$ both cause contraction of the extensor muscles of the legs and back. This action is not on the skeletal muscle but on the spinal cord (Horton & Main, 1965*c*).

Although catatonic stupor occurs in cats following injections of E_1 into the cerebral ventricles, no such effects are seen after $F_{2\alpha}$ even in doses up to 10 times the effective dose of E_1. It is not known whether this is a qualitative difference or merely a difference in sensitivity; higher doses of $F_{2\alpha}$ might have been effective.

On the isolated rabbit jejunum and guinea-pig ileum which contract in response to both E's and F's, it was often observed that the onset of the contraction was quicker and that the maximum height of the response was reached more quickly after E than after F. This difference was particularly marked when we compared $F_{2\alpha}$ with E_1.

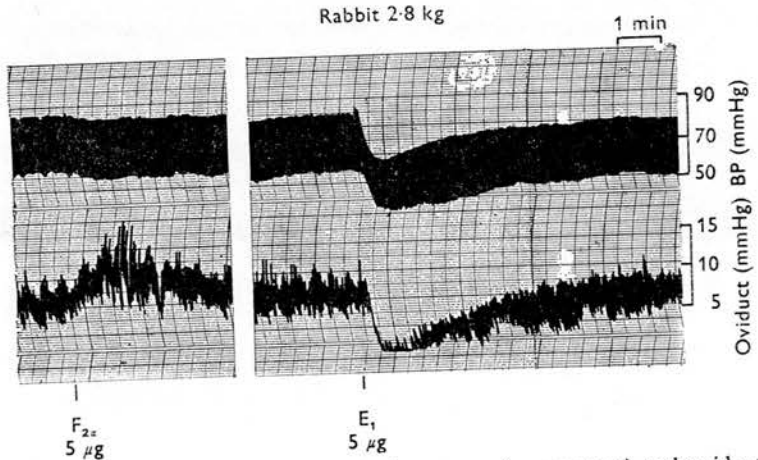

Fig. 3. Record of urethanized rabbit blood pressure (upper trace) and oviduct intraluminal pressure (lower trace). $F_{2\alpha}$ = prostaglandin. $F_{2\alpha}$ and E_1 = prostaglandin E_1, injected intravenously (doses in μg).

Table 2. *Biological activity of prostaglandin F's relative to prostaglandin E's* (= 1)

Preparation	Response	$F_{1\alpha}/E_1$	$F_{2\alpha}/E_2$
Rat uterus	Contraction	0·9	7
Guinea-pig ileum	Contraction	0·02	0·3
Rabbit jejunum	Contraction	2·2	17
Hamster colon	Contraction	0·3	1·2
Cat trachea	Relaxation	0·002	0·03
Rabbit blood pressure	Depression	0·08	0·1
Cat muscle blood flow	Vasodilatation	0·2	< 0·06

Estimates of the relative activities of E's and F's on various preparations are shown in Table 2. There is a large variation between different preparations. On the rabbit jejunum F's are more active than E's, but on the guinea-pig ileum and the cat tracheal chain preparations E's are more active than F's.

Effects on biological activity of changing the configuration of the 9 hydroxyl from cis to trans

When a prostaglandin E is reduced at the 9 position, two stereoisomers are formed, F_α and F_β, the hydroxyls at 9 and 11 being *cis* and *trans* respectively (Fig. 4). Only the *cis* isomers have been isolated from natural

sources. We compared the biological activities of $F_{1\alpha}$ and $F_{1\beta}$ prepared chemically from E_1 and kindly supplied by Dr D. A. van Dorp.

On the five different preparations on which we were able to obtain responses with $F_{1\beta}$, its effects were qualitatively similar to those of $F_{1\alpha}$. $F_{1\alpha}$ varied from 4 times (cat trachea) to 30 times (rabbit jejunum) more active than $F_{1\beta}$ (Table 3).

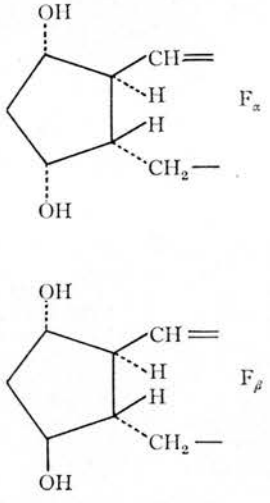

Fig. 4. Formulae to illustrate the difference between the α and β isomers of prostaglandin F_1, F_2 or F_3.

Table 3. *Biological activity of prostaglandin $F_{1\alpha}$ relative to prostaglandin $F_{1\beta}$ (= 1)*

Preparation	Response	$F_{1\alpha}/F_{1\beta}$
Rat uterus	Contraction	24
Guinea-pig ileum	Contraction	20
Rabbit jejunum	Contraction	30
Rat fundus	Contraction	9
Cat trachea	Relaxation	4
Rabbit oviduct *in vivo*	Contraction	> 5
Rabbit blood pressure	Depression	> 5

Effects on biological activity of shortening the carboxylated side chain of prostaglandin E_1 by one methylene group

On ten different biological preparations *nor*-prostaglandin E_1 (Fig. 5) produced responses which were qualitatively similar to those of E_1. *Nor*-E_1 was between a tenth and a third as active as E_1 on most preparations, but on the isolated rat uterus the two compounds were about equi-active (Table 4). They were also of the same order of potency in producing catatonia on injection into the cerebral ventricles of cats, but the number of observations was too few to permit a reliable quantitative estimate to be made.

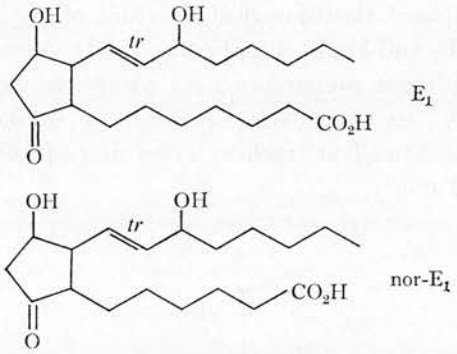

Fig. 5. Formulae of prostaglandin E_1 and *nor*-prostaglandin E_1.

Table 4. *Biological activity of nor-prostaglandin E_1 relative to prostaglandin E_1 (= 1)*

Preparation	Response	*Nor*-E_1/E_1
Rat uterus	Contraction	1·0
Guinea-pig ileum	Contraction	0·1
Rabbit jejunum	Contraction	0·3
Rat fundus	Contraction	0·2
Cat trachea	Relaxation	0·1
Rabbit oviduct *in vivo*	Inhibition	0·1
Rabbit blood pressure	Depression	0·1
Chick	Sedation	0·1
Spinal chick	Potentiation of crossed extensor reflex	0·1

Biological activities of the precursors of the prostaglandins bis-homo-γ-linolenic acid and arachidonic acid

Bis-homo-γ-linolenic acid and arachidonic acid (Fig. 6) were compared with E_1 on eleven biological preparations. They produced effects which were often qualitatively similar to those of E_1, but except on two of the preparations they were of a very different order of activity. *Bis*-homo-γ-linolenic acid and arachidonic acid like E_1 contracted the rabbit jejunum, guinea-pig ileum, rat uterus and rat fundus. In one experiment *bis*-homo-γ-linolenic acid caused a slight inhibition of cat tracheal smooth muscle, and in one out of three experiments it produced a slight inhibition of rabbit oviduct smooth muscle. The essential fatty acids in doses of 200 or 400 μg/chick produced some sedation—an effect equivalent to that produced by 0·5–1·0 μg of E_1.

Neither *bis*-homo-γ-linolenic acid nor arachidonic acid produced cataonic stupor in cats nor increases in skeletal muscle blood flow. On the human blister base preparation, both essential fatty acids produced a sensation of pain in a concentration of 50 μg/ml or more. A response to E_1, 100 μg/ml, could just be detected—higher concentrations were not tested.

Dakhil & Vogt (1962) have shown that some of the smooth-muscle stimulating activity of unsaturated long-chain fatty acids, but not that of the prostaglandins is due to the formation of peroxides. We have not excluded the possibility that the effects of *bis*-homo-γ-linolenic acid and arachidonic acid which we have described are due to peroxides. At first

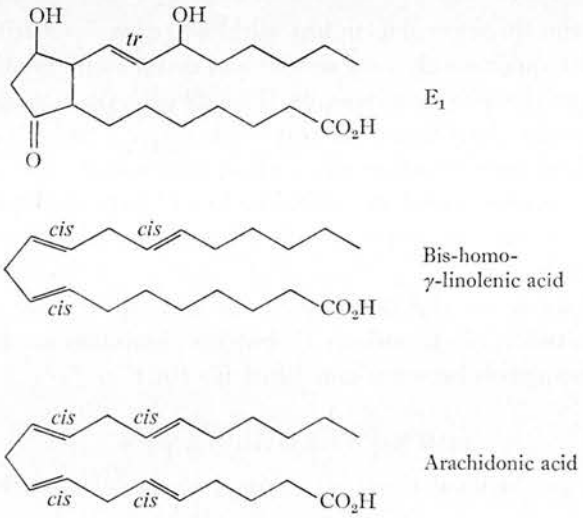

Fig. 6. Formulae of prostaglandin E_1, *bis*-homo-γ-linolenic acid and arachidonic acid.

Table 5. *Biological activity of bis-homo-γ-linolenic acid and arachidonic acid relative to prostaglandin* E_1 (= 1)

Preparation	Response	Bis-homo-γ-1/E_1	Arach./E_1
Rat uterus	Contraction	0·06	0·03
Guinea-pig ileum	Contraction	0·003	0·003
Rabbit jejunum	Contraction	0·1	0·17
Rat fundus	Contraction	0·03	0·015
Cat trachea	Relaxation	0·004	< 0·002
Rabbit oviduct *in vivo*	Inhibition	0·01	0·01
Rabbit blood pressure	Depression	0·005	0·003
Cat muscle blood flow	Vasodilatation	< 0·01	< 0·01
Chick	Sedation	0·0025	0·0025
Cat	Catatonic stupor	< 0·10	< 0·125
Human blister base	Pain	2	2

our solutions were kept under nitrogen, but later they came into contact with air intermittently over a period of several months. There was no appreciable change in biological activity after this contact with air. It seems likely that if peroxidation occurred it was already complete at the time of the first biological tests. Whether or not the biological activity was due to peroxides or to the acids themselves, it is certain that these substances are far less active than the prostaglandins on most biological preparations which we investigated (Table 5).

SUMMARY AND CONCLUSIONS

Seven prostaglandins have been studied. The four with an oxo substituent at the 9 position have qualitatively similar biological properties irrespective of other changes in the molecule such as degree of unsaturation or chain length.

Similarly, the three prostaglandins with a hydroxyl substituent at the 9 position were qualitatively very similar and could easily be distinguished as a group from the 9-oxo compounds. This division on biological grounds corresponds to the classification already made into E's and F's on the basis of their partition between ether and a phosphate buffer.

Bis-homo-γ-linolenic acid and arachidonic acid have weak prostaglandin E-like actions, but it is uncertain whether these actions are due to the acids or their peroxides.

Parallel bioassay on the rabbit jejunum and cat trachea will readily distinguish between an E and an F, but no combination of tissues yet found will distinguish between individual E's (or F's).

ACKNOWLEDGEMENTS

Grants from the Medical Research Council are gratefully acknowledged.

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COMMENT ON THE TWO PRECEDING PAPERS

DORP: In the biosynthesis of homologues such as the *nor*-PGE_1, contamination with endogenous prostaglandins should be avoided. Therefore it is desirable to use an enzyme which is free of endogenous prostaglandin, and that can be done by working with centrifugates and not homogenates.

ÄNGGÅRD: I should like to ask Dr Horton how the cats reacted when the prostaglandin was injected intravenously.

HORTON: In the intact cat there is some diminution of activity with rather large intravenous doses of prostaglandin E_1. In the decerebrate cat there is a marked potentiation of decerebrate rigidity after intravenous prostaglandin and in the spinal cat there is also an increase in muscle tension and a potentiation of the spinal reflexes.

ÄNGGÅRD: Prostaglandins of the E type are metabolised very quickly, and the half-life of 1 μg of prostaglandin E_1 would probably not exceed 1 or 2 min.

VOGT: Atropine reduces some responses to prostaglandins. This may indicate an effect on cholinergic nerve endings. Further, the separation of lipid-soluble acids from amines and peptides by extraction with organic solvents may not be complete; some amines may stick to the acids. We realized this when we acetylated lipid-soluble acids in order to prove the presence of hydroxy groups. The acetylated compounds produced strong contractions which could be blocked by atropine, and were probably caused by acetylcholine formed from contaminating choline.

CLITHEROE: As you mentioned, Dr Ambache, the reduced activity of ricinelaidic acid as compared with ricinoleic acid can be associated with the *trans* double bond. This causes the hydrocarbon chain with the hydroxyl group attached to it to move away from the carboxylic acid group. Similarly one would expect lower activity with 12-hydroxy-stearic acid. In prostaglandins the double bond at position 13 similarly tends to bring the rest of the hydrocarbon chain with a hydroxyl group on it towards the acid end of the molecule. I wonder whether these structural similarities could be possibly associated with a receptor site containing at least three possible points of access.

EDITOR'S NOTE

The term *bis*-homo-γ-linolenic acid is used in this Memoir for all-*cis* eicosa-8,11,14-trienoic acid, as suggested by Dorp (p. 75).

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Biological Activities of Pure Prostaglandins

By E. W. HORTON

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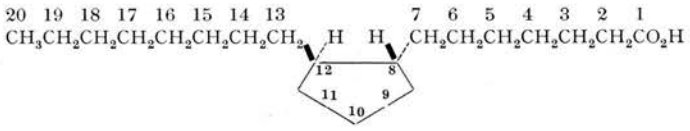
Prostaglandin was the name given by EULER in 1935 to a substance in extracts of human semen which contracts isolated smooth muscle and lowers arterial blood pressure, and which differs from all other known naturally-occurring substances[1–4]. Using prostaglandin-containing extracts of various degrees of purity, EULER and others investigated its biological actions. These results were the subject of a comprehensive review by ELIASSON in 1959[5]. About that time BERGSTRÖM et al. announced the isolation of prostaglandin[6,7], and soon these workers were able to elucidate its chemical structure[8]. It became apparent that prostaglandin was not a single substance but a family of closely-related compounds. Six of these have now been isolated from natural sources, including semen[9–11], vesicular glands[7,12,13], menstrual fluid[14], lung[13,15], brain[16], thymus[17] and iris[18] (Table I). Chemically, the prostaglandins are hydroxy unsaturated C_{20} fatty acids, five of the carbon atoms forming a cyclopentane ring (Figure). Prostaglandin E's have oxo and hydroxy substituents in the ring whereas the prostaglandin F's have two hydroxy substituents. The three E's (and three F's) differ from each other only in their degree of unsaturation (Figure).

The object of the present paper is to review the work which has been done using prostaglandins known to be



Prostanoic acid[10]

Prostaglandin E_1 = 11α, 15-dihydroxy-9-oxo-prost-13-enoic acid[8].

Prostaglandin E_2 = 11α, 15-dihydroxy-9-oxo-prost-5, 13-dienoic acid[12].

Prostaglandin E_3 = 11α, 15-dihydroxy-9-oxo-prost-5, 13, 17-trienoic acid[19].

Prostaglandin $F_{1\alpha}$ = 9α, 11α, 15-trihydroxy-prost-13-enoic acid[8].

Prostaglandin $F_{2\alpha}$ = 9α, 11α, 15-trihydroxy-prost-5, 13-dienoic acid[15].

Prostaglandin $F_{3\alpha}$ = 9α, 11α, 15-trihydroxy-prost-5, 13, 17-trienoic acid[19].

The formulae of six prostaglandins and of the parent acid, prostanoic acid, which has not been isolated from natural sources.

Table I. Distribution of prostaglandins. Prostaglandins have been isolated in pure form from the sources indicated below. The figures refer to concentrations in μg/g (μg/ml in the case of semen). + indicates that the compound has been isolated from that source, but the amount present is unknown; – indicates that the evidence suggests that the prostaglandin is not present

Tissue	Prostaglandin						Reference
	E_1	E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$	$F_{3\alpha}$	
Semen (human)	20	20	22	3	5		9, 10
Semen (sheep)	+						11
Vesicular gland (sheep)	+	+	+	(+)	–	–	7, 12, 13
Menstrual fluid (human)		+			+		14
Lung (human)					+		13
Lung (monkey)					+		13
Lung (ox)					+	+	13
Lung (pig)					+		15
Lung (sheep)	–	+	–	(+)	0.5	(+)	13, 15
Lung (guinea-pig)					+		13
Brain (ox)					0.3		16
Thymus (calf)	0.8	–	–	–	–	–	13, 17
Iris (sheep)					+		18

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chemically pure. No attempt has been made to include results obtained with impure preparations.

Actions on reproductive smooth muscle. Smooth muscle from the female reproductive tract of all species so far investigated responds to prostaglandin. Rat and guinea-pig uteri are contracted by prostaglandins E_1, E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$[20–24], and in addition to a direct stimulant action on guinea-pig myometrium prostaglandin E_2 potentiates subsequent responses to other oxytocic substances[24]. In vitro, the non-pregnant uterus of the rabbit shows little or no response to prostaglandin E_1, but contracts in response to prostaglandin $F_{1\alpha}$[20]; in vivo, spontaneous contractions of the rabbit uterus are inhibited by prostaglandin E_1[25]. Similar inhibitory effects of prostaglandin E_1 have been observed on contractions of the cervix in the anaesthetized rabbit, but the part of the reproductive tract in this species most sensitive to prostaglandin is the oviduct smooth muscle[25,26]. Prostaglandins E_1, E_2 and E_3 injected intravenously reduce the tone and contractions of the oviduct, but prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ usually have the opposite effect, although higher doses of these compounds are required[22,27].

The suggestion[28] that prostaglandins from semen are absorbed from the vagina into the circulation and thence act upon the reproductive tract smooth muscle receives some support from the finding that prostaglandin E_1 is absorbed from the rabbit vagina[26]. Although this is unlikely to be a physiological mechanism in the rabbit in view of the absence of prostaglandin from rabbit semen, nevertheless it is of great interest that prostaglandin can reach the circulation in this way and it would seem to justify an investigation in humans to determine whether a similar process operates after coitus. This could equally well be investigated in sheep, since ram semen, like that of humans, contains prostaglandin. HORTON, MAIN and THOMPSON, however, have shown[25] that injections and infusions of prostaglandin in anaesthetized ewes produce detectable changes in the intraluminal pressure of the oviduct only in amounts greatly in excess of those present in a single ejaculate of ram semen[29]. These observations do not exclude the possibility that seminal prostaglandin acts locally upon vaginal or cervical smooth muscle. Nor do they exclude the possibility that circulating prostaglandin has a more subtle and less easily recordable effect which is of physiological importance. If this is so, the demonstration that a prostaglandin has an effect on the muscle tone of the oviduct of a species may be irrelevant to the question of a physiological mechanism.

These points should be borne in mind when considering results obtained on tissues of the human female reproductive tract in vitro. BYGDEMAN and ELIASSON[30] showed that isolated myometrial strips from non-pregnant females are inhibited by prostaglandins E_1, E_2 and E_3. Prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ usually cause contraction but are rather inactive. PICKLES and HALL[31] obtained similar results, but their preparations were more sensitive to prostaglandin $F_{2\alpha}$ (contracting in the approximate range of 2 to 20 ng/ml), and the responses they obtained with prostaglandin E_2 were sometimes stimulant and sometimes inhibitory. Human Fallopian tubes in vitro are contracted by prostaglandin E_1 at their uterine ends but elsewhere are relaxed like the uterus itself[28].

Although SANDBERG, INGELMAN-SUNDBERG and RYDÉN[28] postulate that prostaglandin is absorbed from the posterior fornix and transported to the uterus and tubes, a simple calculation indicates that the concentrations likely to be achieved by such a process would be low. For example, assuming that 5 ml of semen containing 70 μg of prostaglandin/ml (Table I) is deposited in the vagina of a woman with a blood volume of 5 l, then the maximum concentration of prostaglandin which could be achieved in the circulation would be 70 ng/ml. In order to act upon tissues the prostaglandin would have to pass into the interstitial fluid and so further dilution would occur. This calculation assumes that all the prostaglandin would be absorbed before any had been lost by excretion or inactivation, yet we know that prostaglandin is removed from the blood during its passage through the heart and lungs, and that it is excreted in the urine (see below). The problem of whether or not prostaglandin is absorbed from the vagina in physiologically significant amounts will probably have to be solved by experiments on human subjects.

Little is known about the actions of prostaglandins on smooth muscle of the male reproductive tract. Adrenaline induced contractions of the vas deferens of the rabbit both in vivo and in vitro are inhibited by prostaglandin E_1[32], but the absence of prostaglandin from the semen and male reproductive tract organs of the rabbit suggests that this effect is of no physiological significance.

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Actions on respiratory smooth muscle. MAIN[33] has shown that prostaglandins inhibit respiratory smooth muscle. In experiments on isolated tracheal chains from cats, monkeys, rabbits, guinea-pigs, ferrets, pigs and sheep, prostaglandin E_1 inhibited contractions produced by adding acetylcholine to the organ bath. On preparations which possessed inherent tone the inhibitory action of prostaglandin could be detected without the prior addition of a stimulant drug. The cat isolated tracheal chain was the most sensitive of all the preparations, sometimes responding to as little as 1 ng of prostaglandin E_1/ml. On this tissue prostaglandin E_2 is equi-active with E_1, but E_3 has $^1/_5$ and $F_{1\alpha}$ only $^1/_{500}$ of the activity of E_1. More recently, HORTON and MAIN[27] have shown that prostaglandin $F_{2\alpha}$ is also less active on the cat isolated trachea, having about $^1/_{30}$ of the activity of E_1, but even at high concentrations the response to prostaglandin $F_{2\alpha}$ is always inhibition. ÄNGGÅRD and BERGSTRÖM had previously reported very weak contractions of cat, rabbit and guinea-pig tracheal chains at rather high concentrations of prostaglandin $F_{2\alpha}$ (2.5 μg/ml); prostaglandin $F_{2\alpha}$ had no effect on isolated bronchial chains from cats in concentrations up to 2.5 μg/ml[23].

When the peripheral stump of the vagus nerve is stimulated in the neck or when histamine is injected intravenously, bronchial resistance is increased in anaesthetized rabbits and guinea-pigs as measured by the Konzett and Rössler method. This increased bronchial resistance, or perhaps more accurately 'increased resistance to inflation', is partially antagonized by prostaglandin E_1 injected intravenously in doses of 0.1 μg/kg (guinea-pig) and 1.6 μg/kg (rabbit)[33]. The inhibitory action of prostaglandin E_1 on bronchial resistance in the guinea-pig cannot be demonstrated unless bronchial tone is first increased by vagal stimulation or a bronchoconstrictor drug[21,33], but in the rabbit there is sometimes a decrease in the normal level of bronchial resistance following an injection of prostaglandin E_1. In the cat both prostaglandin E_1 (0.3 μg/kg)[33] and prostaglandin $F_{2\alpha}$ (15 μg/kg)[23] increase resistance to inflation.

Although prostaglandin E's are more potent inhibitors of smooth muscle than prostaglandin F's, $F_{2\alpha}$ is the prostaglandin which occurs most commonly in the lungs (Table I). Perhaps prostaglandin $F_{2\alpha}$ is stored as a less active precursor of prostaglandin E_2 which is the compound of more immediate physiological importance.

Actions on the cardiovascular system. Prostaglandins lower systemic arterial blood pressure when injected intravenously, the E's being more potent than the F's[20,22,23,27,34]. Part at least of this depressor action of the prostaglandins is due to vasodilatation; blood flow through skeletal muscle in the cat is increased by intra-arterial injection of prostaglandins E_1, E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$[22,23,27]. Intravenous infusions of prostaglandin E_1 in two healthy human subjects caused some reduction in cardiac output accompanied by slight tachycardia[35], suggesting that decreased cardiac output may be contributory to the depressor action. Tachycardia was also observed in the cat following an intravenous injection of prostaglandin E_1[33], but intravenous prostaglandin $F_{2\alpha}$ in the cat produces a bradycardia which is abolished by atropine[26]. Prostaglandin E_1 has no effect on the isometric contractions of the spontaneously beating isolated guinea-pig atria, nor does it affect the positive inotropic responses of this preparation to catechol amines[32].

During the period following injection of prostaglandin E_1 intravenously or intra-arterially, the pressor and vasoconstrictor responses to angiotensin, vasopressin, adrenaline and noradrenaline are reduced[32]. This is not due to a simple algebraic summation of opposite effects but appears to represent a decreased responsiveness of the vascular smooth muscle to vasoconstrictor substances for some time after exposure to prostaglandin.

Unlike several naturally-occurring vasodilator substances (for example bradykinin, histamine and acetylcholine), prostaglandin E_1 does not release adrenaline from the adrenal medulla when injected close-arterially[21]. Prostaglandin E_1 also has only a weak action in increasing capillary permeability[21].

The effects of prostaglandins on blood vessels are of doubtful physiological significance. It is improbable that prostaglandins would be released as local metabolites in functional hyperaemia, other more potent vasodilators being readily available in larger amounts. The vasodilator potency of the most active prostaglandins, namely E_1 and E_2, is certainly less than that of bradykinin for example[21]. If prostaglandins ever circulate in the blood under physiological conditions, it seems likely that they would do so at concentrations considerably lower than those necessary to produce a depressor effect (compare the concentrations of vasopressin necessary to produce antidiuresis and vasoconstriction).

Actions on intestinal smooth muscle. Isolated segments of intestinal smooth muscle of most species investigated contract in response to low concentrations of the prostaglandins, the rabbit isolated duodenum and jejunum respond to 0.25 ng prostaglandin $F_{2\alpha}$/ml for example[23,27]. This type of preparation has therefore been widely used for the estimation of prostaglandin. Other sensitive tissues include the guinea-pig ileum, hamster colon, chicken jejunum, chicken rectal caecum and rat jejunum[5,20–23,27,34]. In contrast to tissues on

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which prostaglandins exert an inhibitory effect where prostaglandin E's are more potent than the F series, these intestinal preparations are often more sensitive to the prostaglandin F's. Whether prostaglandins have any local role in the control of intestinal movements which are independent of innervation is a problem which awaits further study.

Actions on the nervous system. Injections of prostaglandins E_1, E_2 and E_3 in doses of 10 to 60 μg into the cerebral ventricles of unanaesthetized cats are followed after a latent period of 20 min or more by signs of sedation and catatonic stupor[36]. The stupor lasts for several hours and the cat shows diminished spontaneous activity for up to 48 h after the injection. On the other hand, the righting reflex is always present and when the cat is disturbed its movements are brisk and show no evidence of ataxia. In marked contrast, prostaglandin $F_{2\alpha}$, the only prostaglandin so far found in tissues of the central nervous system[16], produces no detectable changes in cats following injections into the ventricles in doses equal to or higher than those of prostaglandin E_1 necessary to produce pronounced catatonia[37]. The catatonia and stupor are therefore not due to some non-specific physico-chemical effect of long-chain fatty acids since a simple reduction of the oxo group in the prostaglandin E molecule abolishes (or lessens) its activity.

Using an iontophoretic technique for applying substances to single cells, KRNJEVIĆ (personal communication) detected no significant effect of prostaglandin E_1 on 18 cortical neurones in two cats. There was no excitation of quiescent cells, no excitation or depression of spontaneously active cells, no effect on glutamate firing, no change in response of cholinoceptive cells to firing by acetylcholine and no change in the effectiveness of γ-aminobutyrate as a depressant of glutamate firing.

Some rather transient sedation is the only effect seen following intravenous injection of prostaglandin E_1 (20 μg/kg) in cats. A decrease in spontaneous activity of mice lasting for about 1 h is observed following intravenous or subcutaneous injections (unpublished observations). In the young chick, which is believed to lack a blood brain barrier, prostaglandins are particularly active on the central nervous system. Prostaglandins E_1, E_2 and E_3 in doses as low as 1 μg injected intravenously cause sedation with cessation of spontaneous movements, closure of the eyes and, in higher doses, loss of righting reflexes[36]. In contrast, prostaglandin $F_{2\alpha}$ produces not sedation but a postural defect. The limbs extend or abduct often to extreme degrees, but the legs appear not to be paralysed[37]. All these effects are temporary; the chicks recover completely after 10 min to 2 h depending upon the dose.

Little work has been reported on the actions of prostaglandins on the peripheral nervous system. Prostaglandin E_1 in concentrations from 0.1 to 100 μg/ml when applied to exposed blister bases on the human forearm did not give rise to any sensation of pain or itch[21]. Bradykinin (0.1 μg/ml) and 5-hydroxytryptamine (0.01 μg/ml) were effective in producing a sensation of pain on these preparations.

Part of the stimulant action of prostaglandin E_1 on the guinea-pig isolated ileum appears to be mediated by a nervous pathway since the response is partially antagonized by atropine (0.01 μg/ml) (unpublished observations). Contractions of the cat nictitating membrane produced by pre-ganglionic cervical sympathetic nerve stimulation were unaffected by prostaglandin E_1 injected either intravenously or into the common carotid artery, although relaxations of the membrane after cessation of stimulation were more rapid in the period immediately following prostaglandin administration[32]. There was no evidence in these experiments to suggest that ganglionic transmission is affected by circulating prostaglandin but experiments using more refined techniques are needed in order to be certain of this.

Actions on adipose tissue. Rat epididymal fat pads incubated *in vitro* release glycerol and fatty acids into the medium in response to catechol amines, corticotrophin, glucagon and thyroid stimulating hormone. The presence of prostaglandin E_1 in concentrations of 20 ng/ml or more inhibits the lipolysis induced by these agents[38]. Although prostaglandin E_1 suppresses adrenaline-induced activation of lipase, it does not affect adrenaline-induced activation of phosphorylase, an enzyme also present in adipose tissue. BERGSTRÖM, CARLSON and ORÖ[39] in experiments on anaesthetized dogs have shown that prostaglandins E_1, E_2 and E_3 are very potent also *in vivo* in inhibiting catechol amine-induced lipolysis, although prostaglandin $F_{1\alpha}$ is inactive, at least in doses similar to those of the E's. Doses which were effective in lowering plasma free fatty acid levels also produced a slight fall in blood pressure but the effect on the fatty acids was more prolonged. Increases in blood glucose due to injections of adrenaline were only moderately inhibited by prostaglandin E_1. In contrast to these results in dogs, prostaglandin E_1 infusion in human subjects increases the arterial level of free fatty acids and does not counteract the rise in free fatty acids produced by noradrenaline[13].

Actions on micro-organisms. Long-chain fatty acids inhibit the growth of certain bacteria and yeasts, and the presence of such substances accounts for the antibacterial activity of human sebaceous secretions[40].

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HOLMES[41] has recently shown that prostaglandin E_1, although a long-chain fatty acid, does not have an inhibitory action on the growth of micro-organisms in vitro in concentrations up to 1 mg/ml, whereas undecenoic, lauric and ricinoleic acids were inhibitory against some or all of the organisms tested. These observations would seem to exclude the possibility that prostaglandins provide some form of chemical defense mechanism in the tissues in which they occur.

Metabolism of prostaglandin E_1. Intra-aortic infusions of prostaglandin E_1 inhibit noradrenaline-induced fat mobilization in dogs more effectively than intravenous infusions, suggesting that prostaglandins are either taken up or inactivated by the lungs (and/or heart)[39]. Similar differences have been observed in ewes[25]. SAMUELSSON[42] has reported that when tritium-labelled prostaglandin E_1 is injected subcutaneously in rats, most of the radioactivity appears in the urine and about a one fifth in the faeces.

Miscellaneous observations (unpublished). In the water-loaded ethanol-anaesthetized rat[43], prostaglandin E_1 injected intravenously decreased urine flow but only in doses which lowered arterial blood pressure. The rats responded to 0.01 milliunits of vasopressin, but these antidiuretic responses were unaffected by previous administration of prostaglandin E_1 in doses which produce a slight depressor response. This contrasts with the observation[24] that prostaglandin E_2 potentiates oxytocic responses to vasopressin in vitro, and with the observation that pressor responses to vasopressin are temporarily abolished after prostaglandin E_1[32].

The presence of high concentrations of prostaglandin in human semen (Table I) does not appear to be essential for sperm motility. Two equal portions of a freshly collected sample of human semen were centrifuged and the deposited sperms were re-suspended in artificial seminal plasma. One plasma contained prostaglandin E_1 (100 μg/ml), the other contained no prostaglandin. The total number of motile sperms in the two aliquots was not significantly different even after three such washings when the % motility was still 75% of the initial figure.

The beating of cilia of the epithelium lining the rabbit trachea was not affected by the presence of prostaglandin E_1 (10 μg/ml) as measured by observing the rate of movement of foreign particles under the microscope. The movement of the particles could be blocked by atropine and the rate increased by eserine.

Discussion. The concept that the significance of prostaglandin is confined to the reproductive tract is no longer tenable. Prostaglandins are widely distributed throughout the body and they have an equally wide range of biological activities; indeed all organs from which prostaglandins have been isolated contain some cells (in addition to vascular smooth muscle) which respond to their presence. The thymus may be an exception to this generalization.

The idea that prostaglandins are hormones which circulate and act upon a variety of tissues is also difficult to accept on the present evidence. Prostaglandin E_1, for example, inhibits the tone of vascular, respiratory and reproductive smooth muscle, inhibits the mobilization of fats and, if the dose is adequate, depresses central nervous activities. Under what circumstances would such a combination of diverse effects be likely to occur? It is conceivable, of course, that prostaglandins circulate in concentrations lower than those required to produce the effects described above and that the truly physiological responses to these low concentrations remain to be elucidated.

Another possibility is that prostaglandins act locally, perhaps intracellularly as coenzymes. For example, the prostaglandin E's and F's may function in pairs, the conversion of an F to an E providing two hydrogen atoms for a hydrogenase system and vice versa. Some observations are difficult to explain on this hypothesis. If a prostaglandin E acting as a hydrogen acceptor relaxes smooth muscle then the corresponding F acting as a hydrogen donor might be expected to have the opposite action to E or possibly no action, but not to have the same effect as occurs with respiratory and vascular smooth muscle.

The position may be analogous to that of the adrenal corticosteroids. Many biologically-active steroids have been isolated from the adrenal cortex but only a few are actually secreted, the remainder being precursors in the biosynthetic pathway. If the prostaglandins are similar in this respect, then the F's must be considered as less active precursors of compounds of greater physiological significance (the corresponding E's). It is the F's which are predominantly stored in many tissues. According to this hypothesis $F_{2\alpha}$ in lung is functionally important only as a precursor of E_2, which possibly has some role in the control of respiratory smooth muscle tone. In the brain prostaglandin E_2, having been formed in small amounts from $F_{2\alpha}$, may act upon a similar biochemical pathway in nervous tissue. The dramatic effects seen on intracerebroventricular injection of the E's could be interpreted as due to the presence of a great excess of a compound which is normally present in only minute quantities. On the other hand $F_{2\alpha}$, although it is the compound normally present in larger amounts, is less active and therefore has no effect on intraventricular injection. Furthermore, the rate of conversion of $F_{2\alpha}$ to E_2 is probably too slow for any detectable effects

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due to increased E_2 to be observed following an injection of $F_{2\alpha}$.

The idea that F is a less active precursor of E is, to some extent, supported by the findings in semen. If the E's are the physiologically important compounds, then it is to be expected that E's and not F's would be secreted by the seminal vesicles. In this instance the semen is not a storehouse for prostaglandin (like lung and brain) but a secretion which must contain its prostaglandins in an already active state. Both human and sheep semen contain predominantly prostaglandin E's (Table I).

Table II shows the relative activities of prostaglandin E's and F's on seventeen biological preparations. On those which are inhibited by prostaglandins, E's are always more potent than F's, but on tissues which are stimulated the ratios of activity are more variable, the two prostaglandins tending often to be equiactive. It is of interest in this connection to compare these effects with those of adrenaline and noradrenaline. In general, adrenaline (like prostaglandin E's) has more potent inhibitory actions but both catecholamines (and both prostaglandins) are very active on tissues which are stimulated. A further point of similarity may be that in both cases a more potent inhibitor (adrenaline and prostaglandin E) is formed in the tissues from a less potent inhibitor (noradrenaline and prostaglandin F). The inhibitory and excitatory effects of prostaglandins, like those of the catecholamines, may each have a physiological importance at different sites.

Most of these speculations are based upon insufficient and incomplete evidence. Our knowledge of the distribution, metabolism and actions of the prostaglandins is still fragmentary and certainly does not allow any but the most tentative conclusions to be drawn at the time of writing. The functional significance of the prostaglandins still eludes us.

Table II. Relative biological activities of prostaglandins E's and F's on various preparations. An asterisk indicates that the figure was calculated from results obtained from two sources

Biological preparation	Response	$E_1/F_{1\alpha}$	$E_2/F_{2\alpha}$	Reference
Cat isolated trachea	Inhibition	500	30*	33, 27
Guinea-pig isolated ileum	Contraction	45, 43		20, 22
Chicken jejunum	Contraction	40		20
Cow isolated iris	Contraction	>30		20
Rabbit B.P.	Depressor	>20, 13		20, 22
Chick	Sedation		>15*	36, 37
Rat isolated jejunum	Contraction	12		20
Cat	Stupor		>6*	36, 37
Cat skeletal muscle blood vessels	Dilatation	4.5		22
Hamster isolated colon	Contraction	4.2		22
G.P. isolated uterus	Contraction	3		20
Fat mobilization	Inhibition	>2		39
Chicken rectal caecum	Contraction	1.8		20
Rat isolated uterus	Contraction	0.5, 1.0		20, 22
Rabbit isolated jejunum	Contraction	0.6, 0.45		20, 22
Rabbit isolated uterus	Contraction	<0.5		20
Rabbit oviduct in vivo	Inhibition	(Prostaglandin F's contract)		22, 27

Résumé. Les prostaglandines sont des acides gras non saturés existant à l'état naturel et contenant un anneau de cyclopentane; elles ne diffèrent les unes des autres que par leur degré de non-saturation et la présence dans l'anneau d'un substituant oxo (prostaglandine E) ou hydroxy (prostaglandine F). Les prostaglandines ayant une ou deux liaisons doubles sont plus actives au point de vue biologique que les prostaglandines ayant trois liaisons doubles. La réduction du groupe oxo a un effet prononcé sur l'activité biologique. C'est ainsi que les prostaglandines F inhibent moins que les prostaglandines E le muscle lisse vasculaire, respiratoire et reproducteur et la mobilisation des lipides et causent une sédation et une stupeur moins fortes chez les animaux non anesthésiés. D'autre part, le muscle lisse qui est contracté par les prostaglandines, comme par exemple l'intestin du lapin, est souvent plus sensible aux F qu'aux E. Les prostaglandines F abondent dans les tissues animaux et on suggère qu'elles sont peut-être les précurseurs des E plus actives dans certains organes, par exemple le poumon et le cerveau.

On discute le rôle physiologique éventuel des prostaglandines. Il est, pense-t-on, peu probable que leur importance fonctionnelle soit limitée à la physiologie de la reproduction, bien que cette fonction puisse être importante. On postule que si les prostaglandines circulent sous forme d'hormones, elles le font dans des concentrations plus faibles que celles qui affectent la pression artérielle. Alternativement, les prostaglandines peuvent agir localement, peut-être à l'intérieur des cellules sous forme de co-enzymes. Par exemple, les prostaglandines E et F peuvent fonctionner par paires, la conversion d'une F en E fournissant deux atomes d'hydrogène par système d'hydrogénase et vice versa. L'abondance des prostaglandines et la diversité de leurs effets biologiques suggèrent certainement qu'elles jouent un rôle général de cet ordre dont l'importance est fondamentale.

THIN-LAYER CHROMATOGRAPHY AND BIOASSAY OF PROSTAGLANDINS IN EXTRACTS OF SEMEN AND TISSUES OF THE MALE REPRODUCTIVE TRACT

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By acid ether extraction and thin-layer chromatography, prostaglandins have been separated from biologically active compounds of other chemical groups. The technique does not, however, separate different prostaglandins from each other. The biological activity of the eluates was estimated on the rabbit isolated jejunum and the hamster isolated colon preparations in terms of prostaglandin E_1; the concentrations (thus expressed) of prostaglandin in human semen ranged from 24 to 783 μg/ml. with a mean of 226 μg/ml. No prostaglandins (minimal detectable concentration, 0.5 μg/g) could be detected in the male reproductive organs of several species of laboratory animal.

Bergström and his co-workers have isolated and determined the structure of six prostaglandins (Bergström, Ryhage, Samuelsson & Sjövall, 1962), all of which occur in human semen (Samuelsson, 1963). One or more prostaglandins have also been isolated from sheep prostate glands (Bergström & Sjövall, 1960a,b ; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962), sheep semen (Bergström, Krabisch & Sjövall, 1960), sheep and pig lung (Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962), calf thymus (Bergström & Samuelsson, 1963) and human menstrual fluid (Eglinton, Raphael, Smith, Hall & Pickles, 1963).

In this paper a method is described for the extraction of prostaglandins from tissues and for their separation from biologically active substances of other groups by thin-layer chromatography. After elution, prostaglandin activity was assayed in parallel on the rabbit isolated jejunum and the hamster isolated colon preparations in terms of pure prostaglandin E_1. A preliminary report of this method has been published (Horton & Thompson, 1963).

METHODS

Collection of semen. Human semen was obtained from fertility clinics through the kind co-operation of Dr D. F. Hawkins of University College Hospital, London. Ejaculates were poured into a bottle containing 50 ml. of 0.2 M-phthalate buffer (*p*H 4) and 500 ml. of diethyl ether. The pooled samples so collected were extracted at two-weekly intervals. Ram semen, collected out of season by electro-ejaculation, was kindly provided by Dr H. M. Dott of the Agricultural Research Council's Unit of Reproductive Physiology, Cambridge. Rabbit semen was collected by the method of Macirone & Walton (1938).

Extraction of semen. The samples of human semen were extracted three-times with 250 ml. of diethyl ether. The pooled ether phases were evaporated to dryness on a rotary film evaporator. The residue was weighed and then dissolved in a mixture of methanol and chloroform (1 : 1). Ram and rabbit semen in phthalate buffer were extracted with ether in a similar way.

Extraction of tissues from the male reproductive tract. The seminal vesicles and prostate glands from rabbits, guinea-pigs, hamsters, rats and mice, and the bulb of the penis of the cat and the testis of the ferret were removed from freshly killed animals. The tissues were weighed and then ground up in 0.1 M-phthalate buffer (*p*H 4). After three extractions with two volumes of ether, the pooled ether phases were evaporated to dryness and the residue was dissolved in the chloroform : methanol mixture and chromatographed as described for the semen extracts.

Thin-layer chromatography of extracts. A Desaga Thin Layer Spreader was used to prepare a layer of Silica Gel G (Merck), 250 mμ thick. The plates were then dried in an oven at 120° C for 30 min. The solutions in methanol : chloroform mixture, volumes of 5, 10 and 20 μl., were spotted on to a 10 × 20 cm marker plate, with the origin 1.5 cm from the bottom edge. The developing solvent was a mixture of diethyl ether, petroleum ether (60 to 80° C boiling range), glacial acetic acid and methanol (50 : 40 : 5 : 5), and it was allowed to run to a line 10 cm from the origin. The spots were located under ultra-violet light after spraying with fluorescein and contrasting with bromine vapour. They were then sprayed with 50% sulphuric acid and heated for 15 to 30 min to provide additional confirmation. Pure prostaglandin E_1 was used as a marker. Preparative plates (20 × 20 cm) were run under identical conditions to the marker plates, and the solution in methanol : chloroform mixture was applied at 5 mm intervals. Between 1 and 3 mg of residue were applied to the plate. After development, the

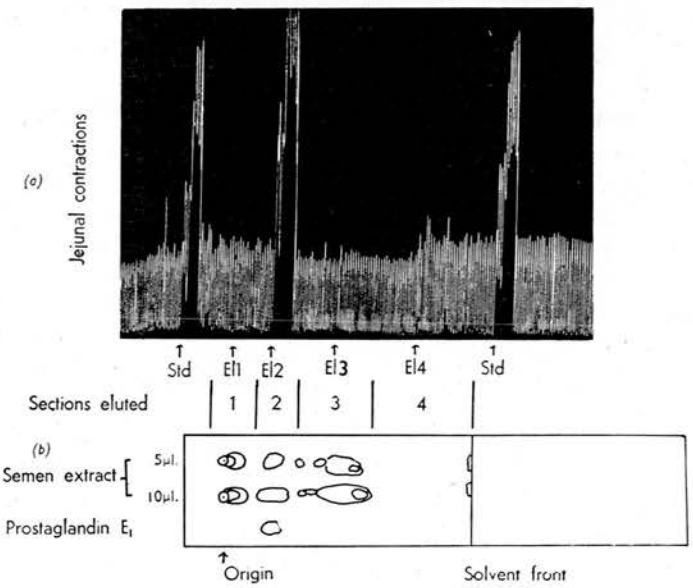

Fig. 1. (*a*) Isotonic contractions of a rabbit's isolated jejunum preparation in a 5 ml. organ-bath containing atropinized (1 μg/ml.) Tyrode solution at 36° C. Responses are shown to the standard prostaglandin E_1 (Std, 800 ng) and to the eluates (diluted 1 in 20, 0.2 ml. samples, El1, etc.) from the four zones of a preparative thin-layer chromatogram of an ether-extract of human semen. (*b*) A tracing of the corresponding marker plate on which 5 and 10 μl. of extract of human semen and of pure prostaglandin E_1 were chromatographed. The origin is on the left and the solvent front on the right of the diagram. The spots on the marker plate were identified under ultra-violet light after treating the plate as described in Methods.

appropriate zones of silica gel, as indicated by the marker plate and shown in Fig. 1, were scraped off. Elution was carried out by shaking the silica gel with methanol. After filtering off the silica gel with a sintered glass funnel, the methanol was removed on a rotary film evaporator and the dry residue taken up in 1 ml. of water for biological assay.

Biological assays. Eluates from the chromatograms of human semen extracts were always assayed in parallel on both rabbit jejunum and hamster colon preparations. Eluates from chromatograms of other tissue extracts were assayed on one preparation only. All estimations are expressed in terms of pure prostaglandin E_1 on the basis of bracketing assays.

Rabbit isolated jejunum. A 3 to 4 cm segment from the duodeno-jejunal region of the small intestine was removed from rabbits which had been stunned by a blow on the head and killed by exsanguination. The tissue was suspended in a 4 ml. organ-bath containing aerated Tyrode solution at 35 to 36° C. A dose cycle of 3 to 5 min was used with 45 to 90 sec contact. Isotonic contractions were recorded on a smoked drum using a frontal writing point lever, with a tension of 1 to 1.5 g and a five-fold magnification.

Hamster isolated colon. A 3 to 5 cm segment of the ascending colon was removed from hamsters, which had been killed by a blow on the head. The procedure was identical to that described for the rabbit jejunum, except that the tension applied was 0.6 to 0.8 g.

RESULTS

Thin-layer chromatography of extracts of human semen. Numerous solvent mixtures were tested as developing solvents in an attempt to separate the prostaglandins from other lipid constituents of human semen by thin-layer chromatography. When the mixture (diethyl ether: petroleum ether: glacial acetic acid: methanol) was used several spots separated, as shown by ultra-violet light. When pure prostaglandin E_1 was run in parallel in this solvent system, it had an R_F value of about 0.25, which corresponded to the position of a discrete spot on the chromatogram of the seminal extracts. On the basis of the chromatographic distribution of the components of the seminal extracts, four zones were marked out on the plates (Fig. 1). The first zone, from 0.5 cm below to 1.25 cm above the origin, contained those substances which moved little, if at all, from the origin. The second zone (from 1.25 to 3 cm) contained the prostaglandins. The third zone (from 3 to 6 cm) contained other lipid components of the seminal extracts, and the fourth zone (from 6 to 10 cm) represented the remainder of the plate up to the solvent front which, in the case of the seminal extracts, usually contained no substances. After developing the preparative plates, the four zones were scraped off and eluted separately. Almost all biological activity of the ether extracts of semen was found in zone 2, which corresponded to the position of the prostaglandin E_1 spot.

The biological activity of residues which had not been chromatographed varied between 84 and 120% of the activity of the corresponding eluate from zone 2. This variation is within the limits of error of the biological assay.

Concentration of prostaglandin in human semen. Two aliquots of each extract were chromatographed on two preparative plates on different days. The eluates from all zones of both plates were assayed on both the rabbit jejunum and the hamster colon preparations. Four separate estimates of the biological activity of the zone of the chromatogram corresponding to the prostaglandin spot were therefore obtained for each sample. In general there was reasonable agreement between the results of the assays (Table 1). The concentration of prostaglandin in fourteen

TABLE 1

CONCENTRATION OF PROSTAGLANDIN IN FOURTEEN POOLED SAMPLES OF HUMAN SEMEN

All samples were run on two thin-layer chromatograms and the zones corresponding to the position of prostaglandin E_1 were eluted and assayed using prostaglandin E_1 as standard. The four results so obtained and the means are given

Volume of semen (ml.)	Weight of residue extracted by ether (mg)	Prostaglandin E_1-equivalent (μg/ml. semen)		
		Rabbit jejunum	Hamster colon	Mean
4·0	18	320, 212	471, 529	383
13·5	270	796, 844	746	783
49·4	134	111, 109	111, 109	110
52	98	32, 41	16, 21	27
3	33	313, 104	835, 42	324
33	183	11, 21	21, 42	24
13	240	47, 30	47, 26	37
10	275	88, 70	88, 70	79
24	195	466, 391	466, 520	461
2·6	32	133, 190	133, 190	162
2·0	322	164, 261	164, 164	188
49	847	192, 240	480, 360	318
29	204	111, 74	37, 37	65
7·0	91	204, 382	115, 130	208
	Total means	213	240	226

pooled samples of human semen, in terms of prostaglandin E_1, ranged from 24 to 783 μg/ml. with a mean of 226 μg/ml.

Concentration of prostaglandin in other tissues and tissue fluids. Sheep semen contained 7.3 μg/ml. of prostaglandin E_1-equivalent and rabbit semen (six samples) less than 0.5 μg/ml. In none of the following tissues could prostaglandin be detected (minimal detectable concentration, 0.5 μg/g); the seminal vesicles and prostate glands of rabbits, guinea-pigs, hamsters, rats and mice, the ferret testis and the bulb of the penis of the cat.

DISCUSSION

Previous estimates of the concentration of prostaglandin in human semen were expressed in terms of arbitrary standards (Asplund, 1947; Eliasson, 1959; Hawkins & Labrum, 1961). Since then prostaglandin E_1 has been isolated in pure form (Bergström & Sjövall, 1960b), and it has therefore been possible, in the present investigation, to express the concentrations as prostaglandin E_1-equivalents in μg/ml. The original standard of Euler has been compared directly with pure prostaglandin E_1 and, on the rabbit isolated jejunum at least, it contains the prostaglandin E_1-equivalent of 4.5 μg/Euler's unit (Bergström, Eliasson, Euler & Sjövall, 1959). Asplund (1947) used this standard for his assays of prostaglandin in human semen, while Eliasson used a standard which was 1.5-times more active. No direct comparison was made between the standard used by Hawkins & Labrum (1961) and either Euler's standard or pure prostaglandin E_1. However, Hawkins & Labrum state that the rabbit isolated jejunum and the guinea-pig isolated ileum preparations respond with small contractions to 0.0005 U/ml. of their standard. Since these

preparations usually respond to a threshold concentration of 8 to 12 ng/ml. (Horton & Main, 1963), the standard of Hawkins & Labrum must have contained the equivalent of about 20 μg of prostaglandin E_1/U. Using these calculated conversion factors, the results of the various workers can be expressed in terms of prostaglandin E_1-equivalents in μg/ml. The results are summarized in Table 2. There is remarkably good agreement between the results of the four investigations.

TABLE 2

COMPARISON OF THE ESTIMATED CONCENTRATIONS OF PROSTAGLANDIN IN HUMAN SEMEN REPORTED BY FOUR GROUPS OF INVESTIGATORS

The results of previous workers have been converted to prostaglandin E_1-equivalents (μg/ml. of semen) using the conversion factor shown in the second column

Reference	Approximate prostaglandin E_1-equivalent of 1 unit (μg/U)	Prostaglandin E_1-equivalent (μg/ml. semen) Mean	Range	No. of samples	Origin of samples
Asplund (1947)	4·5	37	<20–>90	155	Individual ejaculates from fertility clinics
Eliasson (1959)	6·75	81	10–203	16	Individual ejaculates from sixteen infertile patients
		187	74–405	13	Individual ejaculates from three healthy subjects
Hawkins & Labrum (1961)	20	122	34–448	50	Individual ejaculates from fertility clinics
Present investigation	—	226	24–783	14	Pooled samples from fertility clinics

Each sample assayed in the present investigation represented a mixture of ejaculates from several individuals all attending fertility clinics. Eliasson (1959) and Hawkins & Labrum (1961) have produced some evidence that subfertile males may have low seminal prostaglandin levels. The contribution from subfertile individuals to the samples collected is unknown, but all the individuals must be regarded as potentially infertile and some probably were so, and the proportion is likely to have varied from one sample to another. This could partly account for the wide range of concentrations.

In agreement with previous workers (Euler, 1936 ; Bergström *et al.*, 1960), we found that sheep semen contains prostaglandin, though in lower concentrations than does human semen. Our estimate may not present a normal value because the semen was collected by an artificial procedure from rams outside the reproductive season. Rabbit semen and the tissues of the male reproductive tract from the various species investigated contained less than 0.5 μg/g of prostaglandin. These findings also confirm previous reports that prostaglandins are either absent or present only in low concentrations in these tissues (Euler, 1937 ; Eliasson, 1959).

The present method has the advantage that the presence of biologically active substances of a non-lipid nature (for example, choline, histamine, adrenaline and bradykinin) in the final eluates can definitely be excluded. Even if these substances passed into the ether phase during extraction, they would not move from the origin when the chromatogram developed with the solvent mixture used in these experiments. It is likely that most of the activity in the final eluates was due to prostaglandins. However, the method does have the disadvantage that a separate estimate

of the concentrations of the different prostaglandins is not possible. When this investigation was begun the chemical identity of the prostaglandins in human semen was unknown. Later prostaglandin E_1 was isolated from semen (Bergström & Samuelsson, 1962). More recently the presence in semen of the five other prostaglandins has been reported (Samuelsson, 1963). Clearly the next step must be the development of a method for the separate estimation of these six prostaglandins.

We thank Professor S. Bergström for kindly supplying the pure prostaglandin E_1 used in this investigation.

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EFFECTS OF PROSTAGLANDINS ON THE OVIDUCT, STUDIED IN RABBITS AND EWES

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Prostaglandins are widely distributed in animal tissues (Bergström, 1964; Samuelsson, 1964), but they are found in particularly high concentrations in human and ram semen (Bergström, Krabisch & Sjövall, 1960; Eliasson, 1959; Hawkins & Labrum, 1961; Horton & Thompson, 1964; Samuelsson, 1963). It has been suggested that the physiological significance of these high concentrations may be related to the effects which prostaglandins have on female reproductive smooth muscle. Uterine and oviduct smooth muscle is contracted or relaxed by prostaglandins, the effect depending upon the species and upon the type of prostaglandin (Änggård & Bergström, 1963; Bergström, Eliasson, von Euler & Sjövall, 1959; Bygdeman & Eliasson, 1963; Hall & Pickles, 1963; Horton & Main, 1963, 1965; Pickles & Hall, 1963; Sandberg, Ingelman-Sundberg & Rydén, 1963). As a result of coitus in humans and sheep a high local concentration of prostaglandin will occur in the upper vagina; it is likely therefore that this prostaglandin has a direct effect on smooth muscle of the cervix. Effects of prostaglandins on oviduct smooth muscle on the other hand are less likely to be due to such a local action. It has been postulated that prostaglandins are absorbed from the vagina into the systemic circulation and so reach the oviducts in addition to other parts of the female reproductive tract. By this means prostaglandins may aid sperm transport and so increase the chances of conception (Asplund, 1947; Horton, Main & Thompson, 1963; Sandberg *et al.* 1963). The present investigation was undertaken to test first whether prostaglandin can be absorbed from the vagina and secondly whether the amounts reaching the circulation in this way are sufficient to affect the tone of the oviduct.

We have shown that prostaglandin can be absorbed from the rabbit

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vagina, but that the intravaginal concentrations necessary to produce detectable effects on intra-luminal pressure of the oviduct are greatly in excess of those which occur in the rabbit under physiological conditions. We have also shown that the amount of prostaglandin E_1 in ram semen (about 10 μg/ejaculate) is insufficient when injected systematically in ewes to affect oviduct intra-luminal pressure. We conclude that although it is possible for prostaglandin to reach the circulation from the vagina, the physiological significance of the low concentrations likely to be thus achieved has still to be determined.

METHODS

Experiments on anaesthetized rabbits. Female rabbits (2–3·5 kg) were anaesthetized with urethane (1·75 g/kg) injected intraperitoneally. The trachea was cannulated. An external jugular vein was cannulated for intravenous injections. Blood pressure was recorded from a carotid artery with a Statham transducer. The abdomen was opened with a mid-line incision, and an oviduct was identified. A polyethylene cannula was inserted via an incision in the uterine horn through the utero-tubal junction and into the uterine end of the oviduct. It was tied in position taking care not to occlude blood vessels supplying the oviduct. The cannula was attached to a Statham transducer the side limb of which was connected via polyethylene tubing to a syringe placed in a Palmer slow injection apparatus. The infusion system from syringe to cannula contained Tyrode solution and an inflow rate of 27 or 54 μl./min was used. Intra-luminal pressure was recorded with a Grass ink-writing polygraph. The Tyrode solution escaped from the infundibular end of the oviduct into the peritoneal cavity.

For recording intra-luminal pressure of the uterine horn an incision was made at the cervical end of the horn and a cannula was inserted towards the oviduct. A second incision was made in the horn to allow the Tyrode to escape. With rates of infusion of 54–216 μl./min spontaneous contractions of the uterine horn were observed. These were always less frequent and more prolonged than those of the oviduct. In some experiments tension of the uterine horn was recorded isometrically with a Grass force-displacement transducer. The vaginal end of the horn was attached by a thread to a fixed point and the oviduct end to the transducer; the lumen of the horn was not perfused in these experiments.

In order to record the intra-luminal pressure of the cervix a cannula was inserted through an incision at the cervical end of one uterine horn so that its tip lay in the cervical canal. The vagina was opened to expose the external os and so allow the perfusing Tyrode solution to escape freely. Intra-luminal pressure was recorded by the same method as used for the oviduct and uterine horn; the rate of infusion was adjusted until contractions of the cervix were obtained. These were similar in frequency and magnitude to those of the uterine horn.

Intravaginal administration of prostaglandin to anaesthetized rabbits. A polyethylene cannula was introduced into the vagina so that its tip lay about 2 cm from the cervix; it was tied in position internally by a ligature which was passed around the wall of the vagina taking care not to occlude any of the vaginal blood vessels. The uterus was tied off to prevent fluid from passing from the vagina to the uterine lumen. Solutions were administered and withdrawn by means of a syringe attached to a soft polyethylene catheter which was passed down the vaginal cannula until its tip lay in the lumen of the vagina.

Experiment on an unanaesthetized rabbit. In one rabbit an oviduct was cannulated aseptically under pentobarbitone sodium anaesthesia in the usual way. The cannula was brought to the surface via a skin incision on the back of the neck. The abdominal wound was

sutured and the animal allowed to recover. On the following day records of oviduct intraluminal pressure in the unanaesthetized rabbit were obtained by attaching the cannula to a Statham pressure transducer and perfusing with Tyrode as described above.

Experiments on anaesthetized ewes. Adult non-pregnant ewes weighing from 39 to 92 kg were anaesthetized with pentobarbitone sodium injected via an external jugular vein. The dose administered initially to induce anaesthesia varied from 27 to 48 mg/kg in different experiments. The trachea was cannulated immediately. Anaesthesia was maintained by intravenous injections of further small doses of pentobarbitone sodium at intervals varying from 15 to 60 min.

An external jugular vein was cannulated for intravenous injections and a common carotid artery was cannulated for recording arterial blood pressure with a Statham pressure transducer. A fine polyethylene catheter was inserted through a small incision in the right femoral artery and passed retrogradely so that its tip lay either in the thoracic or in the upper abdominal aorta. The exact position of the catheter was verified at the end of the experiment. Intra-aortic injections were made through this catheter. Infusions were made from syringes placed in a Palmer slow injection apparatus.

The intra-luminal pressure of an oviduct was measured with a Statham pressure transducer by the technique described above for similar recordings in rabbits. The Tyrode solution was infused through the lumen of the oviduct at 54 or 108 μl./min.

Hormonal pre-treatment of ewes. The first two ewes were not pre-treated with hormones and in view of the time of year (March/April) they were unlikely to have been in oestrus. The third ewe was pre-treated with stilboestrol, 1 mg/day injected subcutaneously on 4 alternate days for a week before the experiment. The remaining four ewes were pre-treated by the regime described by Gordon (1963) which is designed to bring about the onset of oestrus. Progesterone 50 mg (in olive oil) was injected intramuscularly on days 1, 4 and 7. Pregnant mare's serum (750 units) was injected intramuscularly on day 9 or 10 and the experiment was performed 2 days later. Tests were not carried out to confirm whether oestrus had in fact occurred. Receptivity of the ewe to the ram is probably the most reliable indication but facilities for carrying out such a test were not readily available at the time of this investigation.

Experiments on isolated oviducts. Rabbit oviducts were taken from freshly killed animals, ewe oviducts were taken from ewes which had been used for *in vivo* experiments. All were placed in Tyrode solution. Ewe oviducts were stored at 4°C until use. A complete oviduct was suspended in a 10 ml. organ bath containing Tyrode solution at 37°C. Intra-luminal pressure was recorded with a Statham transducer using the same method as that described for the *in vivo* experiments. The Tyrode solution was infused at 54 or 108 μl./min and escaped from the infundibular end into the organ bath.

Drugs. Drugs and materials used were pentobarbitone sodium (Nembutal, Abbott Laboratories), adrenaline hydrochloride (B.D.H.), acetylcholine bromide (B.D.H.) and progesterone (B.D.H.). We are grateful to Professor S. Bergström of the Karolinska Institute for supplies of prostaglandins E_1 and F_{1a}, to Dr H. M. Dott of the A.R.C. Institute of Animal Reproductive Physiology, Cambridge for the ram semen and to Dr E. D. Nicolaides of the Parke Davis Company, Ann Arbor for synthetic bradykinin.

Preparation and standardization of seminal prostaglandin. Human semen was obtained from fertility clinics through the kind co-operation of Dr D. F. Hawkins of University College Hospital, London. Ejaculates were pooled in 0·2 M-phthalate buffer (pH 4) and extracted with diethyl ether as described previously (Horton & Thompson, 1964). The ether phase was evaporated to dryness and the residue was taken up in a small volume of saline. This concentrated solution of prostaglandin was standardized on the rabbit isolated jejunum against pure prostaglandin E_1. All quantities of this extract are expressed in terms of μg of prostaglandin E_1 equivalent. In the text it is referred to as the crude prostaglandin mixture.

RESULTS

Actions of prostaglandin E_1 on female reproductive tract smooth muscle in the rabbit

In rabbits anaesthetized with urethane, prostaglandin E_1 injected intravenously in doses of 0·5 μg/kg or more lowered the intra-luminal pressure and reduced the size and frequency of spontaneous contractions of the oviducts. In rabbits which had been pre-treated with stilboestrol the oviduct was more sensitive sometimes responding to as little as 0·03 μg prostaglandin E_1/kg. Spontaneous contractions of the uterine horn and cervix were also reduced in size and sometimes cervical contractions were

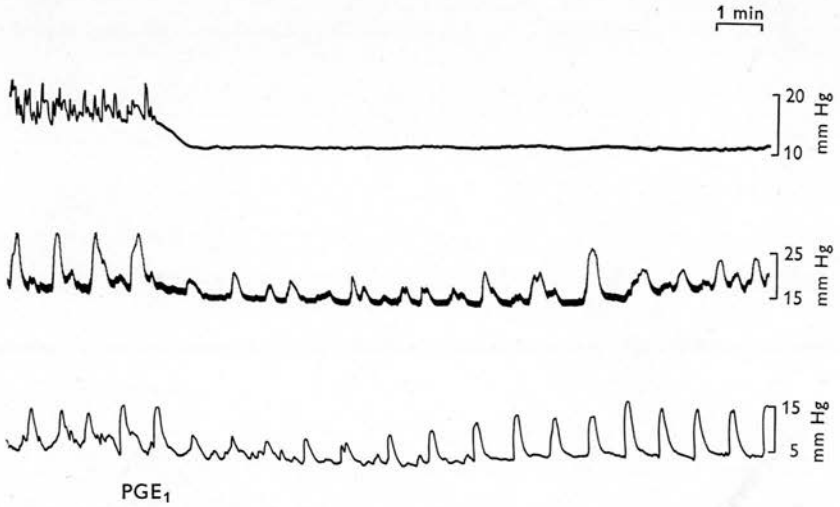

Fig. 1. Rabbit, 2·5 kg, anaesthetized with urethane (1·75 g/kg) injected intraperitoneally. Records of intra-luminal pressure of the oviduct (upper trace), uterine horn (middle trace) and cervix (lower trace). PGE_1 = prostaglandin E_1 (6 μg) injected intravenously.

temporarily abolished. The inhibitory effect on the uterine horn was not always seen but there was never any evidence of uterine contraction following prostaglandin E_1. The oviducts were the most sensitive and most consistent in their response to prostaglandin E_1, whereas the uterine horn was the least sensitive of the three parts of the female reproductive tract investigated (Fig. 1). Inhibition of oviduct smooth muscle tone was therefore used for detecting the presence of prostaglandin E_1 in the blood supplying the female reproductive organs following intravaginal administration. Before proceeding to this experiment, however, it was necessary to show that inhibition of the oviducts following an intravenous injection

of prostaglandin E_1 was a direct action, and not an indirect one, secondary to changes in blood pressure or to a reflex mediated via the central nervous system.

Evidence that prostaglandin E_1 exerts a direct effect on the rabbit oviducts

Evidence in favour of a direct action of prostaglandin E_1 on the rabbit oviducts was obtained by experiments on anaesthetized animals, on pithed animals and *in vitro*. Intravenous doses of prostaglandin E_1 sufficient to

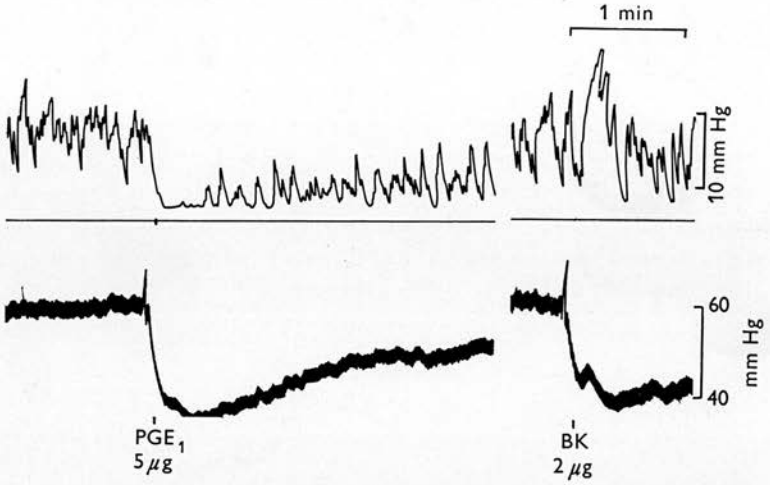

Fig. 2. Rabbit, 2·3 kg, anaesthetized with urethane (1·75 g/kg) injected intraperitoneally. Records of oviduct intra-luminal pressure (upper trace) and arterial blood pressure (lower trace). PGE_1 = prostaglandin E_1 (5 μg); BK = bradykinin (2 μg) injected intravenously.

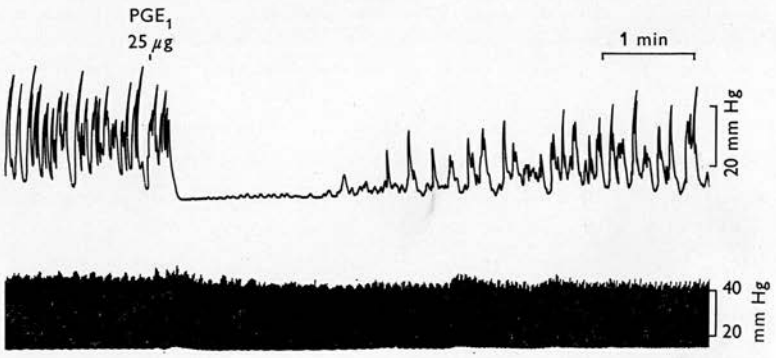

Fig. 3. Rabbit, 2·6 kg, pithed under ether. Records of oviduct intra-luminal pressure (upper trace) and arterial blood pressure (lower trace). PGE_1 = prostaglandin E_1 (25 μg) injected intravenously.

inhibit oviduct tone invariably cause a simultaneous fall of arterial blood pressure in the rabbit (Horton & Main, 1963). It could therefore be argued that the effect on the oviduct was secondary to the depressor response. When acetylcholine or bradykinin was injected intravenously in doses equi-depressor to those of prostaglandin E_1 no inhibitory effect on the oviducts was seen, although bradykinin sometimes caused a transient increase in tone (Fig. 2). Furthermore, in the rabbit, which had had its central nervous system entirely destroyed by pithing, prostaglandin E_1 caused the usual inhibition of the oviducts although the already very low blood pressure was not lowered further (Fig. 3). A fall of blood pressure alone is therefore insufficient to produce changes in tubal tone.

The isolated oviduct of the rabbit suspended in Tyrode solution possessed little inherent tone. However, when adrenaline was added to the bath, the tone increased and was maintained even after the organ bath had been washed out. This tone was reduced by the addition of prostaglandin E_1 in a similar manner to the effect observed *in vivo* (Fig. 4). These experiments suggest that prostaglandin E_1 exerts its inhibitory effect directly upon the oviducts.

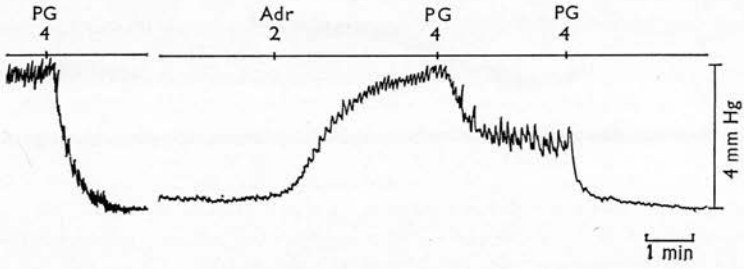

Fig. 4. Rabbit isolated oviduct suspended in a 4 ml. bath containing aerated Tyrode solution at 35°. Record of intra-luminal pressure. PG = crude prostaglandin mixture; Adr = adrenaline; doses in μg. Adrenaline had been added to the bath producing a rise in oviduct tone before the first dose of prostaglandin was added.

In one experiment on an unanaesthetized rabbit prostaglandin E_1 (12 μg) injected intravenously inhibited spontaneous muscular contractions of the oviduct. The duration and intensity of the inhibition were very similar to those observed in the anaesthetized preparation.

Absorption of prostaglandin from the rabbit vagina

One or 2 ml. of prostaglandin solution (50–400 μg/ml.) was administered intravaginally to ten anaesthetized rabbits. After a latent period of 2–10 min varying from one experiment to another the intra-luminal pressure of the oviduct and the arterial blood pressure began to fall. Contractions of the oviduct became less frequent and of smaller magnitude.

In the experiment illustrated in Fig. 5 the intra-luminal pressure of the oviduct fell from approximately 14 to 4 mm Hg and the frequency of contractions fell from 10 to 0·5/min. These effects were long lasting. On washing the vagina with saline both the arterial blood pressure and the intra-luminal pressure and frequency of contractions of the oviduct slowly returned to normal.

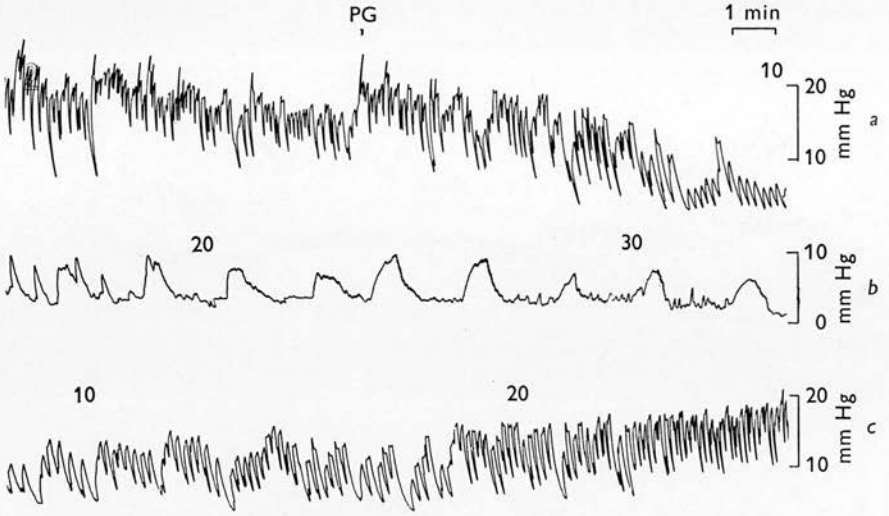

Fig. 5. Rabbit, 3·7 kg, anaesthetized with urethane (1·75 g/kg) injected intraperitoneally. Record of intra-luminal pressure of an oviduct. Between *a* and *b* there was a 5 min interval and between *b* and *c* an 8 min interval. PG = crude prostaglandin mixture (400 μg in 1 ml. saline) administered intravaginally. The vagina was washed out 34 min later (between *b* and *c*). Figures on the trace indicate time in minutes after PG administration and wash-out, respectively.

The threshold concentration of prostaglandin E_1 which produced an effect on intravaginal administration was approximately 50 μg/ml. With lower concentrations falls in oviduct tone could not with certainty be distinguished from spontaneous variations in tone. The crude prostaglandin mixture was used for most of the intravaginal administrations in order to conserve supplies of the pure material. The results were confirmed with pure prostaglandin E_1 in similar dose ranges.

Actions of prostaglandin on the oviduct smooth muscle of the anaesthetized ewe

Intra-luminal pressure of an oviduct was recorded in five anaesthetized ewes. Prostaglandin solutions (both the crude prostaglandin mixture and pure prostaglandin E_1) injected either intravenously or intra-aortically produced changes in intra-luminal pressure. The direction of the change

varied; in three ewes there was an increase in intra-luminal pressure, in two a decrease (compare Figs. 6 and 7). In any one animal the response to prostaglandin was always qualitatively the same throughout the experiment and a similar response was usually obtained subsequently with the isolated oviduct from that animal. There was no obvious correlation between the type of response and either the time of year or the hormonal status of the ewes. The doses required to produce effects, whether increase

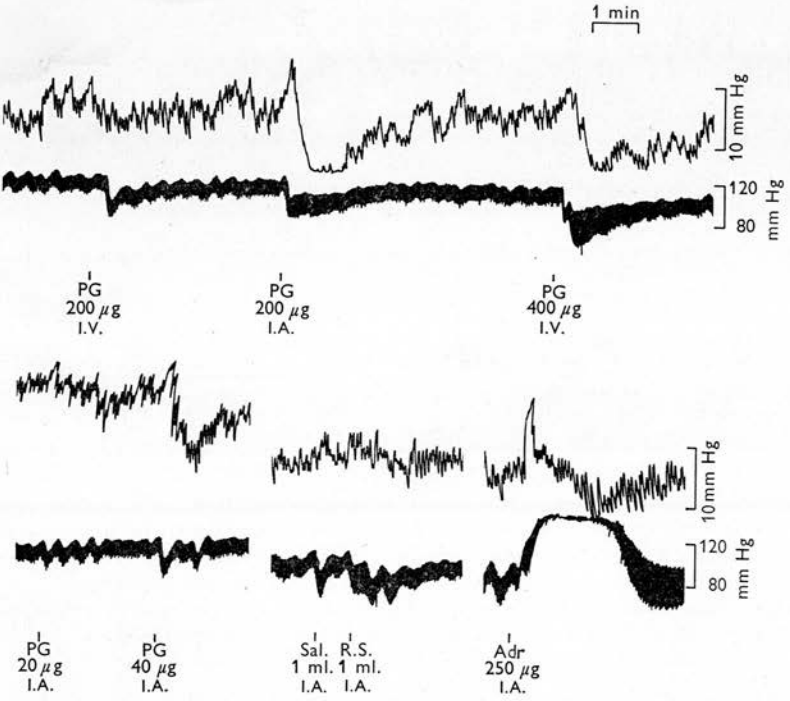

Fig. 6. Ewe (No. 5), 40 kg, anaesthetized with pentobarbitone sodium injected intravenously. In each panel upper trace is a record of oviduct intra-luminal pressure and lower trace arterial blood pressure. PG = crude prostaglandin mixture (in μg) injected intravenously (I.V.) or intra-aortically (I.A.); Sal. = saline; Adr = adrenaline; R.S. = ram semen.

or decrease in tone, did not vary much from one ewe to another (Table 1); approximate thresholds for intra-aortic doses ranged from 0·4 to 2·5 μg/kg, effective intravenous doses were substantially higher (Fig. 6) and so to conserve prostaglandin supplies the intra-aortic route was generally used.

In two ewes (Nos. 5 and 6) prostaglandin was infused intra-aortically in an attempt to mimic more closely the effects of circulating prostaglandins. Infusion rates of 0·7 and 2·2 μg/kg/min respectively had no effect on the intra-luminal pressure of the oviduct, whereas 1·4 and 4·3 μg/kg/min

produced a moderate sized response. In all these experiments a fall in arterial blood pressure was observed. Moreover, the threshold for the depressor response was always below that for effects on the oviducts (see Table 1).

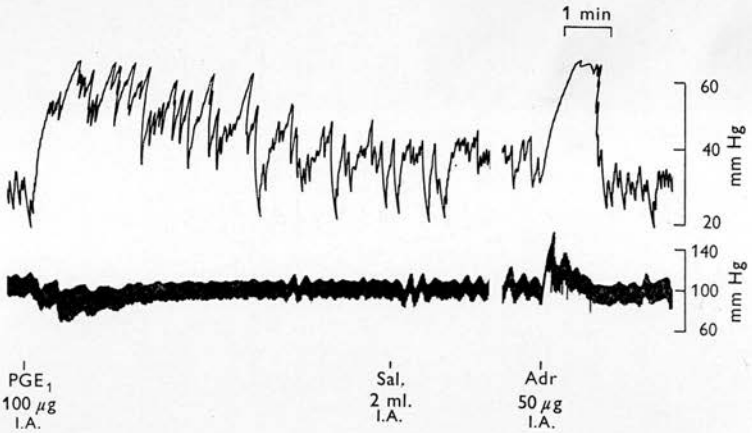

Fig. 7. Ewe (No. 6), 39 kg, anaesthetized with pentobarbitone sodium injected intravenously. Records of oviduct intra-luminal pressure (upper trace) and arterial blood pressure (lower trace). PGE_1 = prostaglandin E_1 (in μg); Sal = saline (ml.); Adr = adrenaline (μg). All injected intra-aortically.

Although the crude prostaglandin mixture was generally used, pure prostaglandin E_1 always produced similar effects. Figure 7 illustrates the rise in intra-luminal pressure of the oviduct accompanied by a fall in blood pressure which followed the intra-aortic injection of 100 μg of prostaglandin E_1. In this ewe the crude prostaglandin mixture also increased intra-luminal pressure.

Effect of ram semen on the anaesthetized ewe

In two ewes (Nos. 4 and 5) the effect of injecting ram semen intra-vascularly was observed. In ewe No. 4, 1 ml. of ram semen injected intra-aortically caused a slight rise in arterial blood pressure but no change in the response of the tone of the left oviduct. Later in the experiment the intra-luminal pressure of the right oviduct was recorded and in this case 1 ml. of ram semen injected intra-aortically caused a slight transient increase in tubal tone similar to the effect seen with prostaglandin itself. This second injection did not affect arterial blood pressure. In ewe No. 5 1 ml. of a fresh batch of ram semen injected intra-aortically caused a slight fall in blood pressure but did not affect tubal tone (Fig. 6*b*). The same amount injected into the external jugular vein caused a fall followed by a rise in blood pressure, but no effect on tubal tone was detected.

TABLE 1. Effects and effective doses of prostaglandin on the intra-luminal pressure of the oviduct *in situ* and *in vitro* and on the arterial blood pressure in ewes

Ewe no.	Wt. (kg)	Hormonal pre-treatment	Month of expt.	Oviduct *in situ*			Approximate threshold depressor doses (μg/kg) on intra-aortic injection	Oviduct *in vitro*	
				Response	Effective intra-aortic dose* (μg/kg)	Intra-aortic infusion (μg/kg/min)		Response	Effective concentrations* (ng/ml.)
1	55	None	March	—	—	—	—	Relaxation (sometimes preceded by contraction)	300–400
2	92	None	April	Contraction	0·4–1·7	—	0·2	Contraction	800
3	56	Stilboestrol	April	Relaxation	0·9–2·1	—	< 0·9	Relaxation (after latent period)	100–200
4	76	Progesterone + P.M.S.	May	Contraction	1·0–4·0	—	< 0·7	No effect	> 1000
5	40	Progesterone + P.M.S.	November	Relaxation (preceded by transient contraction)	1·0–5·0	0·7 no effect 1·4 relaxation 1·4 relaxation	0·7 μg/kg/min (intra-aortic infusion)	Relaxation	2·5–40 (prostaglandin E_1)
6	39	Progesterone + P.M.S.	November	Contraction	2·5–5·0	2·2 no effect 4·3 contraction 4·3 contraction	2·2 μg/kg/min (intra-aortic infusion)	Relaxation (sometimes contraction)	100–4000
7	40	Progesterone + P.M.S.	November	—	—	—	—	Relaxation	80

* The lower figure in each range represents the approximate threshold dose or concentration.

Effect of prostaglandin on the isolated oviduct of the ewe

The isolated oviduct of the ewe unlike that of the rabbit showed spontaneous contractions. In four ewes the response of the isolated oviduct to the crude prostaglandin mixture resembled that of the same tube *in situ*. In ewe No. 4, no responses to prostaglandin could be obtained *in vitro* although it had responded to prostaglandin *in situ*, and it showed the usual increase in tone when adrenaline was added to the organ bath.

The concentrations required to produce a threshold effect *in vitro* ranged from 2·5 to 800 ng/ml. in those preparations which responded to prostaglandin. The preparations which relaxed tended to be more sensitive than those which contracted (Table 1).

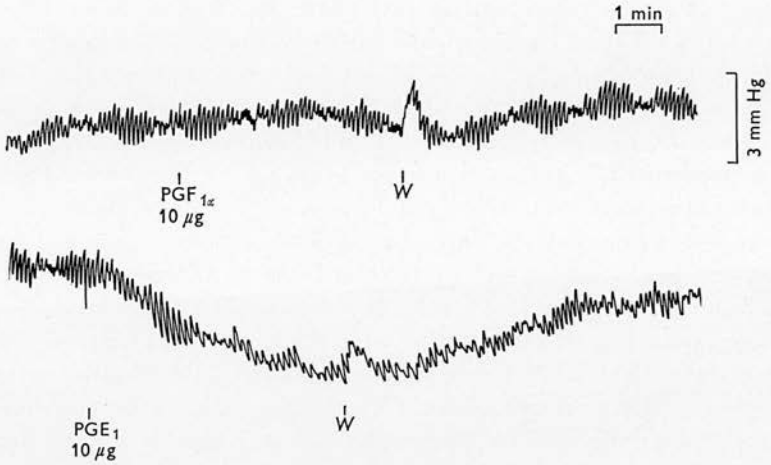

Fig. 8. Ewe (No. 1) isolated oviduct suspended in a 10 ml. bath containing Tyrode solution at 35°. Record of oviduct intra-luminal pressure. $PGF_{1\alpha}$ = prostaglandin $F_{1\alpha}$; PGE_1 = prostaglandin E_1; doses in μg. *W*, organ bath washed out.

On an oviduct taken from ewe No. 1 prostaglandin E_1 (250 to 1000 ng/ml.) like the crude prostaglandin mixture caused a reduction in tubal tone. Prostaglandin $F_{1\alpha}$ (1000 ng/ml.) had no effect on this preparation (Fig. 8).

DISCUSSION

The object of the experiments described in this paper was to test the hypothesis that seminal prostaglandin can be absorbed from the vagina into the circulation in amounts sufficient to affect smooth muscle tone of the oviduct. Prostaglandin E_1 injected intravenously in the anaesthetized rabbit inhibits smooth muscle tone in all parts of the female reproductive tract. Our experiments *in vitro* and on the pithed rabbit strongly suggest

that, on the oviduct at least, this is a direct action and is not due to changes of blood pressure or to effects mediated via the central nervous system.

Our assertion that prostaglandin can be absorbed from the vagina into the circulation is based upon the following evidence. When a solution of prostaglandin was introduced into the vagina there was a fall in systemic arterial blood pressure and a fall in oviduct intra-luminal pressure very similar to that observed following an intravenous injection. Moreover, the effects following intravaginal administration occurred after a latent period and were more gradual in onset than those seen following a single intravenous injection. Such a time lag fits in with the concept of gradual absorption. It might be argued that the effect on the oviduct was mediated in some way by the reproductive tract itself. In all experiments the lumen of the uterus was tied off in at least one place between the vagina and the oviduct so that prostaglandin could not reach the oviduct via the lumen; furthermore, in several experiments the same effects were observed when the oviduct was completely isolated from the remainder of the reproductive tract by dividing the ipsilateral uterine horn between ligatures. The effects on the oviduct could therefore not have been due to a locally mediated reflex of either neurogenic or myogenic origin. It would also be difficult to explain the blood-pressure changes on a basis of a local action of prostaglandin on the reproductive tract. If it is accepted that these effects are due to circulating prostaglandins and it is further accepted that the prostaglandins act directly upon the oviduct or structures intimately associated with it—a nerve plexus, for example—then it must be concluded that prostaglandin can be absorbed from the rabbit vagina into the circulation in amounts sufficient to alter the tone of the oviduct. On the other hand this is unlikely to be a physiological mechanism in the rabbit. Several workers have now shown conclusively that rabbit semen, like the semen of many other laboratory animals but unlike that of man and the ram, is very low in prostaglandin. The amounts present (if any) are certainly too small to affect the tone of the oviduct even if all the prostaglandin were absorbed immediately into the circulation. In fact we have shown that freshly collected rabbit ejaculates on intravenous injection in the rabbit have no detectable effect on the oviduct. Nevertheless, the demonstration that vaginal absorption of prostaglandin can occur is of interest. Unless conditions in the human female and in the ewe are very different from those in the rabbit some absorption of seminal prostaglandin is to be expected following coitus in these species.

Our next step was to investigate a species whose semen was rich in prostaglandin. As prostaglandin E_1 has been isolated from ram semen, the ewe was selected for these experiments. We were able to show that oviduct

intra-luminal pressure could be measured by the same method as that used in the rabbit. On the other hand the effects of prostaglandin varied from one animal to another. In spite of this qualitative difference, the significance of which is unknown, the sensitivity to prostaglandin varied very little. The most striking feature, however, of this part of the investigation was the large amount of prostaglandin necessary to elicit changes in oviduct intra-luminal pressure. Even when intra-aortic injections were made, the amount of prostaglandin which produced a threshold effect on the oviduct of the ewe was 40 μg or about 4 times the total amount present in a single ram ejaculate. When the smaller effectiveness of intravenous administration and still more of intravaginal administration is taken into account it is clearly impossible for absorption from the vagina in the ewe to give rise to concentrations sufficient to affect tubal tone. In view of this result and because of the technical difficulties to be overcome and of the relative scarcity of prostaglandin, vaginal absorption in the ewe was not investigated. We did, however, show that the intra-aortic injection of ram semen (which contained 10 μg prostaglandin/ml.) did not affect the oviduct or even the arterial blood pressure to any significant degree.

These results disprove our original hypothesis. They do not exclude the possibility that prostaglandin is absorbed from the vagina following coitus in sheep but the blood prostaglandin levels likely to be achieved would be far too small to affect smooth muscle tone of the oviduct. Possibly prostaglandins so absorbed may fulfil some other role. It is conceivable that in the unanaesthetized ewe the oviduct is far more sensitive than in the animal under pentobarbitone although this was not the case in the rabbit where sensitivities were of the same order whether the animal was unanaesthetized or under urethane or pentobarbitone. One major snag to the concept of prostaglandins acting as circulating hormones is their potent vasodilator activity. All effects so far reported in the intact animal following systemic injection of prostaglandin have been accompanied by a fall in blood pressure. While that is not to say that there is a cause and effect relation between the one and the other, nevertheless, we consider it unlikely that such effects would under physiological conditions be mediated by a hormone which at the same time lowered arterial blood pressure appreciably.

We conclude then that seminal prostaglandin may be absorbed from the vagina but that the amounts reaching the circulation in this way are too small to affect the tone of the oviduct smooth muscle. Our experiments do not, of course, exclude the possibility that prostaglandins in the semen act locally upon the cervix and the uterus itself from within the lumen of the reproductive tract.

SUMMARY

1. Spontaneous contractions of the oviduct, uterine horn and cervix of the anaesthetized rabbit are inhibited by prostaglandin E_1 injected intravenously.

2. The action on the oviduct is direct; it is not mediated via the central nervous system nor is it secondary to changes in blood pressure.

3. When prostaglandin solutions in concentrations of 50 μg/ml. or more are administered intravaginally in rabbits, absorption into the circulation occurs in amounts sufficient to reduce the tone of the oviduct.

4. Intra-aortic injections of prostaglandin lowered oviduct intra-luminal pressure in two ewes but raised it in three other ewes. In any one animal responses remained qualitatively similar throughout the experiment and a similar response was usually obtained subsequently with the isolated oviduct from that animal.

5. Freshly collected ejaculates of ram semen injected intra-aortically had little or no effect upon the intra-luminal pressure of the oviduct.

6. It is concluded that although prostaglandin can be absorbed from the vagina (of the rabbit) the amounts of prostaglandin in both rabbit and sheep semen are too small to have any significant effect upon oviduct smooth muscle even if complete and rapid vaginal absorption of seminal prostaglandin were to occur.

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ACTIONS OF PROSTAGLANDINS E_1, E_2, AND E_3 ON THE CENTRAL NERVOUS SYSTEM

BY

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ACTIONS OF PROSTAGLANDINS E_1, E_2 AND E_3 ON THE CENTRAL NERVOUS SYSTEM

BY

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Prostaglandins E_1, E_2 and E_3, injected into the cerebral ventricles of unanaesthetized cats, produced sedation, stupor and signs of catatonia. The threshold dose was 3 μg/kg. Slight sedation was also observed following an intravenous injection, but a dose of 20 μg/kg was required. In chicks, intravenous injections of prostaglandins (10 to 400 μg/kg) caused respiratory depression, profound sedation, loss of normal posture and, with the higher doses, loss of the righting reflex.

Brain extracts contain biologically active lipid-soluble substances (Ambache & Reynolds, 1960, 1961 ; Kirschner & Vogt, 1961 ; Toh, 1963), most of which stimulate smooth muscle. The chemical structure of many of these substances is unknown but their biological and physicochemical properties somewhat resemble those of the prostaglandins (see Horton & Main, 1963, for references to early history and formulae of these substances). It was therefore of interest to know whether prostaglandins have any actions on the central nervous system. In the present experiments prostaglandins of the E series produced stupor and prolonged sedation when injected into the cerebral ventricles of cats and sedation when injected intravenously into chicks. The prostaglandins of the E series have been isolated and their structure elucidated in Bergström's laboratory in Stockholm (Bergström & Sjövall, 1960; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962 ; Bergström, Ryhage, Samuelsson & Sjövall, 1962 ; Samuelsson, 1963).

METHODS

Injections into the cerebral ventricles of cats. Collison cannulae (C. F. Palmer, Ltd.) were implanted with sterile conditions into the lateral ventricles of six female adult cats, weighing 2.5 to 3 kg and anaesthetized with pentobarbitone sodium (40 mg/kg), as described by Feldberg & Sherwood (1953). After an interval of not less than 1 week, injections were made through the cannula without anaesthesia. Prostaglandin E_1 was dissolved in sterile 0.9% saline by the addition of solid sodium bicarbonate. Neutral solutions warmed to 37° C were injected slowly under aseptic conditions in volumes of 0.1 or 0.2 ml. and washed in with 0.1 ml. of 0.9% saline. The cats were observed continuously for 2 to 8 hr and intermittently for 48 to 72 hr.

In four cats weighing 1.8 to 3.3 kg, prostaglandin E_1 was injected intravenously without anaesthesia through the superficial vein of the foreleg.

Intravenous injections into chicks. Two- to five-day-old chicks weighing 40 to 50 g were used and the injections were made into the right external jugular vein in volumes of not less than 0.1 ml. and not more than 0.5 ml.

RESULTS

Injections into the cerebral ventricles of cats. The intraventricular injection of 7 to 20 μg/kg of prostaglandin E_1 was followed within 5 to 20 min by sedation and stupor. Spontaneous movement decreased and the cat would sit in a corner of its cage or, if allowed its freedom, would seek out a dark recess usually under a bench, where it would continue to sit for hours, if undisturbed. The cat assumed a characteristic posture with head forward and slightly lowered ; the eyes were closed. The cat showed little interest in its surroundings, it did not resent being picked up and it showed no signs of affection. When taken up and set free, it tended to retire quickly to its former sheltered position. Its movements were not impaired but rapid and fully co-ordinated.

The cat failed to respond to a sudden loud noise or to a bright flash of light. On the other hand, when pressure was applied to a foot-pad the limb was rapidly withdrawn but there was no vocalization, indeed the cats were invariably silent. To a few stimuli, there was a sluggish response ; for example on the introduction of another cat into the room, the eyes slowly opened, the ears pricked up and the head moved in the appropriate direction.

Sometimes there were definite signs of catatonia. This was a late feature which occurred after a latency of at least 40 min and developed gradually. When fully developed, the cat could be placed across the rungs of an inverted stool and would remain in such an unnatural position without moving for periods up to 90 min. In contrast, uninjected cats could not be induced to adopt such a position at all. The catatonic signs lasted up to 4 hr, sedation and stupor up to 24 hr, and even at 48 hr there was sometimes reduced spontaneous activity. The threshold dose which produced sedation and stupor was approximately 3 μg/kg and the effect lasted 4 to 8 hr.

Another effect of the injection of prostaglandin E_1 was moderate dilatation of the pupils lasting 3 to 4 hr ; the pupillary reflexes were, however, normal. There was no evidence of any loss of function of any cranial or spinal nerves. There was no obvious change in respiratory or cardiac rates ; no salivation, lachrymation, vomiting, defaecation or micturition occurred. There was no hyperphagia, indeed cats which had been starved for 24 hr before the injection showed no interest in food during the stupor due to prostaglandin. The injection caused no scratching or other movements.

In one cat prostaglandin E_2 (12 μg/kg) and in another cat prostaglandin E_3 (12 μg/kg) were injected intraventricularly. Sedation, stupor and catatonia developed and the effects resembled those seen following an intraventricular injection of 7 μg/kg of prostaglandin E_1.

In control experiments intraventricular injections of neutral 0.9% saline were followed by transient slight diminution in spontaneous activity.

Intravenous injections into cats. When injected intravenously into unanaesthetized cats in doses up to 10 μg/kg, no effects were detected with prostaglandin E_1. In doses of 20 μg/kg the only effect was a reduction in spontaneous activity lasting about 2 hr. The effect was less than that following an intraventricular injection of 3 μg/kg of prostaglandin E_1.

Intravenous injections into chicks. Immediately following an intravenous injection of prostaglandin E_1, E_2 or E_3 in doses from 10 to 400 μg/kg there was a great reduction

in respiratory rate—sometimes the rate decreased from 60 to 15 breaths/min. In addition there was profound sedation but no signs of catatonia. The chick lay on its side and, with the higher doses (50 to 200 μg/kg of prostaglandin E_1), the righting reflex was lost. There was little spontaneous movement, but in a few chicks during the first 3 min after the injection short-lasting convulsive movements of the legs and wings occurred. The duration of sedation depended upon the dose injected. When sedation subsided the chick slowly resumed its normal posture. The righting reflex was first restored, the chick remaining in a squatting position with its eyes closed. The respiratory rate had by then returned to normal. Gradually the chick resumed the standing posture and finally it would begin to chirp and move about the cage.

During the period of sedation the chicks were invariably silent even when pressure was applied to a toe, although this produced immediate withdrawal of the limb. There was no loss of the corneal reflex and, when the standing posture had been regained, there was no abnormality of gait or lack of co-ordination. Control injections of 0.5 ml. of 0.9% saline produced no detectable changes.

With prostaglandin E_1 the effects of 10 μg/kg lasted about 5 min and those of 200 μg/kg about 75 min. When an estimate of the relative activities of the three prostaglandins was made by measuring the time between injection and the recovery or normal posture, prostaglandin E_1 was 1.5-times more active than E_2 and 4-times more active than E_3.

DISCUSSION

The effects following an intraventricular injection of the prostaglandins E resemble the late effects of intraventricular injection of physostigmine, dyflos, acetylcholine or bulbocapnine seen by Feldberg & Sherwood (1954, 1955). These authors describe the posture of a cat after intraventricular physostigmine as sitting "hunched up without movement ; its eyes half shut or shut and its head slightly inclined forward" ; this description could be used equally for cats injected with prostaglandin. The catatonic stupor produced by the four drugs used by Feldberg & Sherwood, however, was preceded by signs of pronounced excitation—vigorous scratching, licking and washing movements followed by reflex hyperexcitability and tremor. None of these excitatory effects was observed with the prostaglandins.

The effects of intraventricular prostaglandin could be mimicked, but to a small degree only, by intravenous injection of a relatively large dose. Such a dose injected intravenously into anaesthetized cats would cause a substantial fall in blood pressure (Holmes, Horton & Main, 1963), and therefore the possibility cannot be excluded that part of the short-lived sedative effect seen on intravenous injection is secondary to this fall of blood pressure. The effects following intraventricular injection, however, cannot be explained in this way, since by the intraventricular route much smaller doses were effective than on intravenous injection, and the effects were not only sedation but also stupor and catatonia.

As the effects of intravenous prostaglandins in the 2- to 5-day-old chick somewhat resemble those seen in cats on intraventricular injection and are not typical signs of circulatory failure, they also appear to be central nervous effects. Prostaglandins will reach the brain from the blood stream as, in these young chicks, the blood-brain

barrier has not yet developed. It is of interest to note that the relative central depressant activities of the three prostaglandins in chicks were similar to those found in smooth muscle preparations (Horton & Main, 1963).

Ambache & Reynolds (1960, 1961) found a smooth muscle stimulating substance in rabbit brain which they were unable to distinguish from irin, a hydroxy unsaturated fatty acid extracted from the iris. Kirschner & Vogt (1961) prepared three biologically active fractions from horse, rabbit and guinea-pig brains ; two were identified as lysophosphatidic acid and ganglioside respectively, but the third fraction was believed to consist of unsaturated fatty acids. Similarly, Toh (1963) separated three fractions from cat and dog brain extracts ; the third fraction (Toh's substance C) behaved like an unsaturated fatty acid. Ramwell & Shaw (1963) have found an oxytocic ether-soluble acid in brain superfusates. Whether any of these biologically active fatty acids are prostaglandins is unknown. It is significant, however, that Bergström and his colleagues (Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962) were unable to detect any prostaglandin in sheep brain whereas other sheep tissues which they extracted all contained either prostaglandin or prostaglandin-like material. The demonstration of the central actions of prostaglandin described in this paper perhaps justifies a renewed search for it in the central nervous system.

I wish to thank Professor S. Bergström for very kindly supplying the pure prostaglandins used in this investigation.

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DIFFERENCES IN THE EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$, A CONSTITUENT OF CEREBRAL TISSUE, AND PROSTAGLANDIN E_1 ON CONSCIOUS CATS AND CHICKS

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Summary—Prostaglandin $F_{2\alpha}$, a normal constituent of ox brain, produced no obvious effects when injected into the cerebral ventricles of four unanaesthetized cats in doses of 15–100 μg per animal. In contrast the closely related prostaglandin E_1 produced marked sedation and stupor in doses of 20 μg per animal in each of these animals confirming the results obtained previously with this compound. In twenty chicks, intravenous injections of prostaglandin $F_{2\alpha}$ (25–450 μg/kg) caused extension of the limbs and sometimes extension of the neck, followed by a tendency of the limbs to abduct. In lower doses (5–25 μg/kg) abduction of the limbs was the only change observed. Unlike prostaglandin E_1, prostaglandin $F_{2\alpha}$ caused no respiratory depression and little or no sedation in these doses.

INTRODUCTION

PROSTAGLANDINS E_1, E_2 and E_3 produce catatonic stupor in cats when injected into the cerebral ventricles while in young chicks they produce sedation when injected intravenously (HORTON, 1964). SAMUELSSON (1964) has now isolated prostaglandin $F_{2\alpha}$ from ox brain. In view of this, it was of interest to know whether prostaglandin $F_{2\alpha}$ had effects on the central nervous system similar to those reported for the prostaglandin E's.

We have found that prostaglandin $F_{2\alpha}$ has no effect when injected into the cerebral ventricles of cats in doses equal to or greater than those required to produce stupor with prostaglandin E_1. On the other hand in chicks both prostaglandins were active in similar doses but the effects produced were qualitatively different. Whereas prostaglandin E_1 produced sedation, prostaglandin $F_{2\alpha}$ caused extension and abduction of the limbs without sedation.

The chemical relationships between prostaglandin $F_{2\alpha}$ and the prostaglandin E's are shown in Fig. 1. The three prostaglandin E's differ only in the number of double bonds. Reduction of the oxo-group in a prostaglandin E yields two isomeric alcohols, those from prostaglandin E_2, for example, being referred to as prostaglandin $F_{2\alpha}$ and $F_{2\beta}$. The α– but not the β-isomers occur naturally (BERGSTRÖM *et al.*, 1962a; BERGSTRÖM *et al.*, 1962b; SAMUELSSON, 1963).

METHODS

Injections into the cerebral ventricles of cats

Cannulae were implanted under sterile conditions into the lateral ventricles of six adult male cats, weighing 3·0 to 4·6 kg and anaesthetized with pentobarbitone sodium (40 mg/kg),

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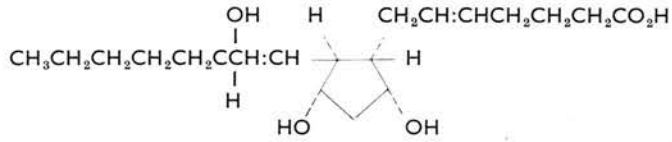
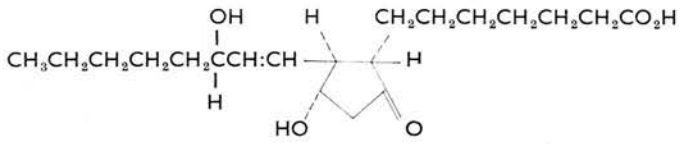
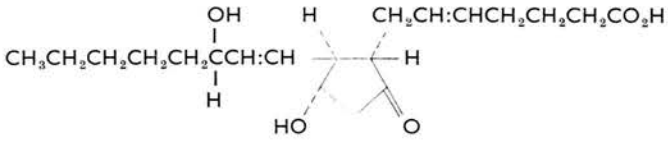


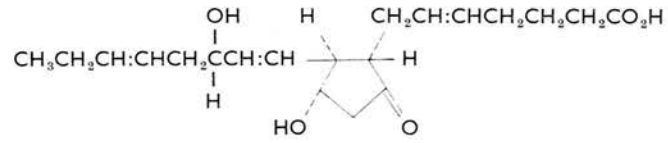
Prostaglandin $F_{2\alpha}$. 9α,11α,15-trihydroxy-prost-5,13-dienoic acid



Prostaglandin E_1. 11α,15-dihydroxy-9-oxo-prost-13-enoic acid



Prostaglandin E_2. 11α,15-dihydroxy-9-oxo-prost-5,13-dienoic acid



Prostaglandin E_3 11α,15-dihydroxy-9-oxo-prost-5,13,17-trienoic acid

FIG. 1. Formulae of prostaglandin $F_{2\alpha}$ and the three prostaglandin E's.

as described by FELDBERG and SHERWOOD (1953). After an interval of not less than 1 week, injections were made through the cannula without anaesthesia. The prostaglandins were dissolved in water by the addition of solid sodium bicarbonate and diluted with 0·9% saline. Neutral solutions were injected in volumes of 0·1 or 0·2 ml and washed in with 0·1 ml of 0·9% saline. The cats were observed for about 8 hr after an injection.

Intravenous injection into chicks

Two to five-day-old chicks weighing 35–50g were used. Injections were made into the right external jugular vein in volumes of not less than 0·1 ml and not more than 0·5 ml. The chicks were observed until about 1 hr after recovery of normal posture.

RESULTS

Injections into the cerebral ventricles of unanaesthetized cats

In cats intracerebro-ventricular injection of prostaglandin E_1 in the dose range 10–60 μg per animal produces a stuporose condition previously described in detail (HORTON, 1964). In order to compare prostaglandin $F_{2\alpha}$ with prostaglandin E_1, the sensitivity of six adult

male cats to prostaglandin E_1 was first tested by injecting 20 μg per animal into the cerebral ventricles. At this dose level, all the cats became stuporose; they sat with their eyes closed or partially closed apparently disinterested in their surroundings. Three showed signs of catatonia. This was diagnosed either when a limb remained in positions in which it was placed or if the cat could be put in abnormal postures (Fig. 2a). Such postures were sometimes retained for periods up to an hour or more.

The four cats which showed the most marked responses to prostaglandin E_1 were injected after an interval of a week or more, with prostaglandin $F_{2\alpha}$ in doses of 15, 20, 60 and 100 μg respectively. Prostaglandin $F_{2\alpha}$, in these doses, produced no obvious effect. The cats showed no signs of sedation but remained alert and interested in their surroundings (Fig. 2b). There was no stupor and no catatonia. Our observations are summarized in Table 1.

TABLE 1. EFFECTS OF PROSTAGLANDINS E_1 AND $F_{2\alpha}$ INJECTED INTO THE CEREBRAL VENTRICLES OF UNANAESTHETIZED CATS

		Prostaglandin E_1		Prostaglandin $F_{2\alpha}$	
Cat number	Weight (kg)	Dose injected (μg)	Effects	Dose injected (μg)	Effects
1	3·1	20	Stupor++ No catatonia	20	Friendly, playful and alert as in pre-injection period.
2	3·3	20	Stupor ++ Catatonia +	60	No difference from pre-injection control period.
3	3·0	20	Stupor + No catatonia		
4	3·2	20	Stupor + No catatonia		
5	4·6	20	Stupor +++ Catatonia +++	15	Alert, no evidence of stupor or catatonia.
6	3·2	20	Stupor ++ Catatonia +	100	No difference from pre-injection control period.*

*In this cat prostaglandin E_1 (20 μg) injected intraventricularly 1 hr after the prostaglandin $F_{2\alpha}$ caused catatonic stupor.

Intravenous injections into chicks

Twenty chicks were injected with prostaglandin $F_{2\alpha}$ in doses ranging from 2·7 to 450 μg/kg, and fourteen other chicks were injected with prostaglandin E_1 (10 to 2000 μg/kg) to confirm previous observations with this compound.

In doses from 25 to 450 μg/kg prostaglandin $F_{2\alpha}$ caused an immediate and extreme extension of the limbs with some dorsiflexion of the neck (Fig. 3a). These effects lasted for from 2 to 10 min. During this period the chick was unable to stand and often it was unable to right itself when placed on its back. When the chick was supported under its body the limbs did not remain extended but were partially flexed. The chick's limbs were unable to support the weight of its body, but the limbs could be moved when support

was provided. There was little or no sedation and there was no depression of respiration; the eyes were open throughout. When the chick regained the standing posture the legs tended to separate until gradually the chick's abdomen came to rest on the bench with its legs in full abduction. This tendency for the legs to abduct lasted for up to 30 min from the time of injection. With the lower dose range (5–25 μg/kg) this was the only effect observed with prostaglandin $F_{2\alpha}$ (Fig. 3b). The threshold dose was about 5 μg/kg.

In contrast, prostaglandin E_1 caused an initial marked but transient slowing of the respiratory rate followed within 30 sec of the injection, by sedation; the righting reflex was lost at the higher dose levels (Fig. 3c and 3d). In three chicks very high doses (1, 1 and 2 mg/kg) of prostaglandin E_1 were injected. The effects were pronounced and lasted for $1\frac{1}{2}$, $1\frac{1}{2}$ and $2\frac{1}{2}$ hours respectively. All the chicks recovered. The threshold dose of prostaglandin E_1 was about 10 μg/kg. The effects of 5 μg/kg could not be distinguished from those following an injection of an equal volume of saline.

No permanent changes were observed following injection of either of the prostaglandins.

DISCUSSION

The presence in brain of lipid-soluble substances of unknown chemical structure but with biological activity similar to the prostaglandins led to the discovery that the pure prostaglandin E's have potent central nervous activity (HORTON, 1964). Shortly after that investigation was completed, SAMUELSSON (1964) reported the isolation of prostaglandin $F_{2\alpha}$ from ox brain. Since prostaglandin $F_{2\alpha}$, and not the prostaglandin E's tested by HORTON (1964), appears to be the natural prostaglandin of brain it was of great interest to know whether it had the same central nervous activities. This investigation shows that it does not. In cats known to respond well to prostaglandin E_1, injection of prostaglandin $F_{2\alpha}$ into the cerebral ventricles produced no effects. This does not exclude the possibility that higher doses might have been effective. The negative result is, however, of great value since it effectively disposes of the criticism that effects produced by intracerebroventricular injection of these fatty acids are due merely to non-specific physical or physicochemical changes within the ventricles. Whereas the degree of unsaturation of the molecule makes little difference to the central nervous activity of the prostaglandin E's (HORTON, 1964), we have now shown that reduction of the oxo substituent of prostaglandin E_2 to form prostaglandin $F_{2\alpha}$ abolishes the ability of the compound to produce sedation and stupor in cats, at least within the same dose range. This finding indicates that a high degree of chemical specificity is necessary for the production of these effects.

The qualitative difference in actions of the prostaglandin E's and prostaglandin $F_{2\alpha}$ in chicks to some extent confirms the differences seen in cats. Whereas the prostaglandin E's produce sedation in chicks somewhat resembling the effects seen in cats but without catatonic stupor, prostaglandin $F_{2\alpha}$ produced little or no sedation in chicks even in doses as high as 450 μg/kg. Quite different effects were observed. With these higher doses the limbs were extended fully but not rigidly. The responses of the chicks to prostaglandins E_1 and $F_{2\alpha}$ superficially resembled those to curare and decamethonium (BUTTLE and ZAIMIS, 1949). Prostaglandins did not however cause a paralysis since active movement of the limbs was always possible. During the sedation due to prostaglandin E_1 pressure applied to a toe was followed by rapid withdrawal of the limb, while in the chick whose legs were extended following prostaglandin $F_{2\alpha}$ the legs could be flexed when the chick was held by its body. These effects of prostaglandin $F_{2\alpha}$ were presumably due to an action on some

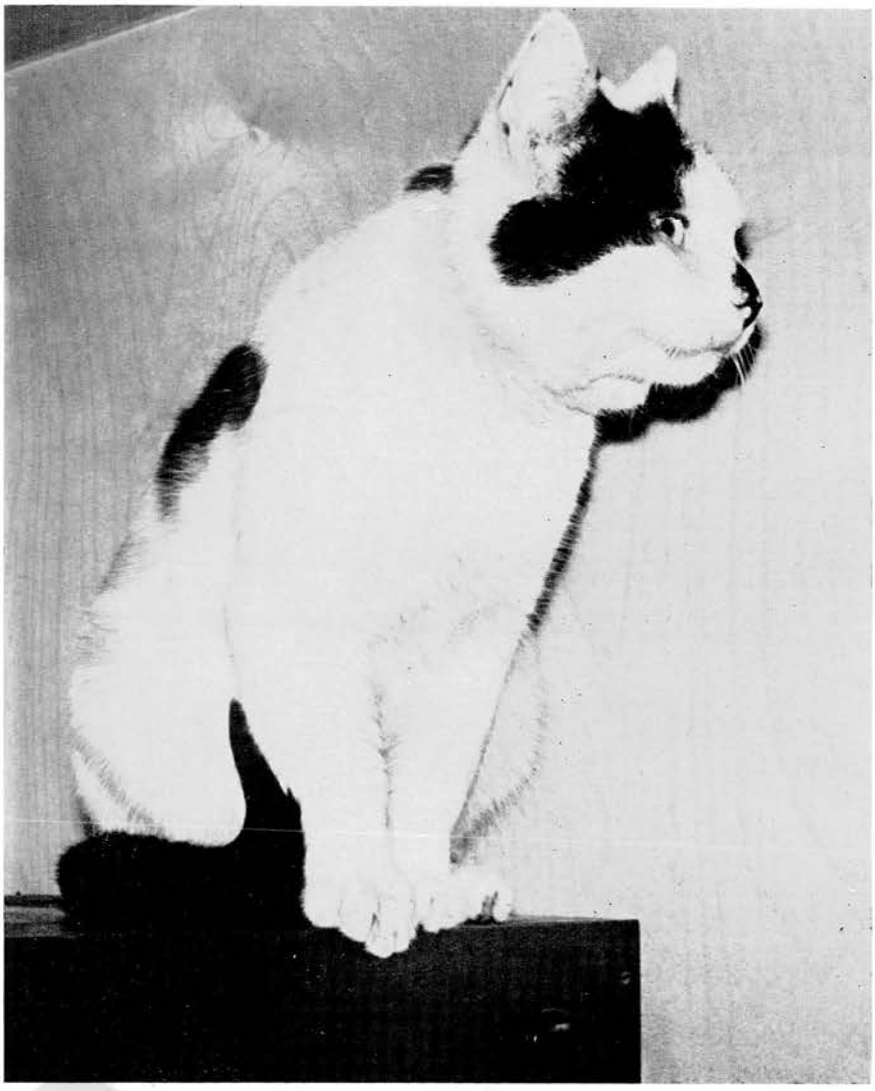


FIG. 2(a). (Cat No. 5) 4·6 kg photographed 1 hr after an injection into the cerebral ventricles of prostaglandin E_1 20 μg. There was a 13-day interval between the two injections.

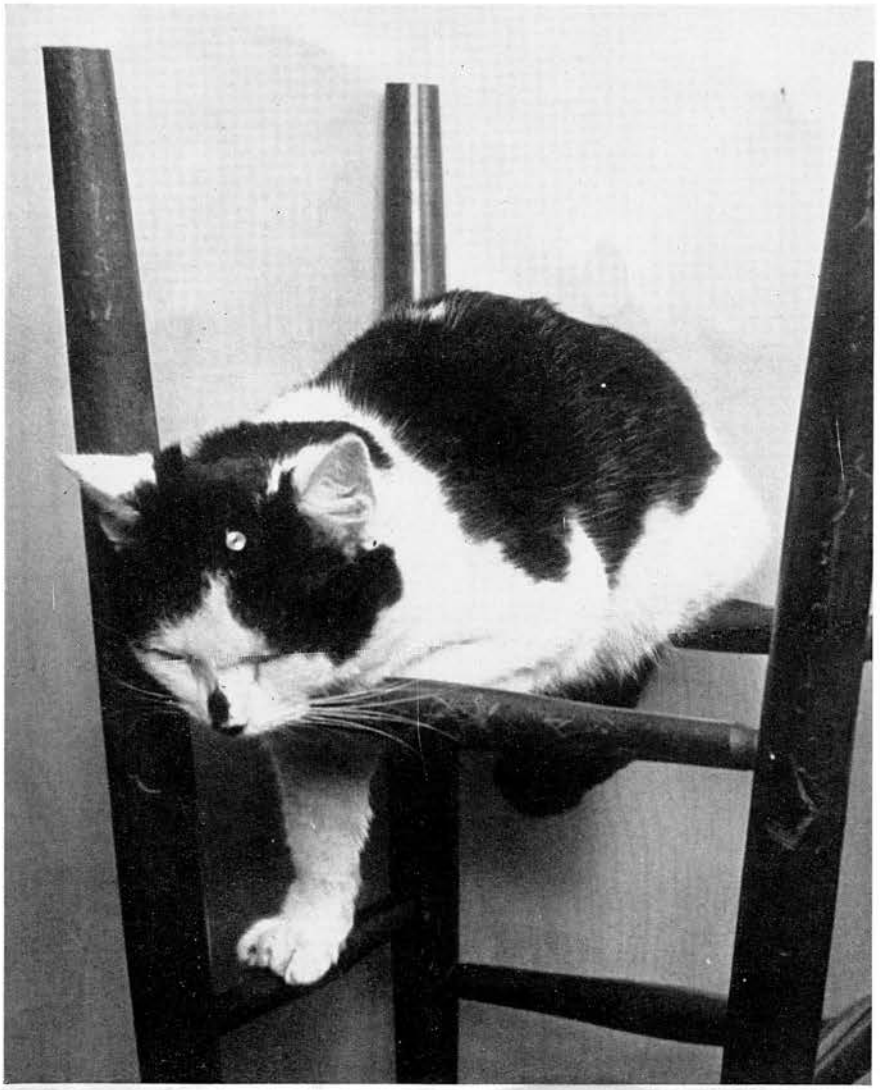


FIG. 2(b). Cat (No. 5) 4·6 kg photographed 1 hr after an injection into the cerebral ventricles of prostaglandin $F_{2\alpha}$ 15 μg. There was a 13-day interval between the two injections.

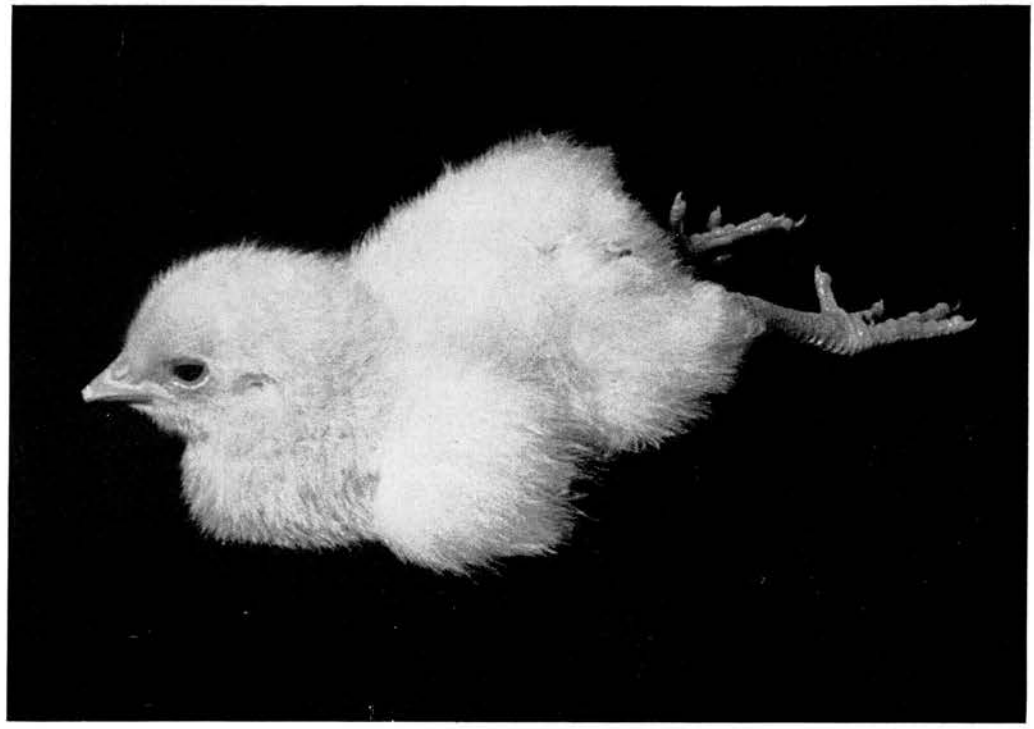
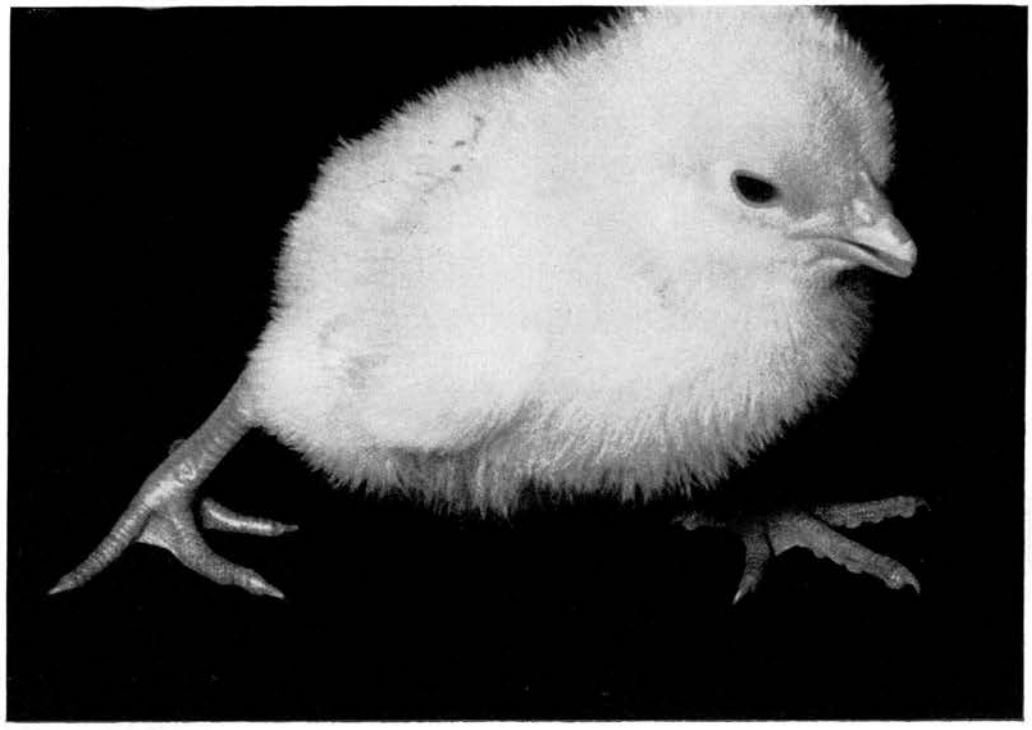


FIG. 3. (a) Chick 40g photographed 1 min after an intravenous injection of 4 μg of prostaglandin $F_{2\alpha}$.

(b) Same chick 22 min later



component of the systems controlling posture. The site of this action remains to be elucidated.

Little is yet known about the distribution of the different prostaglandins in the brain of different species. On the assumption, however, that prostaglandin $F_{2\alpha}$ is a normal constituent of human brain it is of interest to speculate that a derangement of its metabolism might account for certain mental disorders. For example, if prostaglandin $F_{2\alpha}$ in brain is normally formed by reduction of prostaglandin E_2, inhibition or absence of the enzyme system necessary for this conversion might lead to an accumulation of prostaglandin E_2 in cerebral tissue and so might produce a condition of catatonic stupor similar to that seen in cats (HORTON, 1964). Such a defect could account for some cases of catatonia in man.

Résumé—La prostaglandine $F_{2\alpha}$, composante normale du cerveau de boeuf, par injection intraventriculaire dans le cerveau de quatre chats non anesthésiés, n'entraine pas d'effet notable aux doses de 15–100 μg/animal.

Inversément, la prostaglandine E_1, composé très voisin, engendre à la dose de 20 μg/animal, une sédation marquée et de la stupeur chez chacun des animaux. Ces résultats confirment les données obtenues précédemment avec cette substance.

Chez vingt poussins, l'administration intraveineuse de prostaglandine $F_{2\alpha}$ (25–450 μg/kg) détermine l'extension des membres et dans certains cas du cou, suivie d'une tendance à l'abduction pour les membres. A des doses plus faibles (5–25 μg/kg) seule apparaît l'abduction des membres.

Contrairement à la prostaglandine E_1, la prostaglandine $F_{2\alpha}$ ne provoque aucune dépression respiratoire et, aux doses mentionnées, peu ou pas de sédation.

Zusammenfassung—Prostaglandin $F_{2\alpha}$, ein normaler Bestandteil des Ochsen-Gehirns, hatte keine offensichtliche Wirküng, wenn die Impfung in die Gehirnhöhlen (cerebrale Ventrikel) von vier unbetäubten Katzen in Dosen von 15–100 μg/pro Tier vorgenommen würde. Im Gegensatz dazu verursachte das eng verwandte prostaglandin E_1 auffallende Beruhigung und Betäubung in Dosen von 20 μg in jedem dieser Tiere und bestätigte die Ergebnisse die schon früher mit dieser chemischen Substanz erzielt wurden. Die intravenöse Einspritzung von prostaglandin $F_{2\alpha}$ (25–450 μg/kg) verursachte in sehr jungen Hünchen eine Streckung der Glieder und manchmal eine Streckung des Nackens, gefolgt von einer Neigung der Glieder sich vom Körper wegzubewegen (Abduktion). In kleineren Dosen (5–25 μg/kg) wurde nur die Wegbewegung der Glieder vom Körper (Abduktion) beobachtet. Im Gegensatz zu prostaglandin E_1, verursachte prostaglandin $F_{2\alpha}$ in diesen Dosen kein Sinken der Atmung und wenig oder keine Beruhigung.

Acknowledgements—We are indebted to Professor SUNE BERGSTRÖM of the Karolinska Institute for pure samples of the prostaglandins.

Author's address—I. H. M. MAIN, *Department of Physiology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London E.C.*1.

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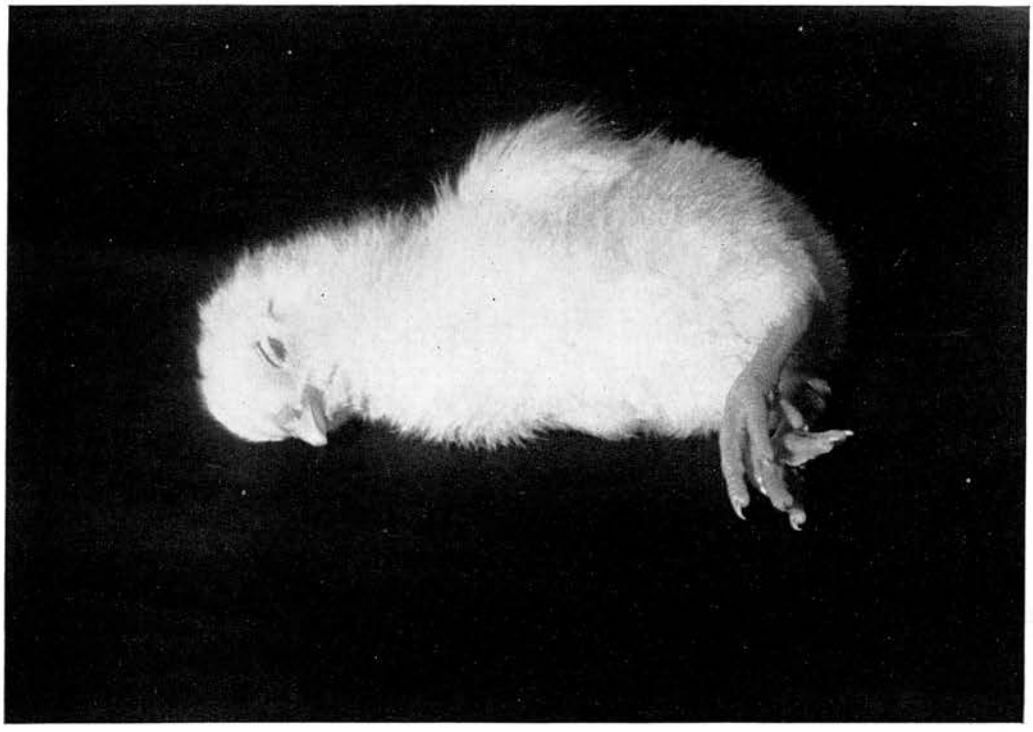
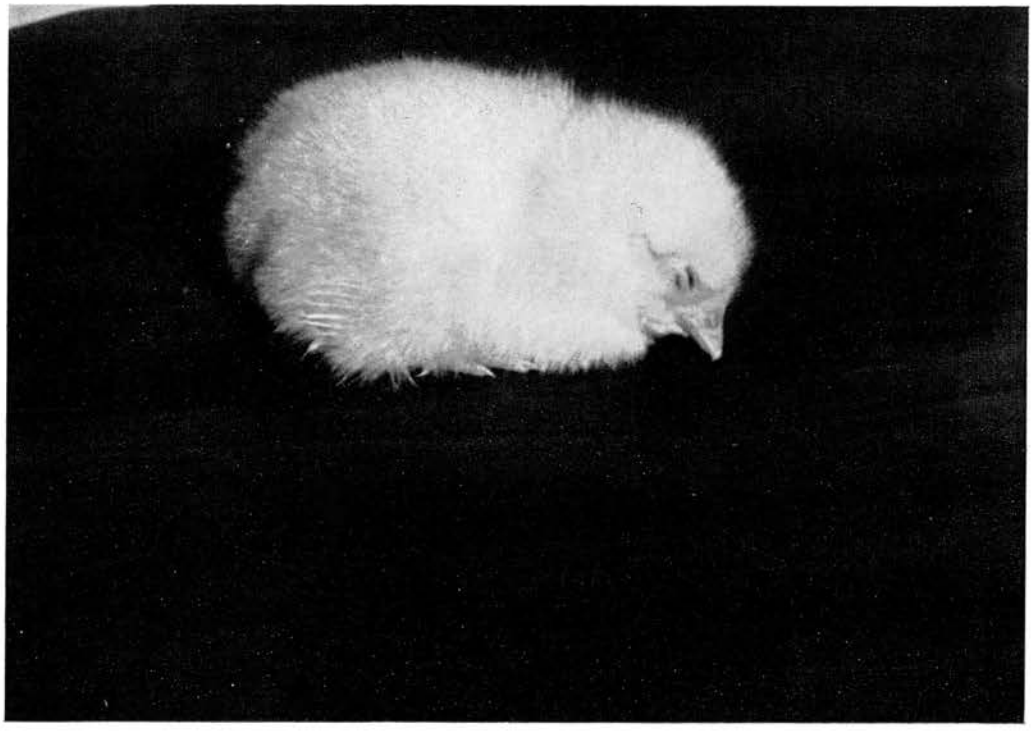


(c) Chick 39g photographed 1 min after an intravenous injection of 2 μg of prostaglandin E_1.

(d) Same chick 30 min later.



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Chez vingt poussins, l'administration intraveineuse de prostaglandine $F_{2\alpha}$ (25–450 μg/kg) détermine l'extension des membres et dans certains cas du cou, suivie d'une tendance à l'abduction pour les membres. A des doses plus faibles (5–25 μg/kg) seule apparaît l'abduction des membres.

Contrairement à la prostaglandine E_1, la prostaglandine $F_{2\alpha}$ ne provoque aucune dépression respiratoire et, aux doses mentionnées, peu ou pas de sédation.

Zusammenfassung—Prostaglandin $F_{2\alpha}$, ein normaler Bestandteil des Ochsen-Gehirns, hatte keine offensichtliche Wirküng, wenn die Impfung in die Gehirnhöhlen (cerebrale Ventrikel) von vier unbetäubten Katzen in Dosen von 15–100 μg/pro Tier vorgenommen würde. Im Gegensatz dazu verursachte das eng verwandte prostaglandin E_1 auffallende Beruhigung und Betäubung in Dosen von 20 μg in jedem dieser Tiere und bestätigte die Ergebnisse die schon früher mit dieser chemischen Substanz erzielt wurden. Die intravenöse Einspritzung von prostaglandin $F_{2\alpha}$ (25–450 μg/kg) verursachte in sehr jungen Hünchen eine Streckung der Glieder und manchmal eine Streckung des Nackens, gefolgt von einer Neigung der Glieder sich vom Körper wegzubewegen (Abduktion). In kleineren Dosen (5–25 μg/kg) wurde nur die Wegbewegung der Glieder vom Körper (Abduktion) beobachtet. Im Gegensatz zu prostaglandin E_1, verursachte prostaglandin $F_{2\alpha}$ in diesen Dosen kein Sinken der Atmung und wenig oder keine Beruhigung.

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The fate of tritium-labelled prostaglandin E_1 injected in amounts sufficient to produce central nervous effects in cats and chicks

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Running title : Fate of prostaglandin E_1

Summary

Tritiated prostaglandin E_1 has been administered to cats and chicks by a variety of routes and its fate studied. After injection into the lateral cerebral ventricle, the carotid and vertebral arteries and the aorta in cats, very little radioactivity was recovered from the central nervous system. Similar results were obtained when the prostaglandin was given intravenously and intra-arterially to chicks. The results of this study confirm work in other species and suggest that prostaglandin E_1 exerts its central pharmacological actions in extremely small concentrations.

Pharmacological actions of prostaglandin E_1 on the central nervous system have been demonstrated in cats by injection into the cerebral ventricles (1 & 2), by intravenous injection (3), by micro-iontophoretic application (4) and by close-arterial injection to the spinal cord (5). Although only relatively small doses are required to produce these effects in cats and also on intravenous injection in young chicks (1, 2 & 3), it has been shown in other species (rats, mice and sheep) that little or no tritium-labelled prostaglandin E_1 reaches the central nervous system following intravenous injection (6, 7 & 8).

In this investigation tritiated prostaglandin E_1 has been injected into cats and chicks by various routes in doses known to produce central nervous effects. The results show that little of the tritiated prostaglandin E_1 is present in the brain or spinal cord when these pharmacological effects are observed.

Methods

Injections into the cerebral ventricles of conscious cats

Collison cannulae were implanted into the lateral ventricles of three cats under pentobarbitone sodium (40mg/kg) anaesthesia (9). After an interval of not less than five days injections were made through the cannula without anaesthesia. Prostaglandin E_1 was dissolved in 0.9% saline. Injections were always of 0.2ml and

were washed in with a further 0.1ml saline. After a predetermined time the animals were anaesthetized with intravenous thiopentone sodium and killed by exsanguination (during anaesthesia the brain was exposed and immediately after death tissue and fluid samples were taken for assay). The brain was divided into various anatomical regions which were extracted separately (see Table 1).

Injection into the left vertebral artery

A cat was anaesthetised with pentobarbitone sodium (40mg/kg i.p.). The chest was opened and the animal was artificially ventilated. The left internal mammary artery was cannulated for retrograde injection into the vertebral artery and the left brachial artery was tied off. A thread was placed around the superior vena cava ready for occlusion and both external jugular veins were cannulated for collection of blood from the head. Prostaglandin E_1 in saline was injected slowly into the internal mammary artery, the superior vena cava being occluded at the start of the injection. The animal was exsanguinated through both external jugular veins. The brain was removed immediately after death.

Injection into the left common carotid artery

A similar injection procedure was followed except that the left lingual artery was cannulated for retrograde injection.

Injection into the abdominal aorta

In a third cat under pentobarbitone anaesthesia, the renal, superior mesenteric, inferior mesenteric and splenic blood vessels were ligated. The intestines, pancreas and spleen were removed. The aorta and inferior vena cava were ligated at their distal ends and the left renal artery was cannulated for retrograde injection into the aorta. Prostaglandin E_1 in saline was injected over a period of one minute into the renal artery. Two minutes after the start of injection the aorta was cannulated and the animal exsanguinated. The spinal cord (L1 to C1) and liver were removed immediately after death.

Intravenous injection into chicks

Two to three-day-old chicks (35-45g) were used. Injections were made into the right external jugular vein in a volume of 0.5ml. The chicks were killed by decapitation, 1 to 2 minutes after injection. The brain, liver and remaining carcass were extracted separately.

Intra-arterial injection into chickens

Chickens (800 - 850g) were anaesthetized with anaesthetic ether and their common carotid arteries were exposed. Injections of prostaglandin dissolved in 0.25 to 0.75ml of saline were made into the right carotid. The chickens were killed by decapitation 30 seconds after injection and their brains removed.

Preparation of samples for assay

Tissue samples were macerated in 96% ethanol and were allowed to stand for 2 hours. The samples were then centrifuged and the supernatant removed. The residue was washed with a further aliquot of 96% ethanol and re-centrifuged. The combined supernatants were taken to dryness under reduced pressure at 50°C. The residue was taken up in either 12ml methanol or the minimum quantity permitting complete solution, which ever was the greater. Blood samples were diluted with an equal volume of $N/_{10}$ hydrochloric acid and partitioned twice with an equal volume of ethyl acetate (10). The combined organic phases were taken to dryness and the residue was taken up in 12ml methanol. Cerebrospinal fluid samples were evaporated to dryness and the residues dissolved in 12ml methanol.

Estimation of tritium in extracts

This was carried out in a Packard Tricarb liquid scintillation counter using the internal standard technique. 4ml of the methanolic tissue extracts were always used with 10ml of a scintillator solution of the following composition: 2,5 - bis - [5' - t - butyl - benzoxazolyl (2')] - thiophene (CIBA), 7g; toluene, 600ml; methylcellosolve, 400ml; naphthalene, 80g. All results are expressed as the mean of at least three ten minutes counts. The tritiated prostaglandin E_1 used in this study had an activity of 138×10^6 d.p.m./mg.

Artificial cerebrospinal fluid

The artificial cerebrospinal fluid was of the following composition: $MgSO_4$, 205mg; $CaCl_2$, 120mg; KCl, 210mg; $NaHCO_3$, 2.3g; NaCl, 7.38g; made up to 1 litre with water.

Results

Injections into the lateral cerebral ventricle of unanaesthetized cats

A mixture containing equal parts tritiated and unlabelled prostaglandin E_1 was used in this study. The total dose injected into the lateral ventricle of each unanaesthetized cat was 50µg prostaglandin E_1 contained in 0.2ml saline.

In the first experiment, where the injection was made into the left lateral ventricle, decreased motor activity was observed 20 minutes after injection and after 1 hour there was definite signs of stupor and some catatonia. One hour and 45 minutes after injection, the catatonia and stupor were still increasing. The animal was then anaesthetized and its ventricular system was perfused with 10ml of artificial cerebrospinal fluid, the perfusate being removed through a needle placed in the cisterna magna. The animal was exsanguinated and the brain, liver and gall bladder and kidneys dissected out. All these tissues were extracted and their tritium content estimated. These are

summarized in Table 1. The largest amount was found in the liver. The kidneys and cerebrospinal fluid contained appreciable amounts but the brain contained very small quantities. This was distributed throughout all the regions (cortical, grey and white matter, caudate nucleus, hippocampus, thalamus, hypothalamus, midbrain, cerebellum, medulla and pons). It is of interest to note that when the liver extract was partitioned between aqueous pH3 and ethyl acetate nearly all the radioactivity went into the aqueous phase which indicated that the tritium was no longer incorporated into prostaglandin but was in the form of a less acidic, water soluble compound which is probably a metabolite.

In the second experiment a similar procedure was followed but the animal was anaesthetized and exsanguinated 5 minutes after injection into the left lateral ventricle. No signs of stupor or catatonia had developed in that time. Similar results to those of the first experiment (Table 1) were obtained with higher levels in the cerebrospinal fluid. Even five minutes after injection into the ventricular system appreciable amounts of radioactivity were recovered from the liver.

In the third cat the injection was made into the right lateral ventricle. Catatonia developed very rapidly and was

marked 20 minutes after injection. After 35 minutes the animal was anaesthetized and exsanguinated. In this experiment the ventricular system was not perfused but any pools of cerebrospinal fluid found during the dissection were removed and each tissue sample was washed with saline to remove any activity present in the cerebrospinal fluid adhering to the tissue surface, but little activity was present in the washings. Similar results were obtained (Table 1).

Intra-arterial injections into anaesthetized cats

In the first experiment 10μg of tritiated prostaglandin E_1 was injected retrogradely into the left internal mammary artery so that it should have passed up the left vertebral artery to the brain. One minute blood samples were taken from the jugular veins until the animal died during the sixth minute after the start of the injection. The brain was removed immediately after death. No area of the brain (forebrain, midbrain, cerebellum, medulla and pons) contained radioactivity equivalent to as much as 0.1μg prostaglandin and one quarter of the injected activity was found in the blood, the majority coming out in the first 3 minutes.

When 10μg of tritiated prostaglandin E_1 was injected retrogradely via the left lingual artery similar results were obtained. Approximately one quarter of the injected activity was recovered from the blood collected during 5 minutes

exsanguination and no part of the brain contained activity equivalent to as much as 0.1μg prostaglandin E_1.

When a similar retrograde injection of 10μg was made into the left renal artery, which should have enabled the prostaglandin to reach the spinal cord, 10% of the injected activity was recovered from the 1½ minute blood samples taken from the aorta. The spinal cord (lumbar and sacral regions) was found to contain activity equivalent to less than 0.1μg prostaglandin E_1. About 10% of the injected activity was recovered from the liver, indicating that not all the hepatic blood vessels had been occluded.

Intravenous injection into chicks

2μg of tritiated prostaglandin E_1 was injected into each of three chicks which all showed marked sedation within 90 seconds of injection. The chicks were killed at the time of peak effort which varied from 1 to 2 minutes. The brain of each chick was assayed separately and their livers were pooled for assay as were the rest of their carcasses. The three brains contained 1.7%, 0.8% and 0.1% respectively of the activity injected. The livers contained 5% and the remaining carcasses 85% of the injected activity.

Intra-arterial injection in chickens

After 1µg of tritiated prostaglandin E_1 was injected into the right common carotid artery of half-grown chicken, 2% of the injected activity was present in its brain. A similar result was obtained when 3µg of tritiated prostaglandin was injected into a second chicken. When 5µg unlabelled prostaglandin E_1 was injected into the right external jugular vein of an unanaesthetized chicken, pharmacological effects, loss of the righting reflex and stupor, similar to those seen in young chicks were observed.

Discussion

The results obtained in this study support Samuelsson's findings in other species that very little radioactivity is found in the central nervous system after intravenous or subcutaneous administration of tritiated prostaglandin E_1 (6, 7 & 8).

Prostaglandin E_1 appears to have little difficulty in crossing the cerebrospinal fluid-blood barrier, since 5 minutes after injection into the lateral ventricle appreciable quantities of activity were found in the liver. Whether this is due to uptake through the blood vessels of the choroid plexus or whether the prostaglandin is transported rapidly through the brain tissue and thence to the cerebral capillaries is unknown. However, from present evidence it appears that by whatever route

prostaglandin E_1 is administered relatively little is taken up by the central nervous system of either the cat or the chick. The midbrain, medulla and pons consistently contained higher quantities of radioactivity after intraventricular injection than other regions. The uptake by these areas is unlikely to be an artefact resulting from contamination of the tissue with cerebrospinal fluid adhering to the surface, as negligible activity was detected in the washings when the pieces of tissue were washed after dissection.

The dose of prostaglandin E_1 employed in each experiment was shown to be sufficient to produce central pharmacological effects. Thus it would seem that only minute amounts of prostaglandin E_1 are required to produce these pharmacological effects on the central nervous system.

The prostaglandin used in this study was tritiated on $C_5 - C_6$ so it is improbable that any active metabolite could be present without radioactivity. The possibility that the central effects observed could be due to a peripheral action of prostaglandin E_1 modifying afferent impulses to the brain can be discounted as very much higher doses are required to produce effects when the drug is given intravenously rather than into the lateral ventricle.

The finding that the radioactivity present in the liver of the cat killed 105 minutes after intraventricular injection

behaved in a different manner from prostaglandin E_1 in the hydrochloric acid-ethyl acetate solvent partition is of interest. All the known immediate metabolites of prostaglandin E_1 would behave in a similar manner to the parent compound in this partition system. Thus it would seem that the activity present in the liver is a simpler, less acidic and water soluble compound; this confirms the results of Samuelsson (6) in the rat after subcutaneous administration of labelled prostaglandin E_1.

The experiments described in this paper extend Samuelsson's observations to the cat and the chick, the species in which prostaglandin E_1 has been shown to have actions on the central nervous system. They further show that even when prostaglandin E_1 is injected more directly towards the central nervous system, only small amounts can be detected in the brain and spinal cord. Furthermore, in the young chick, originally selected for studying central effects of prostaglandin because it is said to lack a blood brain barrier, only minute amounts of prostaglandin E_1 could be detected in the brain following intravenous injections of pharmacologically effective doses.

Acknowledgements

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Table 1

Distribution of prostaglandin E_1 after the injection of 50µg into the lateral ventricles of cats.

The content of each area is given in µg followed by the concentration of ng/g in brackets.

Area	Cats		
	1	2	3
Cerebrum	1.89 (98)	2.92 (132)	<1.0 (<44)
Midbrain	0.10 (100)	0.30 (321)	0.79 (528)
Cerebellum	0.18 (55)	0.70 (194)	1.39 (283)
Medulla & pons	0.25 (125)	0.64 (256)	1.24 (730)
Spinal cord	-	-	0.2 (<<27)
Liver & gall bladder	19.42	0.92	3.00
Kidney	1.08	<0.2	1.18
Lungs	-	0.2	<<0.2
C.S.F.	1.19	11.92	0.79
Blood	-	-	0.47
Washings of brain tissue samples	-	-	<0.2

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Actions of prostaglandins on the spinal cord of the chick

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In unanaesthetized chicks the intravenous injection of prostaglandin $F_{2\alpha}$ (25 μg/kg or more) causes extension of the legs lasting several minutes (Horton & Main, 1965). Experiments have now been made to localize the site of this action. In chicks anaesthetized with chloralose, prostaglandin $F_{2\alpha}$ (30–100 μg/kg) injected intravenously increased gastrocnemius muscle tension, recorded isometrically; the effect was abolished by denervation of the muscle. Prostaglandin $F_{2\alpha}$ had no effect on contractions of gastrocnemius muscle produced by stimulation of the ipsilateral sciatic nerve. These results suggest that prostaglandin $F_{2\alpha}$ acts centrally not peripherally. Since increases in gastrocnemius muscle tension following intravenous injections of prostaglandin $F_{2\alpha}$ (40 μg/kg) were also observed in spinal chicks (Fig. 1), it is concluded that the effect seen in the unanaesthetized chick is either wholly or partly due to an action of prostaglandin $F_{2\alpha}$ on the spinal cord.

We have also observed in the spinal chick that reflex contractions of the gastrocnemius muscle in response to stimulation of the central stump of the contralateral sciatic nerve are potentiated by prostaglandin $F_{2\alpha}$, in doses (1·5–7 μg/kg) too low to affect muscle tension in the absence of stimulation. This action of prostaglandin $F_{2\alpha}$ resembles that of strychnine (Fig. 1), and the two substances are about equally potent on this preparation. Since prostaglandin $F_{2\alpha}$ has been isolated from the central nervous system (Samuelsson, 1964), this high potency on spinal reflexes is of some interest.

Prostaglandin E_1 also potentiated the crossed extensor reflex in the spinal chick in doses of 1·5–5 μg/kg. This result was unexpected since in the chloralosed chick prostaglandin E_1 inhibits (whereas prostaglandin $F_{2\alpha}$ potentiates) the crossed extensor reflex, and in the unanaesthetized chick prostaglandin E_1, unlike prostaglandin $F_{2\alpha}$, causes sedation and no increase in muscle tension (Horton, 1964).

In both the chloralosed and the spinal preparation, prostaglandin $F_{2\alpha}$ caused a rise and prostaglandin E_1 a fall in arterial blood pressure, but these changes were insufficient to account for the central nervous effects. The pressor response to prostaglandin $F_{2\alpha}$ was not abolished by either hexamethonium (10 mg/kg) or phenoxybenzamine (1 mg/kg).

Effects of prostaglandins on the spinal chick will be demonstrated. The

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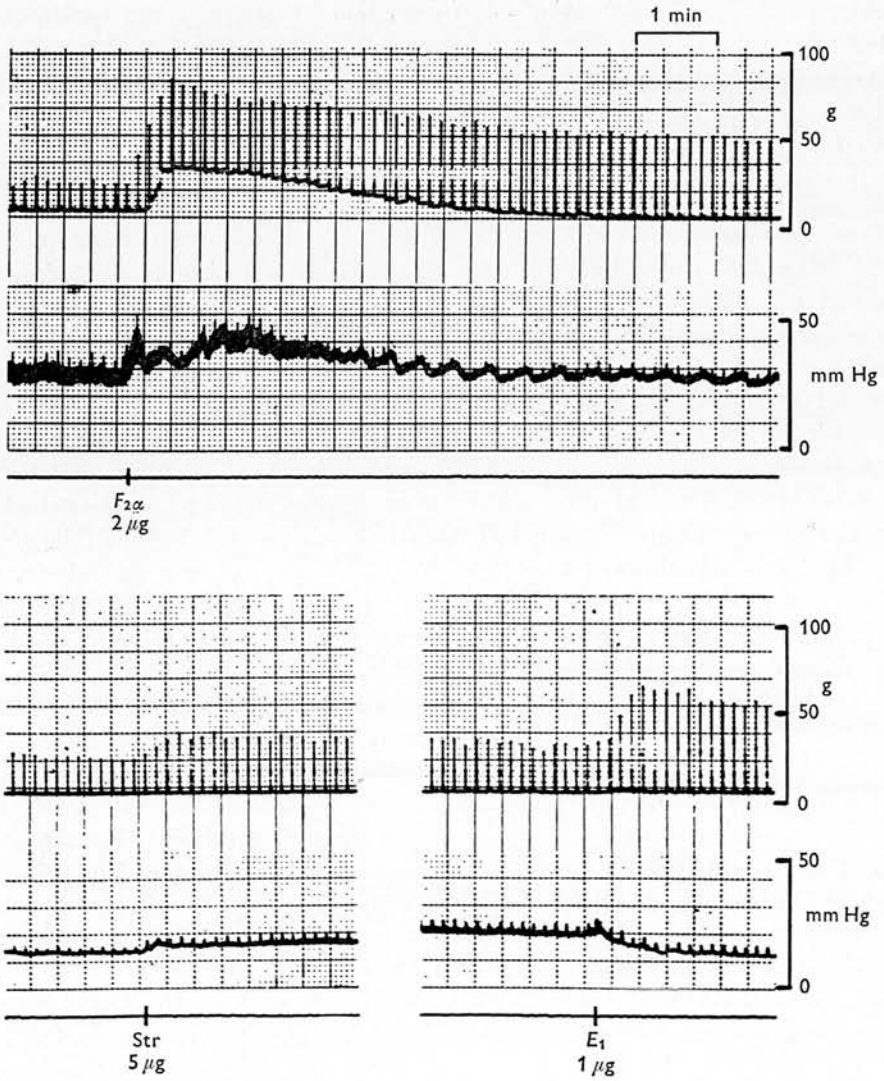

Fig. 1. Spinal chick (decapitated weight 45 g). Upper trace: gastrocnemius muscle tension recorded isometrically. Lower trace: arterial blood pressure (in this trace artifacts occurred due to slight movement of the cannula at the time of each stimulation). Reflex contractions of gastrocnemius muscle were elicited by 5 V pulses of 25 msec duration. $F_{2\alpha}$ = prostaglandin $F_{2\alpha}$; E_1 = prostaglandin E_1 and Str. = strychnine hydrochloride, all injections into the right external jugular vein.

chick is anaesthetized with ethyl chloride and ether, the cord is sectioned between C2 and C4 and the chick is decapitated. Arterial blood pressure is recorded from the left sciatic artery with a Sanborn pressure transducer. Tension of the right gastrocnemius muscle is recorded isometrically with a Grass force-displacement transducer (FT. 03). The central stump of the left sciatic nerve is stimulated once every 10 sec with 5 V pulses of 25 msec duration.

We thank Professor S. Bergström and Dr B. Samuelsson of the Karolinska Institute for supplies of prostaglandins E_1 and $F_{2\alpha}$, and Dr D. A. van Dorp of the Unilever Research Laboratory, Vlaardingen, for prostaglandin E_1.

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FURTHER OBSERVATIONS ON THE CENTRAL NERVOUS ACTIONS OF PROSTAGLANDINS F_{2a} AND E_1

BY

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FURTHER OBSERVATIONS ON THE CENTRAL NERVOUS ACTIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_1

With an Addendum on the Effects of Prostaglandins E_1 and $F_{2\alpha}$ on Systemic Arterial Blood Pressure in Chicks

BY

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(*Received March* 29, 1967)

We have previously reported that prostaglandins E_1, E_2 and E_3 produce catatonic stupor in the cat and sedation in the chick (Horton, 1964), and that prostaglandin $F_{2\alpha}$, the only prostaglandin so far isolated from the brain (Samuelsson, 1964), does not have these central actions (Horton & Main, 1965a). On the other hand, on intravenous injection in the young chick, prostaglandin $F_{2\alpha}$ causes dorsiflexion of the neck and extension of the legs, effects which could be either central or peripheral (Horton & Main, 1965a). It seemed likely that extension of the legs would be conducive to quantitative measurement and this was therefore used as the starting point for further investigation. From experiments reported in this paper, it is concluded that the prostaglandins act upon the spinal cord of cats and chicks. In view of the identification of prostaglandins in central nervous tissues of these species reported in the following paper (Horton & Main, 1967), the possibility that some of these actions may reflect a physiological role of the prostaglandins in the central nervous system must be considered.

Some of the experiments reported here have been demonstrated to the Physiological Society (Horton & Main, 1965b).

METHODS

Experiments on chicks

Chicks of either sex, aged from 2 days to 9 weeks old and weighing from 35 g to 1.3 kg, were anaesthetized with chloralose (60 to 80 mg/kg) or urethane (1.5 to 2 g/kg except in the preliminary experiments described under "Results,") injected intraperitoneally or, for spinal cord transection, with ethyl chloride or an ethyl chloride-diethyl ether mixture. The trachea was cannulated in all experiments and prostaglandins were injected *via* a cannula inserted into the right external jugular vein. Blood pressure was recorded from the left ischiadic artery *via* a polyethylene cannula connected to a Sanborn pressure transducer, using a Sanborn polygraph.

Mid-cervical cord transection was performed as follows. After cannulation of the trachea and right external jugular vein, the common carotid arteries, which lie deep and immediately in front of the vertebral column, were divided between ligatures. Two strong ligatures were passed round the vertebral column at about the level of the second and sixth cervical vertebrae respectively, and a third ligature was passed round the remaining tissues of the neck excluding the tracheal and jugular cannulae. The ligatures were tied and the cord sectioned between the two ligatures around

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the vertebral column at about the level of the fourth cervical vertebra. The head was then removed and subsequently weighed. The chick was artificially ventilated by a Palmer small animal respiration pump with a frequency of 36 or 48/min and delivering a volume of 2 ml./50 g chick.

Muscle tension was recorded isometrically from the freed tendon of a gastrocnemius muscle using a force-displacement transducer (Grass FT.03, 0–200 g springs). The limb was fixed to a cork board by pins inserted through the lower femur and tibia. Muscle contractions were elicited either by stimulating the cut peripheral stump of the ipsilateral sciatic nerve or, reflexly, by stimulating the cut central stump of the contralateral sciatic nerve maximally (5 V square wave pulses, 1–25 msec duration, 0.05–0.5/sec). Lower eyelid tension was recorded by a thread attached to the centre of the lid margin ; in these experiments the head was immobilized. Body temperature was maintained by means of a heated operating table and an overhead warming lamp. In some but not all experiments, rectal temperature was recorded using a small thermometer.

The cerebral hemispheres were exposed by removing the overlying thin cartilaginous area of skull with fine scissors. The dura was then incised on both sides, avoiding the midline from which bleeding occurred readily. The spinal cord was exposed from the mid-thoracic to the lumbar region. Exposure of the lower lumbar region proved difficult owing to the anatomical arrangement of the avian pelvis.

Experiments on cats

Cats of either sex weighing 0.75 to 5.1 kg were either anaesthetized with chloralose (80 mg/kg injected intraperitoneally or intravenously), decerebrated at the inter-collicular level or spinalized at the level of the second cervical vertebra (with destruction of the brain) under ethyl chloride-ether anaesthesia. Spinal cats were artificially ventilated. Blood pressure was recorded from a carotid artery using a Sanborn pressure transducer. Intravenous injections were made into an external jugular vein.

Close-arterial injection to the gastrocnemius muscle. The artery supplying the gracilis muscle was cannulated with fine polyethylene tubing connected to a three-way tap. Retrograde injections were made into the femoral artery.

Recording of muscle tension. Muscle tension was recorded isometrically from the freed tendons of hind limb muscles (gastrocnemius, quadriceps and hamstring) using force-displacement transducers (Grass FT.03, 0–2 kg springs). Contractions of the gastrocnemius muscle were elicited by stimulating the cut peripheral stump of the ipsilateral sciatic nerve (5 V square wave pulses, 1 msec duration), or by direct stimulation of the muscle (20–50 V pulses, 1 msec duration) through silver wire electrodes looped round each end of the muscle. Crossed extensor reflexes were elicited from the gastrocnemius or quadriceps muscles by stimulating the cut central stump of the contralateral sciatic nerve either with single stimuli (5 V pulses, 0.1–1 msec duration) or with trains of stimuli at 20/sec lasting 5–10 sec. The patellar reflex was elicited by an electrically operated Palmer knee-jerk hammer.

Inhibition of the crossed extensor reflexes recorded from the quadriceps muscle was achieved by stretching the ipsilateral hamstring muscle, the tendon of which was connected to a force-displacement transducer. By moving the transducer through a standard distance a constant stretch could be repeatedly applied to the muscle.

Chronic denervation of the gastrocnemius muscle. The cat was anaesthetized with pentobarbitone (40 mg/kg injected intraperitoneally). Under aseptic conditions a sciatic nerve was located *via* a small skin incision on the posterior surface of the limb. A segment of nerve 1 in. long was removed, the wound closed and the cat allowed to recover. Responses of the denervated muscle were investigated 3 weeks later.

Spinal cord exposure. In decerebrate or spinal cats the spinal cord was exposed dorsally from L4 to S3. The dura was incised and reflected. In some experiments exposure was carried out under ether anaesthesia before spinalization to reduce reflex excitability. Cotton wool impregnated with a warm solution of the substance under investigation was applied to the exposed surface.

Hind limb de-afferentation. The dorsal roots from L4 to S3 on one side were cut. This abolished the crossed extensor reflex elicited by stimulating the central stump of the cut sciatic nerve on the side of the de-afferentation but not that elicited by stimulating the cut sciatic nerve centrally on the contralateral side.

RESULTS

The site of action of prostaglandin $F_{2\alpha}$ in chicks

Effects of prostaglandin $F_{2\alpha}$ on anaesthetized chicks

Preliminary experiments were made to determine whether $F_{2\alpha}$ would produce extension of the legs in the anaesthetized chick as we had observed previously in the unanaesthetized chick (Horton & Main, 1965a).

Prostaglandin $F_{2\alpha}$ (40 μg/kg) was injected intravenously in 6 two-day old chicks. Two chicks had been injected intraperitoneally with 0.9% sodium chloride solution, 2 with urethane (1.25 g/kg) and 2 with urethane (2.25 g/kg). In the unanaesthetized control chicks, $F_{2\alpha}$ caused extension of the legs lasting between 1 and 2 min, accompanied by dorsiflexion of the head and neck. The eyes were wide open. This was followed by a period during which the chick could stand with legs abducted. In the chicks lightly anaesthetized with urethane (1.25 g/kg), in which the righting reflex was absent and the eyes were shut but the withdrawal reflex to toe pinching was present, dorsiflexion of the neck and extension of the legs were observed following the intravenous injection of $F_{2\alpha}$; the eyes opened during the period when the legs were extended. In the chicks more deeply anaesthetized with urethane (2.25 mg/kg), the withdrawal reflex was absent and the intravenous injection of $F_{2\alpha}$ had no effect.

These results showed that it would be possible to use the anaesthetized chick to locate the site of action of $F_{2\alpha}$, provided that the anaesthesia was not too deep.

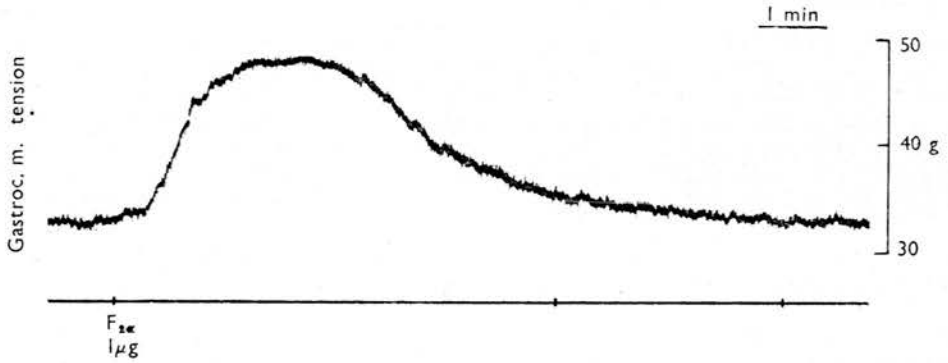

Fig. 1. Chicken (550 g) anaesthetized with urethane (1.7 g/kg). Record of gastrocnemius muscle tension recorded isometrically. Response to prostaglandin $F_{2\alpha}$ (1 μg) injected intravenously.

Effects of prostaglandin $F_{2\alpha}$ on gastrocnemius muscle tension

In chicks anaesthetized with urethane or chloralose, $F_{2\alpha}$ increased gastrocnemius muscle tension measured isometrically (Fig. 1). The increase in tension was similar in onset and duration to the extension of the legs observed in unanaesthetized chicks. The contraction began 10 to 30 sec after the injection and lasted up to 10 min. There was no obvious decrease in sensitivity to $F_{2\alpha}$ (on a weight basis) with age. The sensitivity to $F_{2\alpha}$ was similar in chicks anaesthetized with either urethane or chloralose. In 9 chicks

the lowest effective dose of $F_{2\alpha}$ ranged from 2 to 100 μg/kg. However, tachyphylaxis to successive doses of $F_{2\alpha}$ often developed, especially when the interval between doses was short. Assessment of threshold doses was therefore difficult.

The increase in gastrocnemius muscle tension in response to $F_{2\alpha}$ was accompanied often by defaecation and sometimes by opening of the eyes. The latter effect is illustrated in Fig. 2. In this experiment tension of the lower eyelid was recorded isometrically and the increase in eyelid tension in response to $F_{2\alpha}$ paralleled the increase in gastrocnemius muscle tension.

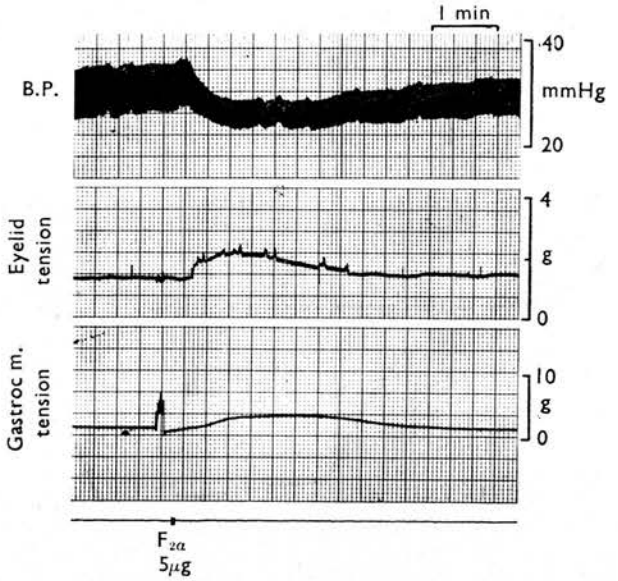

Fig. 2. Chick (85 g) anaesthetized with urethane (2.0 g/kg). Upper trace: systemic arterial blood pressure. Middle trace: lower eyelid muscle tension. Lower trace: gastrocnemius muscle tension. Responses to prostaglandin $F_{2\alpha}$ (5 μg) injected intravenously.

Effects of prostaglandin $F_{2\alpha}$ on systemic arterial blood pressure

In 4 chicks anaesthetized with urethane, in which gastrocnemius muscle tension and ischiadic arterial blood pressure were recorded simultaneously, increases in gastrocnemius muscle tension following an intravenous injection of $F_{2\alpha}$ were accompanied by changes in blood pressure which varied from one chick to another; in one chick there was a rise, in a second a fall and in the other 2 chicks a rise followed by a fall in blood pressure. In view of this it seems unlikely that either a rise or a fall of blood pressure could have been the cause of the increase in gastrocnemius muscle tension produced by $F_{2\alpha}$. Furthermore, similar rises or falls in blood pressure produced by injecting adrenaline or acetylcholine did not alter gastrocnemius muscle tension.

Effect of mid-cervical cord transection on the response to prostaglandin $F_{2\alpha}$

In 8 chicks whose spinal cord had been sectioned in the mid-cervical region (decapitated weight 31–71 g), $F_{2\alpha}$ in doses of 9 to 166 μg/kg, given intravenously, increased gastrocnemius muscle tension.

Effect of acute denervation on the response to prostaglandin $F_{2\alpha}$

In one unanaesthetized chick the left sciatic nerve was sectioned after infiltration of the overlying skin with amethocaine. Fifteen minutes after section an intravenous injection of $F_{2\alpha}$ (330 μg/kg) resulted in the usual dorsiflexion of the neck accompanied by extension of the right (innervated) limb. There was no extension of the left (denervated) limb.

In the anaesthetized or spinal chick $F_{2\alpha}$ also failed to produce a contraction if the gastrocnemius muscle was acutely denervated. In the experiment on a spinal chick illustrated in Fig. 3, both sciatic nerves were exposed and the tension of both gastrocnemius muscles

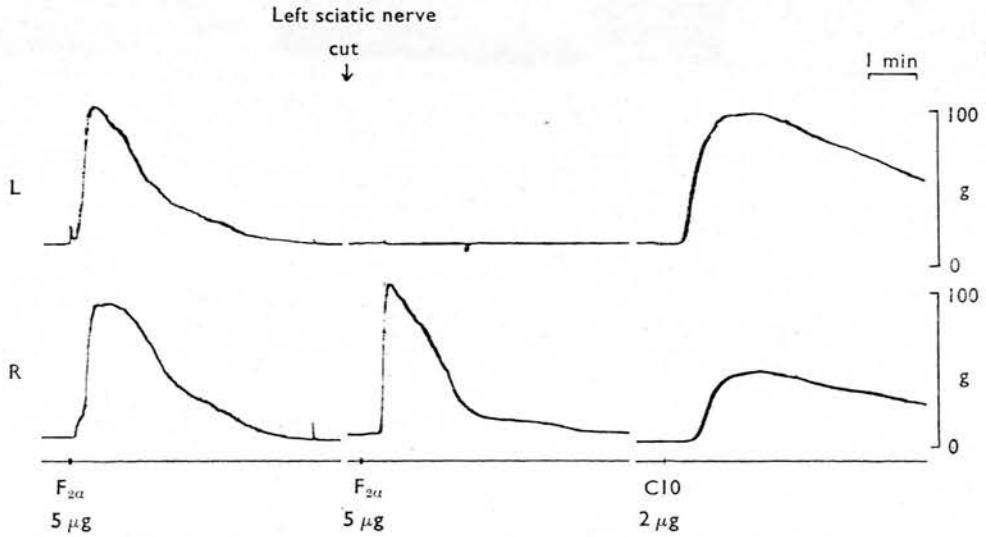

Fig. 3. Spinal chick (decapitated weight 32 g). Gastrocnemius muscle tension recorded isometrically (upper trace: left leg; lower trace: right leg). Responses to prostaglandin $F_{2\alpha}$ (5 μg) and decamethonium iodide (C10, 2 μg) injected intravenously. Between the first and second panel the left sciatic nerve was cut. There was an interval of 60 min between each injection.

was recorded. $F_{2\alpha}$ (156 μg/kg) injected intravenously increased the tension of both the left and the right gastrocnemius muscles. After the left sciatic nerve had been sectioned the same dose of $F_{2\alpha}$ increased the tension of the right gastrocnemius muscle only. To avoid tachyphylaxis the interval between doses was 1 hr. Blood pressure was not recorded in this experiment since cannulation of an ischiadic artery would have interfered with the limb blood flow and in the 2-day old chick no satisfactory alternative artery was available for cannulation. Adrenaline (16 μg/kg) was injected intravenously midway between the doses of $F_{2\alpha}$ to ensure that the blood pressure was maintained. After denervation decamethonium iodide (63 μg/kg) contracted both gastrocnemius muscles indicating that the denervated gastrocnemius muscle was still capable of contracting in response to drugs injected intravenously.

These experiments show that the response of the gastrocnemius muscle to $F_{2\alpha}$ is not due to a direct action on the muscle or an action on the neuromuscular junction.

The site of action of prostaglandins $F_{2\alpha}$ and E_1 in decerebrate and spinal cats

Experiments on decerebrate cats

Although in the unanaesthetized cat prostaglandin $F_{2\alpha}$ injected either intravenously (15 μg/kg) or into the cerebral ventricles (15–100 μg) has no obvious effect, it was observed that in the decerebrate cat $F_{2\alpha}$ injected intravenously increases gastrocnemius muscle tension as it does in the intact and spinal chick. However, in the decerebrate cat, unlike in the chick, E_1 also increases gastrocnemius muscle tension on intravenous injection. These actions are illustrated in Fig. 4. In 3 decerebrate cats the approximate threshold dose of $F_{2\alpha}$ was 5 to 7 μg/kg and in 7 decerebrate cats the approximate threshold dose of E_1 ranged from 5 to 15 μg/kg. In 2 cats E_1 in doses of 8 and 17 μg/kg failed to increase gastrocnemius muscle tension, although the fall in arterial blood pressure was the same as in the other cats. Tachyphylaxis to E_1 sometimes developed.

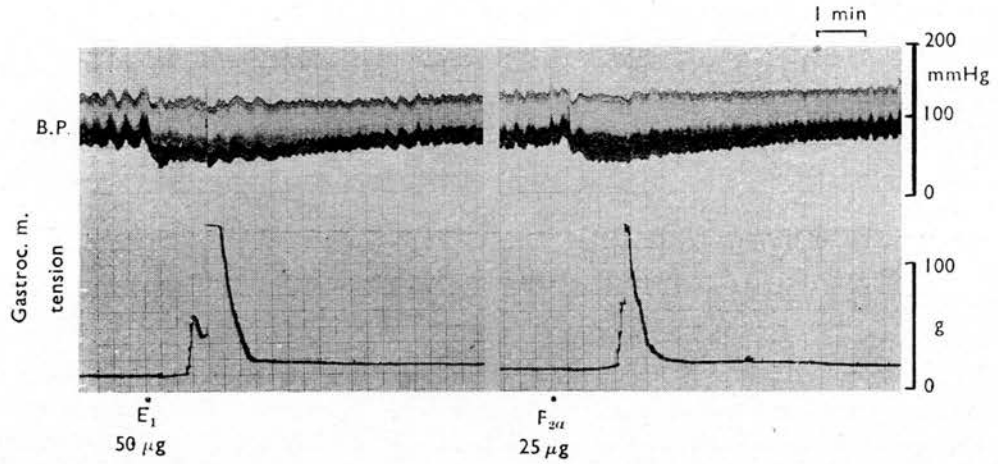

Fig. 4. Decerebrate cat (3.4 kg). Upper trace: carotid blood pressure. Lower trace: gastrocnemius muscle tension. Responses to intravenous injections of prostaglandin E_1 (50 μg) and prostaglandin $F_{2\alpha}$ (25 μg).

The interval between the injection and the onset of contraction varied from 25 to 100 sec. Sometimes the contractions of the gastrocnemius muscle were accompanied by arching of the back, extension of the forelimbs and on two occasions walking movements. Decerebrate rigidity was potentiated. E_1 and $F_{2\alpha}$ always caused a fall in systemic arterial blood pressure sometimes accompanied by an increase in ventilation rate. There was no obvious correlation between the magnitude of the depressor response and the contraction of the gastrocnemius muscle. Larger depressor responses produced by intravenous injection of acetylcholine (10 μg/kg) had no effect on gastrocnemius muscle tension.

In view of the apparent similarity in response of the gastrocnemius muscle in the decerebrate cat to E_1 and $F_{2\alpha}$, and because of the shortage of $F_{2\alpha}$, subsequent studies on the site of this action of the prostaglandins in the cat were carried out with E_1.

In the decerebrate cat acute denervation of the gastrocnemius muscle prevented the increase in tension following an intravenous injection of E_1. On close-arterial injection

into a hind limb in doses similar to those effective by the intravenous route, E_1 had no effect on gastrocnemius muscle tension. Higher intra-arterial doses sometimes increased tension but only after a longer latent period than after intravenous injection, and the response was bilateral. These results show that the increased gastrocnemius muscle tension following intravenous injection of E_1 in the decerebrate cat is not due to an action on skeletal muscle or the neuromuscular junction.

Experiments on spinal cats

In 3 spinal cats E_1 in threshold doses ranging from 5 to 40 μg/kg increased gastrocnemius muscle tension, the time course of the contraction being similar to that in the decerebrate cat. In 3 other spinal cats E_1 in doses from 16 to 58 μg/kg had no effect on gastrocnemius muscle tension.

The response of the gastrocnemius muscle to E_1 in the spinal cat was abolished by denervation of the muscle but not by dorsal root (L4 to S3) section. Close-arterial injections of E_1 into the hind limb of the spinal cat, in doses similar to those effective by the intravenous route, had no effect on gastrocnemius muscle tension, thus confirming the findings in decerebrate cats that this is not a peripheral action of prostaglandin E_1.

In one experiment a direct action of E_1 on the spinal cord was demonstrated by topical application. An increase in gastrocnemius muscle tension occurred when E_1 (500 μg/ml.) was applied to the dorsal surface of the lumbar (L6–7) cord. This effect could be elicited repeatedly. Application of saline had no effect. Concentrations of 100 μg/ml. or less of E_1 had no effect.

Action of prostaglandin E_1 on skeletal muscle

The experiments show that the increase in gastrocnemius muscle tension is not due to an action of E_1 on the muscle itself. In higher doses E_1 injected close-arterially to the muscle had an inhibitory effect on muscle twitches. In a spinal cat E_1 (29 μg/kg) injected close-arterially inhibited contractions of the gastrocnemius muscle in response to electrical stimulation of the sciatic nerve. In another experiment, on a chloralosed cat, contractures of the chronically denervated gastrocnemius muscle in response to close-arterial injections of acetylcholine (0.18 μg/kg) were also inhibited by E_1 (23 μg/kg) injected close-arterially. This effect lasted throughout the remainder of the experiment. On the other hand, contractions due to direct electrical stimulation were not significantly affected (Fig. 5).

Effects of prostaglandins $F_{2\alpha}$ and E_1 on spinal reflexes in cats and chicks

During the experiments described above a crossed extensor reflex was sometimes elicited as a test of the integrity of the spinal cord. It was observed that prostaglandins usually potentiated this reflex in both the chick and the cat. This action of prostaglandins on spinal reflexes has been investigated further and the results are described below.

Spinal chicks

In some of the early experiments on spinal chicks no crossed extensor reflex could be elicited, while in other experiments the reflex appeared only after an interval of 30–60 min. In later experiments, an intravenous injection of 0.9% sodium chloride solution or

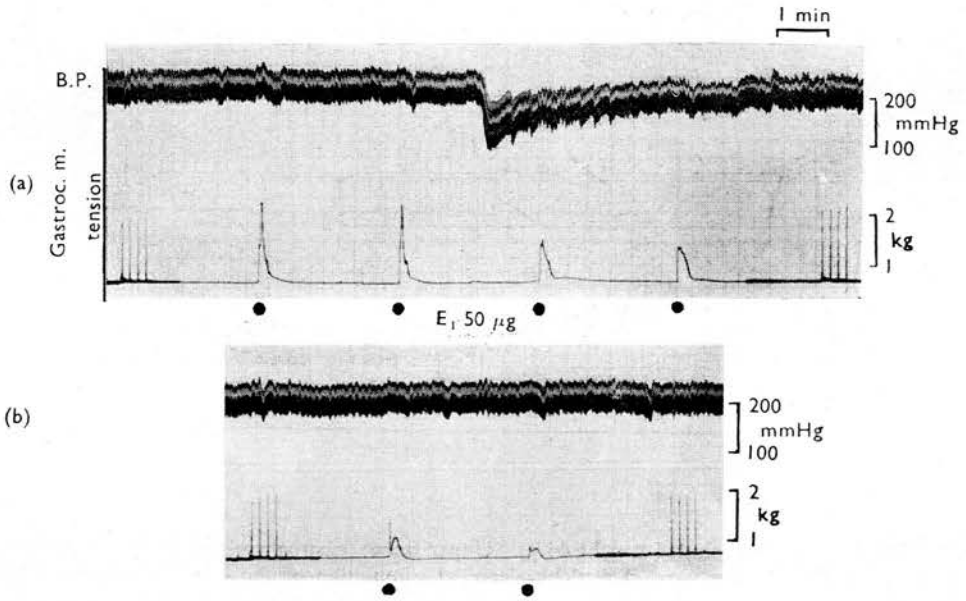

Fig. 5. Cat (2.2 kg) anaesthetized with chloralose (80 mg/kg). Upper trace: carotid arterial blood pressure. Lower traces: muscle tension recorded from a chronically denervated gastrocnemius muscle; twitches were elicited by direct electrical stimulation of the muscle; contractures were elicited by close-arterial injection of acetylcholine (0.4 μg) as indicated by the dots. The interval between (a) and (b) was 12 min. Effects of prostaglandin E_1 (50 μg) injected close-arterially.

adrenaline (5–10 μg/kg) was given immediately after section of the spinal cord, and in these experiments reflexes were present from the outset.

In the spinal chick, reflex contractions of the gastrocnemius muscle in response to stimulating the central stump of the cut contralateral sciatic nerve were potentiated by $F_{2\alpha}$ in doses too low to affect muscle tension in the absence of stimulation. The potentiation began 5–10 sec after the injection, reached a maximum within 30–60 sec and usually lasted 3–30 min according to the dose injected. The crossed extensor reflex was elicited in 16 spinal chicks weighing from 42 to 141 g. In every case $F_{2\alpha}$, in doses ranging from 2 to 150 μg/kg potentiated the response. With the higher doses of $F_{2\alpha}$, a contraction of the gastrocnemius muscle was observed in addition to the potentiation of spinal reflexes (Fig. 6), and tachyphylaxis to both responses frequently occurred.

Prostaglandin E_1 also potentiated the crossed extensor reflex in spinal chicks in threshold doses (1.5 to 5 μg/kg) similar to those of $F_{2\alpha}$. Higher doses of E_1 caused transient potentiation of reflexes and convulsive movements of the chick, but this was followed by inhibition of the reflex possibly due to the lowering of blood pressure. Unless adrenaline was injected to restore the blood pressure, death often occurred after these higher doses of E_1 in the spinal chick.

When gastrocnemius muscle twitches were elicited by stimulation of the intact ipsilateral sciatic nerve, intravenous injections of $F_{2\alpha}$ and E_1 did not affect the size of the muscle

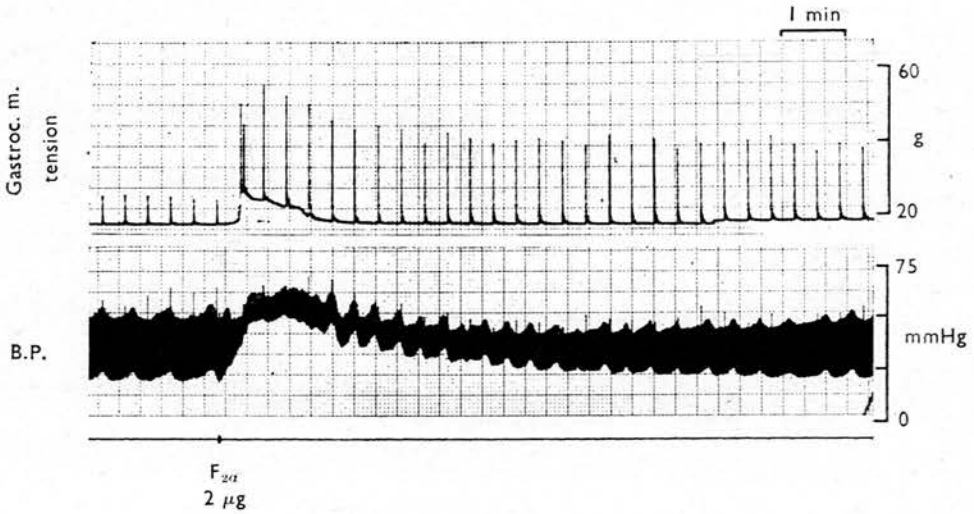

Fig. 6. Spinal chick (decapitated weight 44 g). Upper trace: gastrocnemius muscle tension, crossed extensor reflex twitches were elicited by electrical stimulation of the contralateral sciatic nerve. Lower trace: systemic arterial blood pressure. Response to prostaglandin $F_{2\alpha}$ (2 μg) injected intravenously.

twitches, either in doses which potentiated spinal reflexes or even in doses (of $F_{2\alpha}$) high enough to cause contraction of the ipsilateral gastrocnemius muscle. These results indicate that the potentiation by prostaglandin of the crossed extensor reflex is central not peripheral.

The action of $F_{2\alpha}$ and E_1 on the crossed extensor reflex of the spinal chick resembled strychnine. No valid comparison of potency of prostaglandins and strychnine could be made since the dose-response curves were not parallel. The threshold dose of strychnine was usually of the order of 10 μg/kg. The maximum potentiation which could be achieved with higher doses of strychnine was greater than that which could be produced with the prostaglandins, and the effect of strychnine lasted longer.

Anaesthetized chicks

The crossed extensor reflex could be elicited readily in chicks anaesthetized with chloralose but not in chicks lightly anaesthetized with urethane. Furthermore, small doses of urethane (0.2 g/kg) reduced or abolished the crossed extensor reflex in chloralosed chicks.

In 9 experiments on chloralosed chicks from 2 days to 9 weeks old weighing 35 g–1.25 kg, $F_{2\alpha}$, in doses ranging from 4–15 μg/kg, potentiated the crossed extensor reflex. In chloralosed chicks E_1 either had no effect or inhibited the crossed extensor reflex in doses (1–8 μg/kg) similar to those which potentiated the reflex in the spinal chick.

Neither E_1, nor $F_{2\alpha}$, when applied topically to the dorsal surface of the lumbar region of the spinal cord, had any effect on the crossed extensor reflexes. In one experiment E_1 (100 μg/ml.) had no effect whereas strychnine (100 μg/ml.) caused marked potentiation.

Topical application of E_1 (50–100 μg/ml.) to the exposed cerebral hemispheres had little or no effect on the reflex in the chloralosed chick except in one experiment in which the reflex was reduced by E_1 (100 μg/ml.). The possibility that this action may not have been a local one but was secondary to absorption into the systemic circulation cannot be entirely excluded. The effect was accompanied by a slight fall in arterial blood pressure.

Decerebrate and spinal cats

Prostaglandin E_1 potentiated the crossed extensor reflex in decerebrate and spinal cats in doses of 1–20 μg/kg and 10–32 μg/kg respectively. Figure 7 shows an experiment on a spinal cat in which E_1 caused a potentiation of the crossed extensor reflex which lasted 30 min. Sometimes potentiation of the reflex was preceded by a slight inhibition which coincided with the maximum lowering of the blood pressure (20–30 sec.). Prostaglandin E_1 had no detectable effect on the patellar reflex, in doses which potentiated the crossed extensor reflex (Fig. 7).

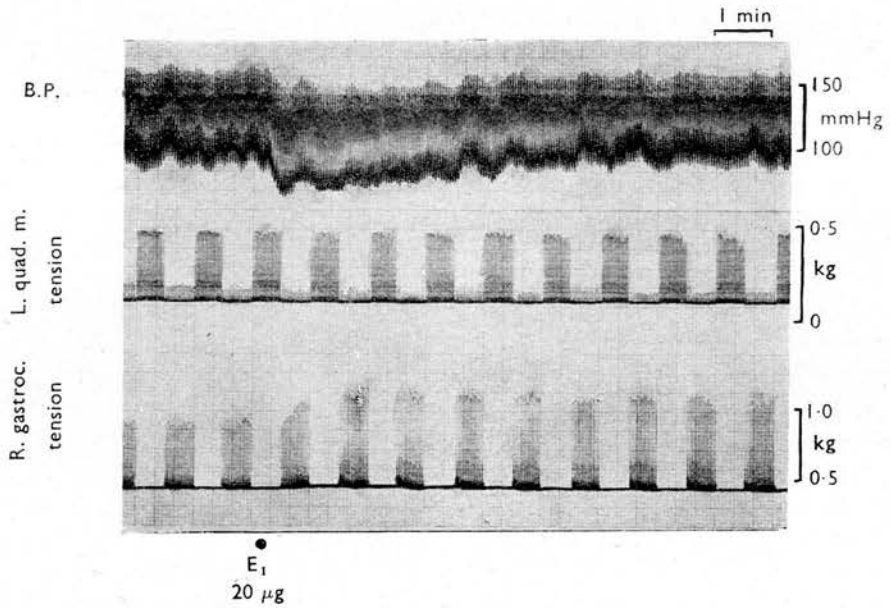

Fig. 7. Spinal cat (3.1 kg). Upper trace: carotid arterial blood pressure. Middle trace: left quadriceps muscle tension, patellar reflex twitches. Lower trace: right gastrocnemius muscle tension, twitches were elicited by stimulating the contralateral sciatic nerve. Effect of prostaglandin E_1 (20 μg), injected intravenously.

Since E_1 did not potentiate the twitches of the gastrocnemius muscle in response to ipsilateral nerve stimulation, it is concluded that the potentiation of spinal reflexes in the cat is due to an action on the spinal cord. This could be due to facilitation of the firing of motor neurones. Such an action could be either by direct facilitation, or by inhibiting pre- or post-synaptic inhibitory mechanisms. A few experiments were carried out on spinal cats in an attempt to find out which mechanism might be responsible.

In one experiment, the crossed extensor reflex, recorded from the left quadriceps muscle, was elicited by repetitive stimulation of the contralateral sciatic nerve for periods of 10 sec at 1 min intervals. A constant stretch applied to the left hamstring muscle during each reflex response resulted in marked inhibition. E_1 (30 μg/kg) potentiated the crossed extensor reflex but did not affect the degree of inhibition. The tension developed in the hamstring muscle during stretching was also increased by E_1.

In another experiment inhibition of the crossed extensor reflex by electrical stimulation of the ipsilateral peroneal nerve was unaffected by E_1.

These results suggest but do not prove that reflex potentiation of E_1 does not result from inhibition of inhibitory mechanisms.

Topical application of E_1 to the spinal cord of spinal cats, in concentrations of 20–100 μg/ml. did not affect spinal reflexes. In one experiment referred to above contraction of the gastrocnemius muscles and potentiation of crossed extensor reflexes followed the application of higher concentrations of E_1 (500 μg/ml.) to the dorsal surface of the cord in the L6–L7 region.

DISCUSSION

Evidence that prostaglandin $F_{2\alpha}$ acts on the spinal cord of the chick

Our experiments confirm that extension of the legs observed in the chick after an intravenous injection of $F_{2\alpha}$ (Horton & Main, 1965a) is due to contraction of the extensor muscles such as the gastrocnemius muscle. By recording the increase in tension of this muscle we have been able to locate the site of this action of $F_{2\alpha}$. Since the response to $F_{2\alpha}$ is abolished by acute denervation of the muscle, it must be centrally mediated. Since it is readily elicited in the decapitated chick the brain cannot be necessary for the mediation of this effect, nor can it be a reflex response mediated by pathways which depend upon the integrity of the brain stem. Reflex contraction of the gastrocnemius muscle due to $F_{2\alpha}$ acting upon spinal afferent nerves is unlikely because under light urethane anaesthesia electrical stimulation of the cut central stump of the sciatic nerve failed to elicit reflex contractions of the contralateral gastrocnemius muscle whereas $F_{2\alpha}$ injected intravenously did produce a contraction of the muscle. It is, therefore, concluded that $F_{2\alpha}$ increases gastrocnemius muscle tension by an action on neurones within the chick spinal cord. Confirmation of this conclusion by topical application of $F_{2\alpha}$ to the exposed spinal cord has not yet been obtained, possibly because the concentrations applied were too small. Potentiation by $F_{2\alpha}$ of the crossed extensor reflex in the chloralosed and spinal chick is compatible with an excitatory or facilitatory action on spinal cord neurones or of inhibition of inhibitory pathways.

Although $F_{2\alpha}$ acts upon the spinal cord of the chick, the possibility that it also acts on the brain cannot be excluded.

Evidence that prostaglandin E_1 acts on the spinal cord of the cat

Increases in gastrocnemius muscle tension in the spinal cat in response to intravenous injections of E_1 could not be imitated by injecting the prostaglandin close-arterially to the muscle. Furthermore, acute denervation of the muscle abolished the response to intravenous E_1 showing that the action of the prostaglandin cannot be upon the skeletal

muscle or the neuromuscular junction, but must be centrally mediated. Reflex contractions of the gastrocnemius muscle mediated *via* the brain could not of course account for the positive responses obtained in the spinal animal, but reflex contractions due to E_1 acting upon spinal afferent nerves are not excluded. A direct action of E_1 on the cord is more likely, because, in one experiment, contractions of the gastrocnemius muscle were, elicited by topical application of E_1 to the spinal cord. We conclude that prostaglandin E_1 in the cat, like $F_{2\alpha}$ in the chick, can increase gastrocnemius muscle tension by an action on the spinal cord.

Since the effect of E_1 was observed equally on both gastrocnemius muscles after unilateral section of the dorsal roots, it could not be due to facilitation of γ-motoneurone firing alone. We conclude that E_1 facilitates the firing of α-motoneurones either directly or indirectly. The facilitation is probably due to stimulation of excitatory pathways since inhibition of inhibitory pathways was not observed after prostaglandin administration. This problem must be investigated further using electrophysiological techniques.

Responses to E_1 observed in the decerebrate cat could be accounted for by an action on the spinal cord. The question of whether E_1 also excites hind-brain descending facilitatory pathways remains unsettled, though Avanzino, Bradley & Wolstencroft (1966) have shown that medullary reticulospinal neurones respond to prostaglandins applied iontophoretically and that the predominant response is excitation. Increases in gastrocnemius muscle tension in response to E_1 were obtained more readily in the decerebrate than the spinal animal. Whether this could be explained on the basis of additional contributions from the stimulation of descending facilitatory pathways which have their origin in the hind-brain is a matter for further experiment.

The evidence concerning the site of action of $F_{2\alpha}$ in the cat is incomplete. Certainly $F_{2\alpha}$ had a similar action to E_1 on the decerebrate cat and it may be supposed that it also exerts its effect partly, at least, on the spinal cord. Avanzino *et al.* (1966) showed that $F_{2\alpha}$ fires off reticulospinal neurones in the medulla and Duda & McPherson (personal communication) have observed that $F_{2\alpha}$ has actions on the spinal reflexes in the chloralosed cat, when injected into the abdominal aorta.

Our experiments on reflexes in both the spinal chick and the spinal cat support the conclusion that prostaglandins have facilitatory action on the firing of α-motoneurones, though the inhibitory action of E_1 on the crossed extensor reflex in the chloralosed chick is suggestive that prostaglandins may also be inhibitory or excite inhibitory pathways in certain circumstances. These observations have received some support from the recent experiments of Duda & McPherson (personal communication) on the chloralosed cat using electrophysiological recording techniques.

Possible physiological implications of these results are discussed more fully in the following paper.

SUMMARY

1. Prostaglandin $F_{2\alpha}$ on intravenous injection in chicks increases gastrocnemius muscle tension. Experiments were made to locate its site of action.

2. The effect was observed in the decapitated chick but was abolished by denervation of the muscle. It could be elicited in the urethanized chick in which reflex contractions

were blocked. It is concluded that the site of this action of $F_{2\alpha}$ in the chick is upon the spinal cord.

3. In spinal cats prostaglandin E_1 injected intravenously increased gastrocnemius muscle tension also by an action on the spinal cord. The effect was abolished by denervation of the muscle but not by dorsal root section. Close-arterial injection of E_1 to the gastrocnemius muscle did not elicit a contraction but did inhibit muscle twitches and acetylcholine-induced contractures. In one experiment prostaglandin E_1 applied topically to the spinal cord induced a contraction of the gastrocnemius muscle.

4. Decerebrate rigidity in the cat was potentiated by prostaglandins $F_{2\alpha}$ and E_1.

5. Crossed extensor reflexes were potentiated by prostaglandin $F_{2\alpha}$ in the spinal chick and by E_1 in the spinal cat. The patellar reflex in the spinal cat was little affected by prostaglandin E_1. In the chloralosed chick $F_{2\alpha}$ potentiated but E_1 inhibited the crossed extensor reflex.

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ADDENDUM

Effects of prostaglandins E_1 and $F_{2\alpha}$ on systemic arterial blood pressure in chicks

During experiments on chicks described in this paper, arterial blood pressure was usually monitored. In all preparations E_1 had a depressor effect. In spinal preparations $F_{2\alpha}$ had a pressor effect but in anaesthetized chicks the response to $F_{2\alpha}$ was more variable; sometimes pressor, sometimes depressor, and sometimes biphasic (pressor followed by depressor). The results are summarized in Table 1.

The pressor action of $F_{2\alpha}$ in spinal chicks was not abolished by phenoxybenzamine (10 mg/kg) which abolished responses to noradrenaline nor by hexamethonium (10 mg/kg) or pronethalol (10 mg/kg).

TABLE 1

ACTIONS OF PROSTAGLANDINS ON THE ARTERIAL BLOOD PRESSURE OF ANAESTHETIZED AND SPINAL CHICKS

		Number of experiments			
				Arterial blood pressure response	
Preparation	Prostaglandin	Total	Pressor	Pressor–depressor	Depressor
Spinal	$F_{2\alpha}$	14	14	0	0
	E_1	9	0	0	9
Chloralose anaesthesia	$F_{2\alpha}$	14	8	5	1
	E_1	8	0	0	8
Urethane anaesthesia	$F_{2\alpha}$	10	3	4	3
	E_1	15	0	0	15
All anaesthetized chicks	$F_{2\alpha}$	24	11	9	4
	E_1	23	0	0	23

In 3 experiments on the isolated chicken heart perfused by the method of Langendorff, $F_{2\alpha}$ had no action on the force or rate of contraction in doses higher than those which had a pressor action on intravenous injection in the spinal chick. In 2 experiments E_1 (25 and 50 μg) injected into the perfusion fluid increased the force of contraction but smaller doses had no effect.

The results suggest that the pressor action of $F_{2\alpha}$ is not mediated by the sympathetic nervous system and does not result from a direct action on the heart.

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THE EFFECTS OF PROSTAGLANDINS E_1, $F_{1\alpha}$ AND $F_{2\alpha}$ ON MONOSYNAPTIC REFLEXES

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SUMMARY

1. Experiments, using electrophysiological recording techniques, were carried out to confirm the report that prostaglandins affect spinal reflexes in cat.

2. Ventral root potentials evoked by stimulation of the ipsilateral dorsal root were recorded. Those with a latent period corresponding to a monosynaptic pathway were used primarily in this investigation.

3. Prostaglandin E_1 (3·5–17·8 μg/kg) injected into the aorta reduced the amplitude of monosynaptic responses in six out of ten cats. The effect began 15 min after injection and lasted over 3 hr. In one cat E_1 caused potentiation of the reflex and in three cats there was no effect.

4. Prostaglandin $F_{1\alpha}$ (2·4–3·5 μg/kg) inhibited the monosynaptic response in four cats but in one of these a subsequent large dose (19·6μg/g/kg) greatly potentiated the reflex.

5. Prostaglandin $F_{2\alpha}$ (1·4–17·8 μg/kg) injected into the aorta was followed by significant but variable changes in monosynaptic response. In one experiment an intravenous injection (30·3 μg/kg) was followed by a long-lasting potentiation of the reflex response.

6. It is concluded that prostaglandins, two of which have previously been identified in cat brain extracts, have pronounced and long-lasting effects on monosynaptic spinal reflexes.

INTRODUCTION

Prostaglandins have been shown to have pharmacological actions on the brain and spinal cord (Horton, 1964; Horton & Main, 1965; Avanzino,

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Bradley & Wolstencroft, 1966; Horton & Main, 1967*a*; Holmes & Horton 1968*a*). In the present investigation, the actions of three prostaglandins have been studied on spinal reflexes by intra-arterial injection close to the spinal cord.

METHODS

Twenty-two cats weighing between 2·8 and 4·0 kg were used. Anaesthesia was induced with a mixture of halothane ('Fluothane' I.C.I., ¾ of Goldman's vaporizer scale) in nitrous oxide (3·0 l./min) and oxygen (0·3 l./min), and maintained with chloralose (70 mg/kg) injected intravenously. To avoid the need for additional anaesthetic during the hours of observation, an additional dose (25 mg/kg) of chloralose was given 4–6 hr after the first dose when the dissection was complete but before observations were made.

The trachea was cannulated in all experiments. Blood pressure was recorded from a carotid using a polyethylene catheter connected to a pressure transducer (Statham model no. P23Db) and then through a carrier amplifier to a potentiometric pen recorder.

The spinal cord was exposed dorsally in the lumbar region and the dura opened. Reflex responses recorded from a ventral root, were evoked by stimulation of the ipsilateral dorsal root of the same lumbar segment. Both ventral and dorsal roots were cut peripherally.

The recordings were obtained from platinum electrodes under liquid paraffin maintained at a temperature of 36–38° C. The dorsal roots were stimulated and the responses recorded by methods previously described (Evans & McPerson, 1958; 1959).

To attain higher concentrations of the prostaglandins in the spinal cord, the solution was injected intra-arterially. A fine nylon catheter was inserted into the aorta via a renal artery with its tip just below the origins of the renal arteries; it was tied at the insertion point in the renal artery. During the administration of prostaglandins the most distal part of the aorta was occluded with an elastic loop placed around the aorta and in order to prevent injected prostaglandins from passing into the blood vessels of the hind limbs. Other branches of the aorta were not occluded. The aortic occlusion lasted throughout the administration of the prostaglandins and ended approximately 20 sec after the injection was completed.

Throughout the operative procedures a mixture of 5 % (w/v) dextrose and 0·9 % (w/v) saline (Evans Medical Ltd.) was infused intravenously at a rate of about 0·5–1 ml./min. The urinary bladder was kept empty throughout all experiments by means of an indwelling catheter.

Prostaglandin E_1 is 11α,15-dihydroxy-9-oxo-prost-13-enoic acid. The material used, except in three experiments, was 99 % pure specially prepared and kindly supplied by Dr D. A. van Dorp of the Unilever Research Laboratories, Vleardingen, The Netherlands. Prostaglandin $F_{1\alpha}$, 9α,11α,15-trihydroxy-prost-13-enoic acid was also supplied by Dr D. A. van Dorp. Prostaglandin $F_{2\alpha}$,9α,11α,15-trihydroxy-prost-5,13-dienoic acid was kindly supplied by Dr John E. Pike of the Upjohn Company, Kalamazoo, U.S.A.

RESULTS

Occlusion of the aorta lasting 1–2 min raised systemic arterial blood pressure and in about half the experiments increased the amplitude of the monosynaptic reflex responses. After the end of occlusion, reflex responses returned to their pre-occlusion levels within 4–6 min. Such an effect is illustrated in Fig. 1. The potentiation was significant ($P < 0·01$) for the first 2 min after the start of occlusion. When prostaglandins were injected during the occlusion similar short-lived potentiation was observed. This is

illustrated in Fig. 2. In this experiment the initial potentiation can be attributed to the occlusion because it was already maximal when the prostaglandin was injected.

Prostaglandins lowered arterial blood pressure but even in the largest doses used, the depressor effect lasted less than 20 min.

Effect of prostaglandin E_1 on monosynaptic reflexes. Prostaglandin E_1 (3·5–17·8 μg/kg) significantly inhibited monosynaptic reflexes in six out

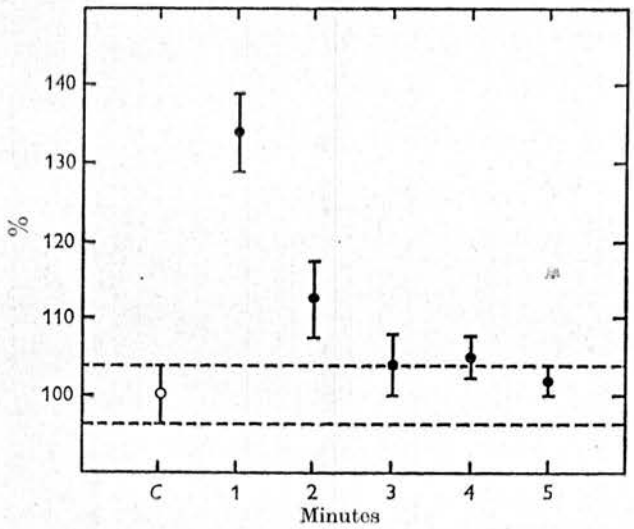

Fig. 1. The effect of aortic occlusion on the amplitude of a monosynaptic reflex. C = control (pre-occlusion) value of the response. During the first minute the aorta was occluded for 30 sec. Ordinate: % change in amplitude. Abscissa: time in min.

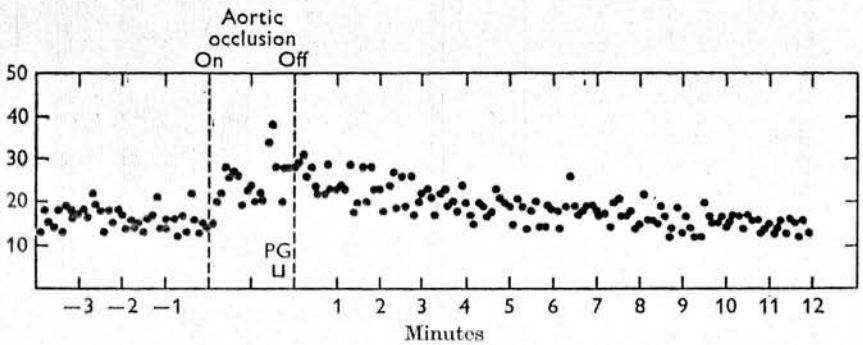

Fig. 2. Monosynaptic reflex responses before, during and immediately after aortic occlusion and injection of prostaglandin E_1 into the aorta (at PG). The occlusion was released 20 sec after the injection. Ordinate: amplitude of the response in arbitrary units. Abscissa: time in min.

of ten cats (Fig. 3; Table 1). The effect began between 15 and 30 min after injection into the aorta and lasted at least 3 hr. In three experiments there was no significant change in reflex responses with doses of 3·3, 3·5 and 15·6 μg/kg during the period of observation following injection ($1\frac{1}{2}$, 2 and 3 hr respectively). In one experiment the reflex response was potentiated following the injection of prostaglandin E_1 (1·5 μg/kg).

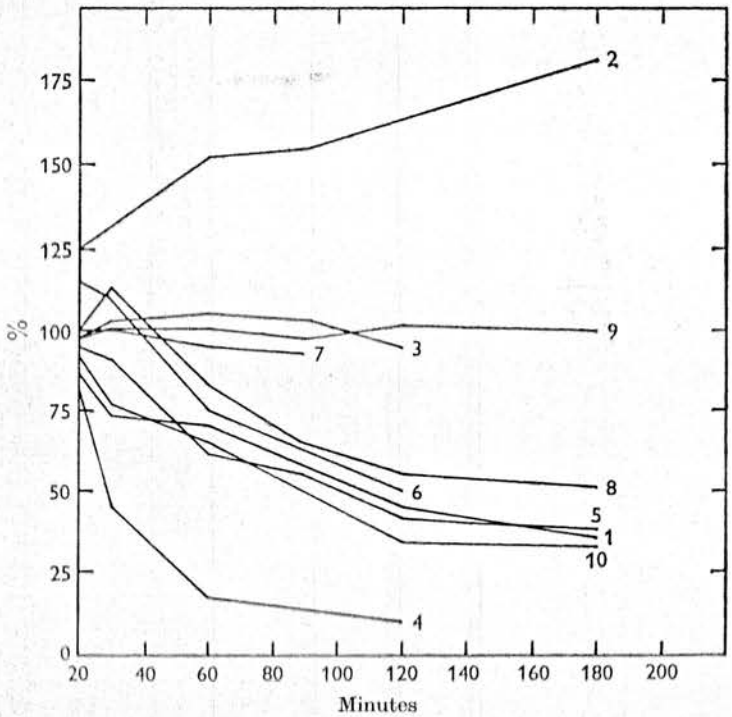

Fig. 3. Results of ten experiments (see Table 1) showing the effect of prostaglandin E_1 injected into the aorta on monosynaptic reflex responses. Changes during the first 20 min which might be attributable to aorta occlusion or to vascular effects have not been plotted. Ordinate: % change in amplitude of the response. Abscissa: time in min after the injection.

The effect of prostaglandin E_1 (3·5 μg/kg) in reducing the size of the monosynaptic reflex responses is shown in more detail in Fig. 4. The reduction in amplitude started 15 min after injection of prostaglandin E_1 and at that time the arterial blood pressure had already returned to its previous level. The reflex responses even 4 hr after the injection were less than 50]% of the pre-injection control values. In this experiment there was no significant change in the size of a polysynaptic reflex response over the 4 hr period (Fig. 4).

Effect of prostaglandin $F_{1\alpha}$ on spinal reflexes In four cats, prostaglandin $F_{1\alpha}$ (2·4–3·5 μg/kg) caused a reduction in monosynaptic responses (Fig. 5; Table 2). These effects lasted up to 3 hr. In one experiment (No. 4)

TABLE 1. Weight of cats and dosages of prostaglandin E_1, used in experiments summarizedin Fig. 3.

No.	Sex	Weight (kg)	Cumulative dose of prostaglandin E_1: Total amount (μg)	Cumulative dose of prostaglandin E_1: μg/kg
1*	Male	3·0	1·2	0·4
			3·7	1·2
			7·0	2·3
2*	Female	4·0	6·0	1·5
3	Female	2·8	10·0	3·5
4	Female	2·8	10·0	3·5
5	Female	2·8	10·0	3·5
6	Female	2·8	10·0	3·5
7*	Female	3·0	10·0	3·3
8	Female	2·8	10·0	3·5
			20·0	7·1
9	Female	3·2	50·0	15·6
10	Female	2·8	50·0	17·8

* 95 % pure prostaglandin E_1 was used in these experiments. In the remaining experiments 99 % pure prostaglandin E_1 was used.

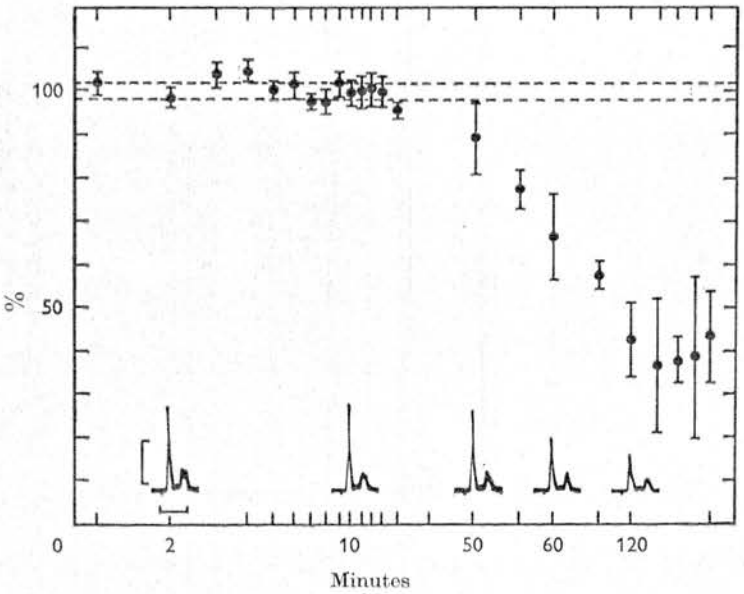

Fig. 4. The effect of prostaglandin E_1 (10 μg) injected into the aorta on monosynaptic reflex responses. Samples of the action potentials at 2, 10, 50, 60 and 120 min after the injection are illustrated. Ordinate: % change in amplitude of monosynaptic reflex response. Abscissa: time in min (log scale) after the injection.

prostaglandin $F_{1\alpha}$ (3·5 μg/kg) caused a small decrease in response over a 3 hr period. A subsequent injection of $F_{1\alpha}$ (19·6 μg/kg) in this cat was followed by a very significant potentiation of the response reaching a peak after 90 min. This potentiation lasted over 3 hr (Fig. 6). In other experiment (No. 1) a diminution in monosynaptic responses was accompanied by a large increase in polysynaptic response (Fig. 5).

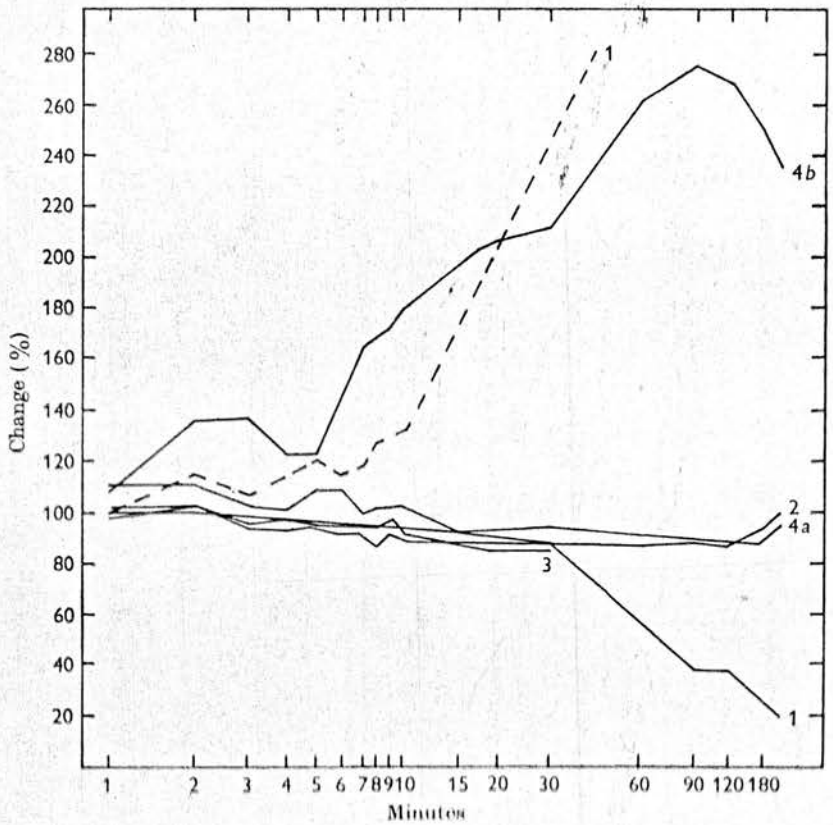

Fig. 5. Results of four experiments (Table 2) showing the effects of prostaglandin $F_{1\alpha}$ on monosynaptic reflex responses and one polysynaptic reflex response (Expt. no. 1, interrupted line). In Expt. no. 4, the effect of 10 μg prostaglandin $F_{1\alpha}$ (4*a*) and a subsequent dose of 55 μg (4*b*) over 3 hr later is illustrated. Ordinate: % change in amplitude of reflex responses. Abscissa: time in min after the injection.

Effect of prostaglandin $F_{2\alpha}$ on spinal reflexes. In seven out of eight cats prostaglandin $F_{2\alpha}$ (1·4–30·3 μg/kg) caused a significant change in the monosynaptic reflex response within 30 min of the injection. Five of these experiments are illustrated in Fig. 7; Table 3. In three of these there was potentiation and in two inhibition 30 min after injection. Changes in

response were still significant ($P < 0{\cdot}001$) $2\frac{1}{2}$ hr later, though in four of the five experiments the direction of the change was opposite to that at 30 min.

The results of the remaining three experiments in which larger doses of prostaglandin $F_{2\alpha}$ were used, are shown in Fig. 8. In one experiment (cat no. 7) $F_{2\alpha}$ (17·8 μg/kg) caused a great diminution in monosynaptic responses. In another cat (No. 6) the same dose had no effect over a 3 hr

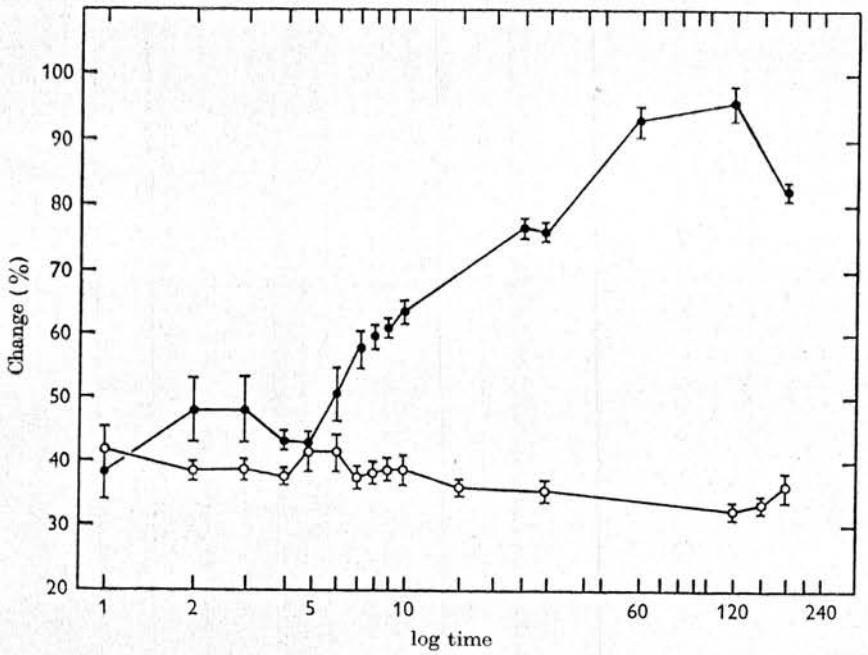

Fig. 6. The results of experiment four from Fig. 5 shown in greater detail. The open circles represent reflex responses measured over a 3 hr period following an intra-aortic injection of 10 μg prostaglandin $F_{1\alpha}$. The filled circles represent reflex responses measured later in the same cat after an injection of 55 μg prostaglandin $F_{1\alpha}$. Ordinate: % change in amplitude of reflex response. Abscissa: time in min after the injections (log scale).

TABLE 2. Weights of cats and dosages of prostaglandin $F_{1\alpha}$ used in experiments summarized in fig. 5

No.	Sex	Weight (kg)	Cumulative dose of prostaglandin $F_{1\alpha}$: Total amount (μg)	μg/kg
1	Male	3·3	5·0	1·5
			8·0	2·4
2	Male	2·8	10·0	3·5
3	Female	2·8	10·0	3·5
4	Female	2·8	10·0	3·5
			65·0	23·2

period. In a third cat (No. 8), $F_{2\alpha}$ (30·3 μg/kg) was injected intravenously and in this experiment there was a potentiation of the monosynaptic response lasting over 3 hr.

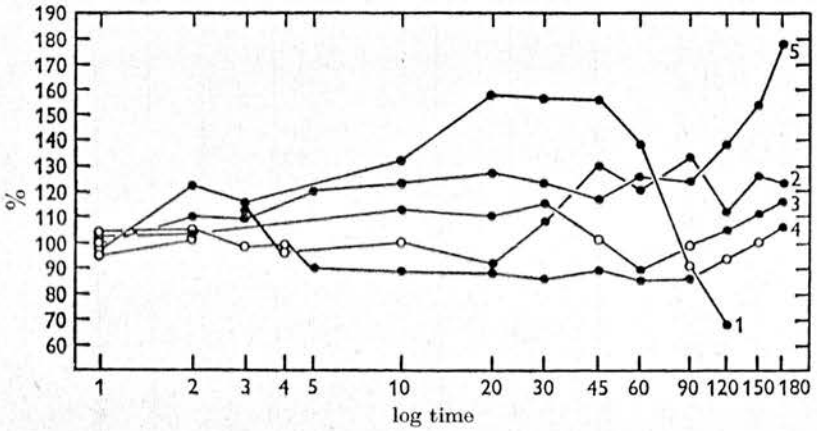

Fig. 7. Results of five experiments (Table 3) showing the effect of prostaglandin $F_{2\alpha}$ (1·4–3·5 μg/kg) on monosynaptic reflex responses. The filled circles represent responses which were significantly different ($P < 0{\cdot}01$) from those of the control periods. Ordinate: % change in amplitude of reflex response. Abscissa: time in min after the injection (log scale).

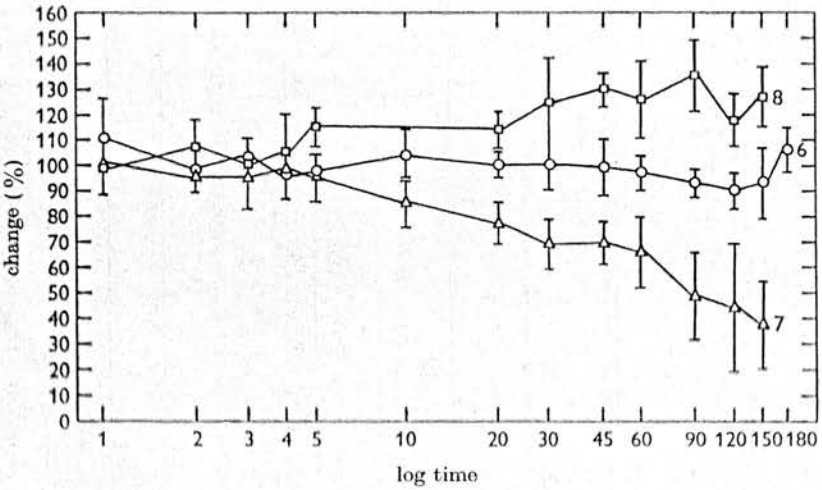

Fig. 8. Results of three experiments (Table 3) showing the effects of prostaglandin $F_{2\alpha}$ (17·8–30·3 μg/kg) on monosynaptic reflex responses. In Exp. 8 the drug was injected intravenously. Ordinate: % change in monosynaptic reflex responses. Abscissa: time in min after injection (log scale).

TABLE 3. Weights of cats and dosages of prostaglandin $F_{2\alpha}$ used in experiments summarized in Figs. 7 and 8

			Cumulative dose of prostaglandin $F_{2\alpha}$	
No.	Sex	Weight (kg)	Total amount (μg)	μg/kg
1	♀	2·7	4·0	1·4
2	♀	3·4	10·0	2·9
3	♀	3·3	10·0	3·0
4	♀	2·9	10·0	3·4
5	♀	2·8	10·0	3·5
6	♀	2·8	50·0	17·8
7	♀	2·8	50·0	17·8
8	♀	2·8	85·0	30·3

DISCUSSION

The results of this investigation confirm previous reports that prostaglandins have central nervous actions (Horton, 1964; Horton & Main, 1965; 1967*a*; Avanzino *et al.* 1966). There are, however, apparent differences in detail which require discussion.

Effect of cardiovascular changes on spinal reflexes. During the intra-arterial injection of prostaglandins, the lower abdominal aorta was occluded in an attempt to direct more of the prostaglandin towards the spinal cord. This intra-arterial technique was based upon that described by Bülbring, Burn & Skoglung (1948) and by Homstedt & Skoglund (1953). The occlusion itself produced facilitation of the monosynaptic reflex response, lasting not more than 6 min. The actions of prostaglandins were slower in onset and far longer-lasting; they could thus be distinguished easily from the effects of occlusion. Effects observed within 5 min of prostaglandin injection and aortic occlusion should be disregarded.

Prostaglandins, particularly E_1, are vasodilators, and usually lowered arterial blood pressure following injection. Even with the highest doses injected, the blood pressure had returned to its pre-injection level within 20 min in all experiments and usually the depressor effect was of far shorter duration. It is clear that the changes in spinal reflexes have an entirely different time of onset and duration than the depressor response.

Actions of prostaglandin E_1. In six out of ten experiments prostaglandin E_1 caused a lasting and pronounced diminution in monosynaptic reflex responses. In seven experiments the prostaglandin E_1 was 99% pure and in five of these, there was inhibition of the reflex. The only experiment in which E_1 facilitated the reflex was with a rather less pure (95%) form of E_1 but the number of experiments is too few to permit the conclusion that this result was due to an impurity in the prostaglandin.

These results in the chloralosed cat contrast with those of Horton &

Main (1967*a*) in the spinal cat, in which the patellar reflex was unaffected by E_1 injected intravenously. It may be that the negative results of Horton & Main (1967*a*) correspond to the three negative results obtained in this investigation. There are, however, other possible explanations. In the present experiments the brain of the cat was intact and so it is conceivable, especially in view of the slow onset of the effects, and even though the prostaglandin was injected towards the spinal cord, that E_1 was acting not on spinal neurones but on supraspinal centres with descending pathways which impinge on the neurones of the spinal monosynaptic reflex arc.

A second possibility is that E_1 blocks, at the spinal level, descending facilitatory pathways which impinge upon the motoneurone. An inhibitory action on the reflex would then only be revealed when these descending pathways and their (supraspinal) centres were intact. Such an action might be expected to inhibit both monosynaptic and polysynaptic pathways. Although few measurements of polysynaptic reflexes were made, E_1 certainly did not affect the response in one experiment in which the monosynaptic reflex was inhibited.

There is an interesting parallel between the contrasting results in chloralosed and spinal cats and the results in chloralosed and spinal chicks. The crossed extensor reflex in the chloralosed chick is inhibited by E_1 whereas in the spinal (decapitated) chick the reflex is potentiated by E_1 (Horton & Main, 1967*a*). A similar explanation of the differences may apply in the two species.

The three negative results may have been due to insufficient dosage but the one instance of potentiation is less readily explained. Avanzino *et al.* (1966) reported qualitative differences in the response of different brain stem neurones, but qualitative differences were never found in spinal cats by Horton & Main (1967*a*).

Actions of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$. The actions of $F_{1\alpha}$ in general resembled those of E_1 on monosynaptic reflexes, but in one experiment facilitation of the reflex was observed. This experiment is of some interest because a small dose of $F_{1\alpha}$ caused a slight but significant reduction in reflex response lasting $2\frac{1}{2}$ hr. At that point an additional larger dose was administered which caused potentiation lasting a further 3 hr.

The actions of $F_{2\alpha}$ are more difficult to understand. Either potentiation or inhibition of the reflex occurred in the first 30 min following injection; these changes were highly significant when compared with the small variation in control responses. The variation in the responses from significant potentiation to significant inhibition in the remaining $2\frac{1}{2}$ hr seems to be a characteristic response to $F_{2\alpha}$. Fluctuations of this magnitude were never observed in control experiments nor were they observed in

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23

Reprint from Nobel Symposium 2

Prostaglandins

Edited by

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Central nervous actions of the prostaglandins and their identification in the brain and spinal cord

By E. W. Horton and I. H. M. Main

Department of Physiology, Medical College of St. Bartholomew's Hospital, London, E.C.1.

Observations on unanaesthetized cats and chicks

In unanaesthetized cats prostaglandins E_1, E_2 and E_3 injected into the cerebral ventricles in doses of 10 to 60 μg produce stupor usually accompanied by catatonia. These effects are not observed after intravenous injection of prostaglandin E_1 or prostaglandin $F_{2\alpha}$, nor do they occur following intraventricular injection of $F_{2\alpha}$ (Table 1). During the stupor which lasted sometimes for 24 hr or more, the cats showed diminished spontaneous activity and a lack of interest in their surroundings, but there was no impairment of movement and no analgesia or anaesthesia. Catatonia was diagnosed when the cat could be placed in abnormal postures (Fig. 1), or when a limb could be displayed by the observer into an abnormal position which was retained for several seconds or even minutes, before the limb was slowly returned to a more natural position.

On intravenous injection in 2–8 day-old chicks E_1, E_2 and E_3 (10–200 μg/kg) produce sedation with loss of the righting reflex in higher doses, whereas $F_{1\alpha}$ and $F_{2\alpha}$ (25–450 μg/kg) produce extension of the neck and legs without any signs of sedation (Fig. 2) (4, 5, 7).

Localisation of the site of action of $F_{2\alpha}$ in the chick and E_1 in the spinal cat

The extension of the legs observed after $F_{2\alpha}$ could be measured in the anaesthetized chick. In chicks lightly anaesthetized with chloralose or urethane an

Table 1. *Effects of prostaglandins on injection into cerebral ventricles.*

Cat No.	E_1 Dose μg	E_1 Response: Stupor	E_1 Response: Catatonia	$F_{2\alpha}$ Dose μg	$F_{2\alpha}$ Response: Stupor	$F_{2\alpha}$ Response: Catatonia
1	20	+ +	−	20	—	—
2	20	+ +	+	60	—	—
3	20	+ +	+ +	15	—	—
4	20	+ +	+	100	—	—

Fig. 1. Cat 2.6 kg photographed in a catatonic posture 75 min. after an injection into the cerebral ventricles of prostaglandin E_3, 30 μg. This posture was maintained for 8 min. before the cat climbed slowly down.

intravenous injection of $F_{2\alpha}$ (2 μg/kg) was followed by an increase in tension of the gastrocnemius muscle (Fig. 3). The effect could not be obtained in deeply anaesthetized chicks suggesting that it was mediated via the central nervous system. Similar responses were obtained with $F_{2\alpha}$ in the decapitated chick

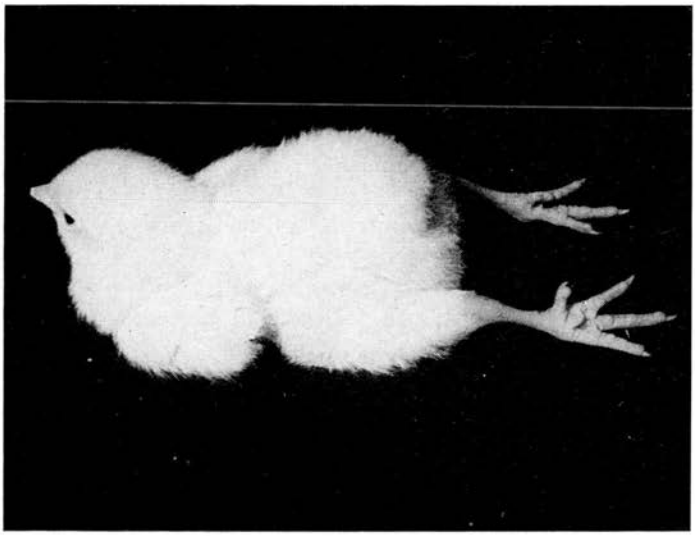

Fig. 2. Chick 39 g photographed 1 min after an intravenous injection of prostaglandin $F_{2\alpha}$, 4 μg.

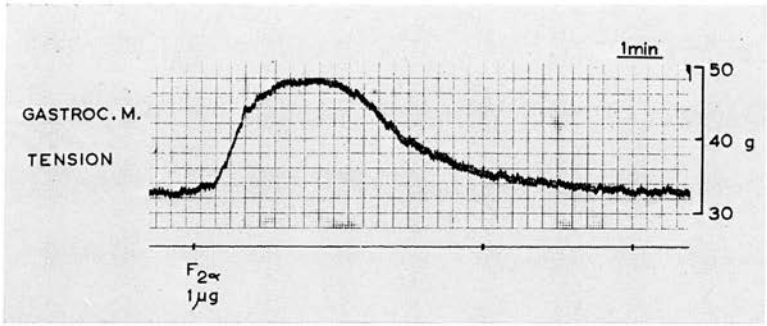

Fig. 3. Chicken 550 g anaesthetized with urethane (1.7 g/kg). Effect of 1 μg of prostaglandin $F_{2\alpha}$ injected intravenously on gastrocnemius muscle tension recorded isometrically. (Horton and Main, British Journal of Pharmacology. In the press.)

spinalized in the mid-cervical region, indicating that the brain is not necessary for the mediation of this effect. The response was abolished by acute denervation of the gastrocnemius muscle showing that $F_{2\alpha}$ does not produce its effect by an action on the neuromuscular junction or on the skeletal muscle itself (Fig. 4).

Similar effects were observed with both $F_{2\alpha}$ (5–30 μg/kg) and E_1 (5–50 μg/kg) in the decerebrate and spinal cat (Fig. 5). The contraction of the gastrocnemius muscle in response to intravenous prostaglandins was abolished by cutting the sciatic nerve but not by cutting the dorsal roots. When E_1 was injected close-arterially to the muscle either in the innervated, acutely denervated or

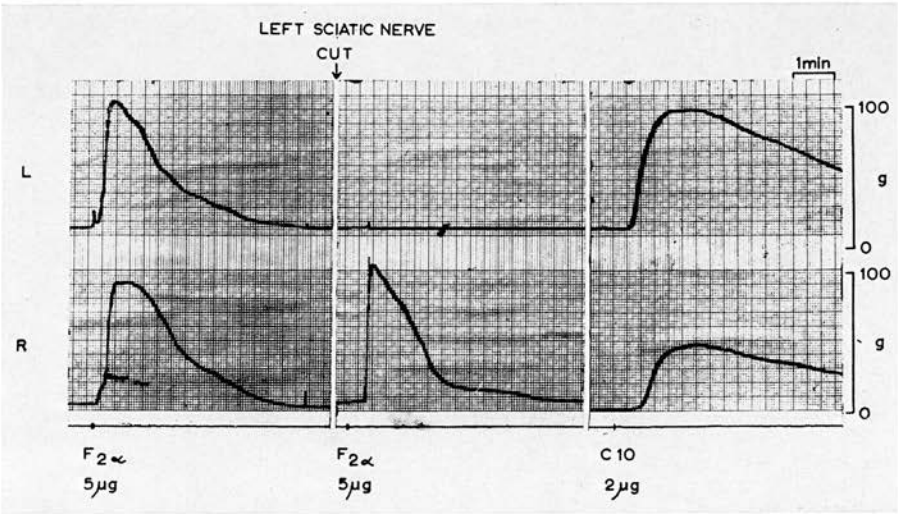

Fig. 4. Decapitated chick (32 g) spinalized in the mid cervical region. Gastrocnemius muscle tension recorded isometrically (upper tracing left leg, lower tracing right leg). Responses to intravenous injections of prostaglandin $F_{2\alpha}$ (5 μg) and decamethonium iodide (2 μg). Between the first and second panel the left sciatic nerve was cut. There was an interval of 60 min. before each injection. (Horton and Main, British Journal of Pharmacology. In the press.)

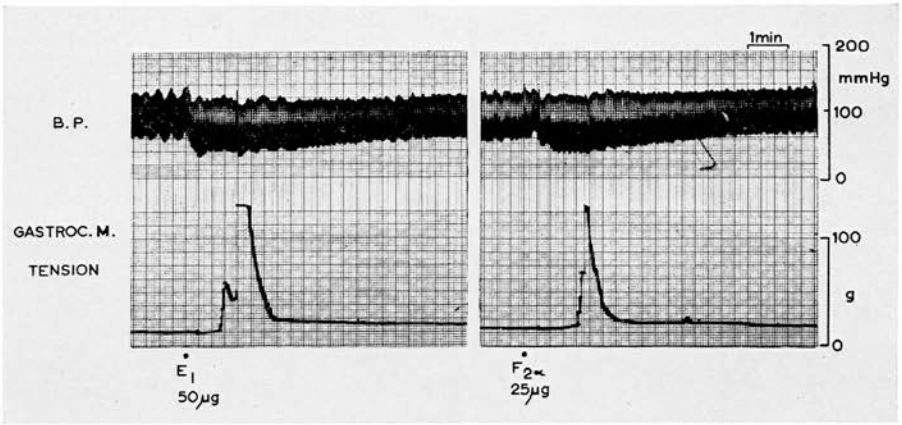

Fig. 5. Decerebrate cat (3.4 kg). Upper trace carotid arterial blood pressure, lower trace gastrocnemius muscle tension. Responses to intravenous injections of prostaglandin E_1 (50 μg) and prostaglandin $F_{2\alpha}$ (25 μg). (Horton and Main, British Journal of Pharmacology. In the press.)

chronically denervated preparation, contraction of the gastrocnemius did not occur. From these experiments it is concluded that $F_{2\alpha}$ in the chick and both E_1 and $F_{2\alpha}$ in the cat produce contraction of the gastrocnemius by an action on the spinal cord, and since contraction can be elicited in the de-afferentated preparation it is possibly due to facilitation of α-motoneurones.

Actions of prostaglandins on spinal reflexes

In the spinal chick and spinal cat crossed extensor reflexes are potentiated by intravenous injections of $F_{2\alpha}$ and E_1 (Figs. 6 and 7). The effect was observed

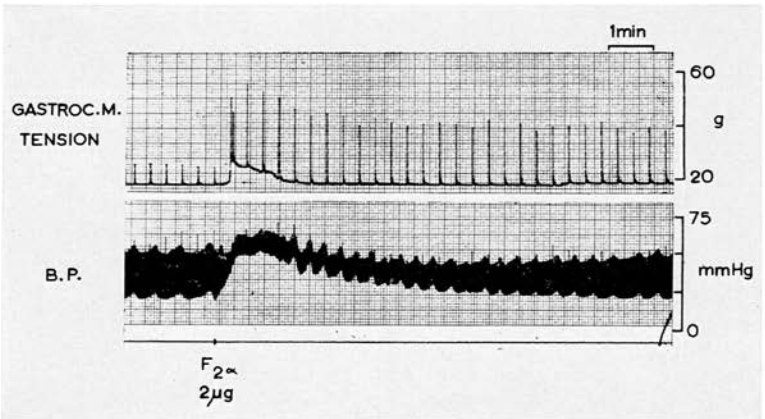

Fig. 6. Decapitated chick (44 g) with a mid cervical spinal transection. Upper trace gastrocnemius muscle tension, muscle twitches were elicited by electrical stimulation of the contralateral sciatic nerve; lower trace systemic arterial blood pressure. Responses to prostaglandin $F_{2\alpha}$ (2 μg) injected intravenously. (Horton and Main, British Journal of Pharmacology. In the press.)

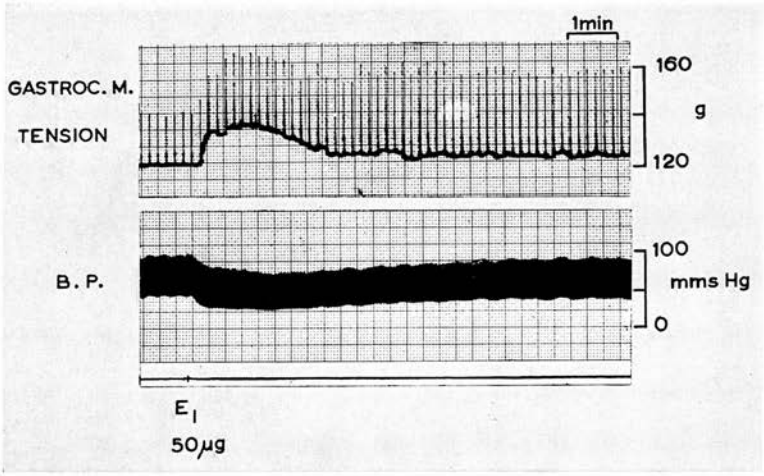

Fig. 7. Spinal cat (2.4 kg). Upper trace gastrocnemius muscle tension, muscle twitches were elicited by electrical stimulation of the central stump of the contralateral sciatic nerve. Lower trace carotid arterial blood pressure. (Horton and Main, British Journal of Pharmacology. In the press.)

following smaller doses than those necessary to produce contraction of the gastrocnemius and was more prolonged. The monosynaptic patellar reflex in the cat was sometimes potentiated by E_1.

Abolition of tremor by prostaglandins

In the lightly anaesthetized chick (urethane) or cat (pentobarbitone, with intact brain) E_1 (3 μg/kg) injected intravenously reduced gastrocnemius muscle tension and abolished tremor or shivering which is commonly observed during the recovery from these anaesthetics in these species (Fig. 8). This is in marked contrast to the increase in gastrocnemius tension observed in the spinal and decerebrate cat. In the chick the abolition of tremor also occurred when E_1 was applied to the cerebral hemispheres suggesting an action on higher centres. However, pentobarbitone tremor in the cat could not be abolished by injection of E_1 (30 μg) into the cerebral ventricles but only by intravenous injection.

Actions of prostaglandins on systemic arterial blood pressure

In the spinal chick $F_{2\alpha}$ (2 μg/kg) had a pressor effect (Fig. 6), but in chicks anaesthetized and with intact brains $F_{2\alpha}$ had a depressor effect sometimes preceded by a transient pressor phase. E_1 was depressor in both types of preparation. The pressor effect of $F_{2\alpha}$ in spinal chicks was not abolished by hexamethonium or phenoxybenzamine, and therefore does not appear to be mediated via the sympathetic nervous system.

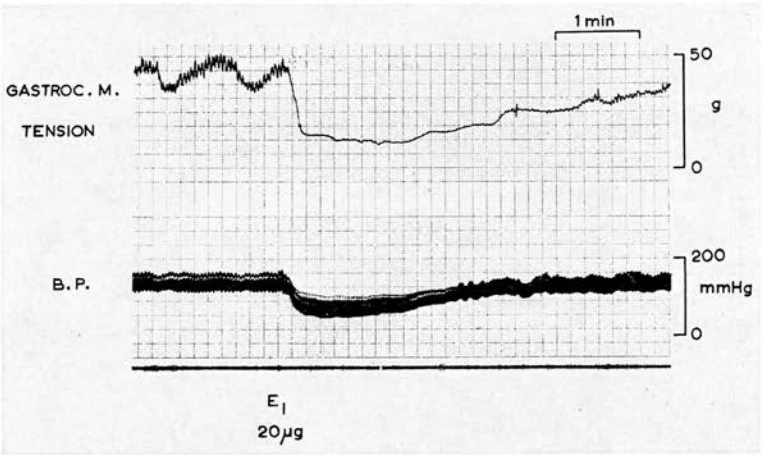

Fig. 8. Cat 3.2 kg anaesthetized with sodium pentobarbitone. Upper trace: gastrocnemius muscle tension. Lower trace: carotid arterial blood pressure. Responses to prostaglandin E_1 (20 μg) injected intravenously. (Horton and Main, British Journal of Pharmacology. In the press.)

Identification of prostaglandins in central nervous tissues

In 1964 Samuelsson (11) announced the isolation of $F_{2\alpha}$ from ox brain. We have used his procedure in order to identify the prostaglandins in cat brain, chicken brain and chicken spinal cord. On silicic acid chromatography peaks of biologically-active material corresponding to prostaglandins E and F were found with all three extracts (Figs. 9 and 10). Fractions from each peak were separately pooled and purified further on silica gel plates using the AI solvent system

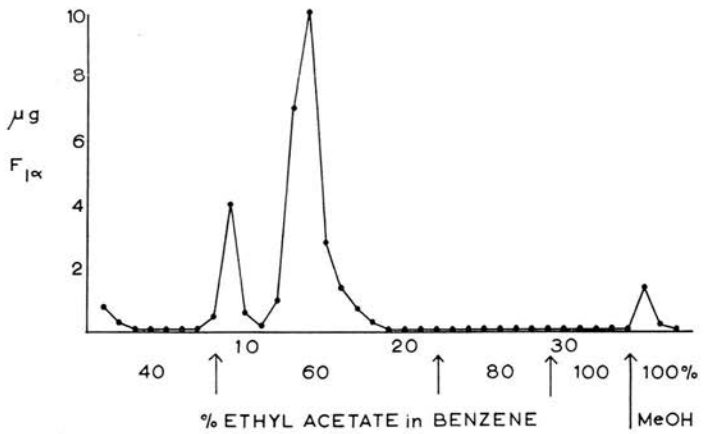

Fig. 9. Chromatography of an extract of cat fore brain on a 2 g. column of silicic acid. Elution rate 0.8–1.0 ml/min. 10 ml fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of $F_{1\alpha}$. Abscissa: fraction number. (Horton and Main, British Journal of Pharmacology. In the press.)

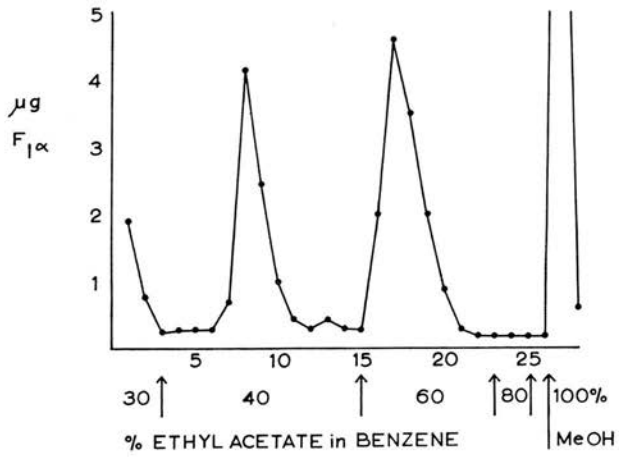

Fig. 10. Chromatography of pooled chicken brain extracts-details as Fig. 9. (Horton and Main, British Journal of Pharmacology. In the press.)

of Gréen and Samuelsson (3). Further evidence about the types of prostaglandin present was obtained by thin layer chromatography on $AgNO_3$-impregnated plates and by parallel biological assays on several tissues (Table 2). By this means we have shown that cat forebrain (supra-collicular) contains $F_{2\alpha}$ and an unidentified E, and that both chicken brain and chicken spinal cord contain $F_{2\alpha}$ and E_2 (8).

Discussion and conclusions

Our pharmacological experiments indicate that prostaglandins act at various sites in the central nervous system. That these actions are upon the neurones themselves is suggested by the results of Avanzino, Bradley and Wolstencroft (1, 2), who have shown that prostaglandins can excite (or sometimes inhibit) central neurones on micro-iontophoretic application. Although we have found prostaglandins in the brain of the cat and the chicken we have no evidence that they are actually in the neurones—they might for example be in the glial cells.

Table 2. *Parallel biological assays of material eluted from zone 2 of a thin layer chromatogram (AI solvent system) of cat brain extract in terms of prostaglandin* $F_{1\alpha}$ *and* E_1.

Preparation	$F_{1\alpha}$	E_1
Guinea pig ileum	1.0	1
Rat fundus	1.0	3
Cat trachea	1.0	3
Jird colon	0.6	9
Rat uterus	0.4	75
Rabbit jejunum	1.2	94

The observation of Ramwell and Shaw (9, 10) that prostaglandins are released from the somato-sensory cortex of the cat on sensory nerve stimulation is suggestive however of a neuronal origin. Taken together these observations might be thought to support the hypothesis that the prostaglandins are central nervous transmitters but they could equally be modulators of such transmitters. For example, prostaglandins could be released from the depolarized post synaptic membrane temporarily preventing either further release or action of the transmitter. Investigations into the cellular and subcellular distribution of prostaglandins in the central nervous system may help in solving this problem.

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IDENTIFICATION OF PROSTAGLANDINS IN CENTRAL NERVOUS TISSUES OF THE CAT AND CHICKEN

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IDENTIFICATION OF PROSTAGLANDINS IN CENTRAL NERVOUS TISSUES OF THE CAT AND CHICKEN

BY

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Brain extracts from several species contain biologically active lipids whose physicochemical properties resemble those of the prostaglandins. Ambache reported the presence in rabbit brain of a smooth muscle-stimulating unsaturated hydroxy fatty acid (Ambache & Reynolds, 1960, 1961; Ambache, Reynolds & Whiting, 1963). Kirschner & Vogt (1961) prepared chloroform–methanol extracts of horse brain and separated three biologically active fractions, one of which consisted of unsaturated fatty acids. Toh (1963) prepared an extract of dog and cat brain, the biological activity of which was due to unsaturated fatty acids. The biological activity of these extracts may have been due, at least in part, to prostaglandins. A prostaglandin has so far been isolated from the brain of only one species, the ox (Samuelsson, 1964), though Coceani & Wolfe (1965) have reported the presence of a prostaglandin-like substance in cat brain using Samuelsson's extraction procedure.

In view of the pharmacological actions of prostaglandins on the central nervous system of cats and chicks (Horton, 1964; Horton & Main, 1965a & b, 1966a, 1967a), we have attempted in this investigation to identify prostaglandins in central nervous tissues of these species. Preliminary reports of this work have been published (Horton & Main, 1966b, 1967b, and c).

METHODS

Chemical Procedures

Collection and ethanol extraction of cat brain

Adult cats of both sexes were anaesthetized with ethyl chloride and ether. All brain tissue above the intercollicular level was removed by classical decerebration technique. The tissue was weighed and either stored at about −10° C for subsequent homogenization or homogenized immediately with a pestle and mortar in 96% aqueous ethanol and sand. The homogenate was stirred for 4 hr at room temperature in 96% aqueous ethanol (4 ml./g of tissue). The mixture was filtered or centrifuged or both, and the residue was re-extracted with 0.5 vol. 96% aqueous ethanol. The combined aqueous ethanol filtrates or supernatants were evaporated to dryness under reduced pressure at 40–45° C using a rotary evaporator. The dried residue was stored at −20° C.

Collection and ethanol extraction of chicken brain and spinal cord

Adult chickens (*Gallus domesticus*) of both sexes were exsanguinated by section of the large blood vessels in the neck. Whole brains and spinal cords were dissected out immediately and

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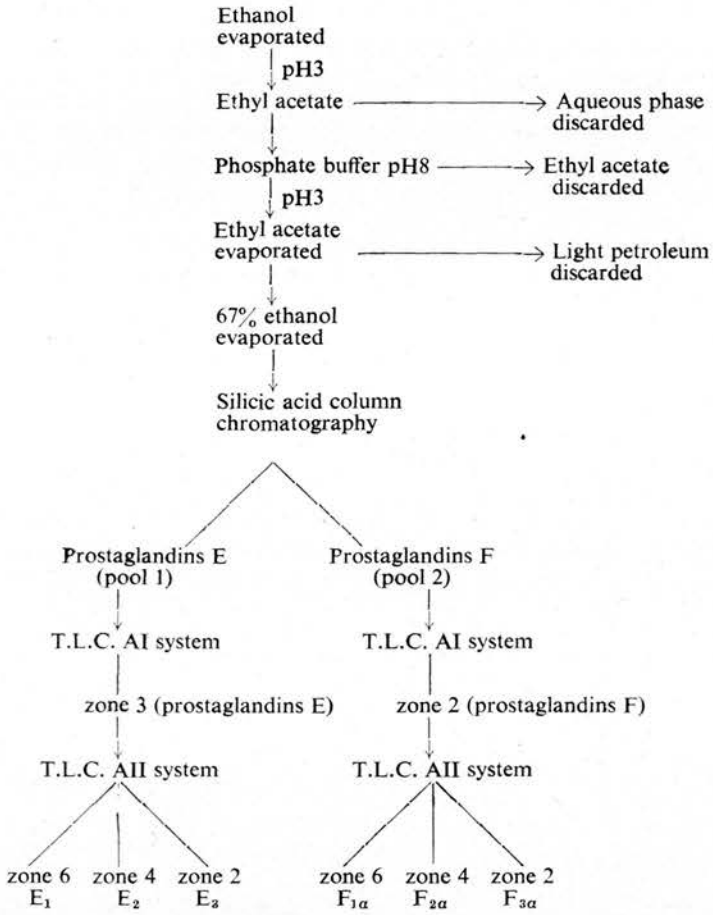

Fig. 1. Outline of the extraction procedure used for the identification of prostaglandins in brain.

weighed. The ethanol extraction procedure was the same as that used for cat brain; the chicken brains and spinal cords were extracted separately. The dried residues were stored at −20° C.

The subsequent extraction procedure was that described by Samuelsson (1964) and was the same for cat brain, chicken brain and chicken spinal cord (Fig. 1).

Silicic acid chromatography

The dried residue from the ethanol extraction was dissolved in an equal volume of ethyl acetate and 0.1 N hydrochloric acid. After separation the acid aqueous phase was re-extracted with 1 vol. ethyl acetate. The combined ethyl acetate phases were concentrated to a smaller volume on a rotary evaporator. The ethyl acetate was extracted twice with 1 vol. pH 8 phosphate buffer solution. The aqueous phases were pooled and acidified to pH 3 by drop-wise addition of concentrated hydrochloric acid. The acid aqueous solution was then extracted twice with ethyl acetate. The ethyl acetate phases were pooled and evaporated to dryness. The residue was partitioned between equal volumes of 67% aqueous ethanol and light petroleum (B.P. 40–60° C). The aqueous ethanol was evaporated to dryness and subsequently chromatographed on a column of silicic acid.

Silicic acid chromatography

Two grammes silicic acid (Bio-Rad Laboratories, Calif.) was activated by heating for 1 hr at 115° C before use. It was washed twice with heavy petroleum (B.P. 60–80° C) and then suspended

in an ethyl acetate : benzene (30:70) mixture. Columns 80 mm in length and of cross-sectional diameter 8 mm reducing to 4 mm were prepared. The dried residue from the solvent partition procedure or a mixture of purified prostaglandins was dissolved in 1 ml. ethyl acetate:benzene (30:70) and applied to the column drop-wise with a Pasteur pipette. The flask was washed three times with ethyl acetate: benzene and the washings applied to the column.

The column was developed with increasing concentrations of ethyl acetate in benzene. Ten or 20 ml. fractions were collected under reduced pressure with a flow rate of 0.8 to 1.2 ml./min. The fractions were evaporated to dryness immediately after they had been collected and the residues were dissolved in distilled water for assay on the rabbit isolated jejunum. Approximate estimates of the biological activity in each fraction were made as soon as possible after collection, thus monitoring the progress of the chromatography and allowing changes in eluant to be made at appropriate times.

The concentrations of ethyl acetate in benzene used to develop the column were 30, 40, 60, 80 and 100% (v/v), followed finally by 100% methanol to remove the most polar substances from the column. All fractions were assayed more accurately at the end of the experiment and those with biological activity were stored frozen for further investigations.

Thin layer chromatography

Plates of dimensions 200×50 mm coated with Silica Gel G (E. Merck) were used throughout. In some preparative experiments the silica gel was 0.5 mm in thickness but a thickness of 0.25 mm was otherwise routinely used. Silica gel plates impregnated with 4% silver nitrate were prepared by suspending the silica gel (30 g) in a 2% solution of silver nitrate (60 ml.). The plates were activated in an oven at 115–120° C for 1 hr and stored over self-indicating silica gel until used. A strip approximately 3 mm in width was removed from each edge of the silica gel before use. Prostaglandins and brain extracts were dissolved in methanol and applied to the plate with a Agla microsyringe using a length of narrow bore polyethylene tubing (capacity 30 μl.) attached to the syringe needle.

Solvent systems used were the AI and AII systems described by Gréen & Samuelsson (1964). The AI system contains benzene:dioxane:acetic acid 20:20:1, and the AII system contains the less polar (upper) phase of a mixture of ethyl acetate:acetic acid:methanol:2,2,4-trimethylpentane:water 110:30:35:10:100 which had been allowed to equilibrate for 2 hr. All solvents except dioxane were redistilled before use.

Cylindrical glass developing tanks of internal diameter 6 cm and of height 21.5 cm were used. The walls were lined with Whatman No. 1 filter paper impregnated with the solvent mixture. The tanks were sealed with glass lids using silicone grease. Marker plates using pure prostaglandin solutions were prepared by spotting on volumes of not more than 30 μl. of stock (methanol) solutions of the prostaglandins (0.1 or 1.0 mg/ml.). With preparative plates a series of spots (or a continuous band) of the extract dissolved in methanol was applied to the origin. Preparative and marker plates were run simultaneously in the same tank.

Plates were usually developed until the solvent had reached between 11 and 14 cm from the origin. The position of the solvent front was marked on removing the plate from the tank. The plates were dried and marker plates were sprayed with 10% phosphomolybdic acid in ethanol. On heating at 100° C for 10 min grey-blue spots developed on a greenish-yellow background, showing the position of the prostaglandins. About 1 μg of prostaglandin $F_{1\alpha}$ and about 10 μg of prostaglandins E_1 and E_2 could be detected by this method.

Preparative plates were divided into zones corresponding to the Rf values of prostaglandins on the marker plate. With the AI system zones corresponding to prostaglandins E and F were separated ; with the AII systems the prostaglandin E fraction was further separated in to E_1, E_2 and E_3, and the prostaglandin F fraction into $F_{1\alpha}$, $F_{2\alpha}$, and $F_{3\alpha}$. Due to shortage of material, marker plates could not be run routinely with $F_{2\alpha}$. The position of $F_{2\alpha}$ on the preparative plate was therefore calculated from the observed Rf value of $F_{1\alpha}$ on the marker plate using the relative Rf values of $F_{1\alpha}$ and $F_{2\alpha}$ reported by Gréen & Samuelsson (1964). In a control experiment $F_{2\alpha}$ was applied to a preparative plate and the predicted $F_{2\alpha}$ zone contained all the biological activity. The

position of the E_3 and $F_{3\alpha}$ zones were also calculated since these prostaglandins were not available for chromatography.

The separated zones were shaken with 3 or 5 ml. methanol and centrifuged. The deposit was re-extracted with methanol and the combined methanol extracts evaporated to dryness. The residue was dissolved in either 0.9% sodium chloride solution or Tyrode solution so that the Ag^+ ions were precipitated as AgCl. This precipitate did not interfere with assays on the rabbit jejunum. In some cases the dried residue from the methanol extraction of these $AgNO_3$ plates was dissolved in 0.1N HCl (which precipitated the Ag^+) and extracted with ethyl acetate. The ethyl acetate was evaporated to dryness, and the residue dissolved in saline or Tyrode solution for assay.

Preparation of $F_{2\alpha}$ from E_2

Reduction of E_2 by sodium borohydride to yield $F_{2\alpha}$ and $F_{2\beta}$ was carried out as described by Bergström, Krabisch, Samuelsson & Sjövall (1962).

Sodium borohydride 1 mg was added to a solution of 400 μg E_2 in 1 ml. methanol at 0° C. The mixture was allowed to stand for 20 min at 0° C and then for 20 min at room temperature. Ten millilitres of 0.1N HCl were then added and the aqueous phase was extracted twice with ether. The combined ether extracts were washed with water and taken to dryness.

The residue was dissolved in methanol and chromatographed on silica gel in the AI solvent system. About 5% of the extract was applied to a marker plate which, after development, was sprayed with phosphomolybdic acid and heated. Two spots appeared, one (Rf 0.32) corresponding to $F_{2\alpha}$ and the other (Rf 0.25) to $F_{2\beta}$. The remainder of the extract was applied to a preparative plate which, after development, was divided into zones corresponding to $F_{2\alpha}$ and $F_{2\beta}$. The zones were eluted with methanol and taken to dryness. The material from the $F_{2\alpha}$ zone was rechromatographed in the AI solvent system to eliminate traces of $F_{2\beta}$ which were present. The biologically active material in the $F_{2\alpha}$ zone from this second chromatogram was standardized on the isolated rabbit jejunum and guinea-pig ileum against pure $F_{2\alpha}$.

Biological preparations

Biological assays

The rabbit isolated jejunum was used routinely for the estimation of biological activity in the various fractions, using prostaglandins E_1 and $F_{1\alpha}$ as the standards for comparison. The concentrations of prostaglandins in brain was calculated from the relative activities previously determined (Table 1).

TABLE 1

BIOLOGICAL ACTIVITY OF PROSTAGLANDINS E_2, E_3, $F_{1\alpha}$ and $F_{2\alpha}$ RELATIVE TO PROSTAGLANDIN E_1 ON RABBIT ISOLATED JEJUNUM

(data from Horton & Main, 1963, 1965, 1966)

E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$
1·5	1·0	2·2	26

Smooth muscle preparations in vitro

Segments of various organs were suspended in a 4 ml. or 10 ml. organ-bath. Longitudinal contractions were recorded either isotonically with a frontal-writing lever on a smoked drum, or isometrically with a force-displacement transducer (Grass F.T.03) on a Sanborn polygraph. A dose cycle of 4 to 6 min with a contact time of 45–90 sec was used for all preparations except the cat trachea.

Rabbit jejunum

Proximal jejunum, from rabbits weighing 1–3 kg, was suspended in Tyrode solution at 37° C, gassed with air or 5% carbon dioxide in oxygen.

Guinea-pig ileum

Terminal ileum from guinea-pigs weighing 200–400 g was suspended in Tyrode solution at 37° C, gassed with air or 5% carbon dioxide in oxygen.

Rat uterus

A segment of a uterine horn from rats weighing 150–250 g which had been injected subcutaneously, 18 hr previously, with stilboestrol (100 μg), was suspended in de Jalon solution at 30° C, gassed with air.

Jird colon

Segments of ascending colon from Meriones weighing 100–150 g were suspended in Tyrode solution at 37° C, gassed with air.

Rat fundus

Longitudinal strips of the fundus from rats weighing 200–350 g. were prepared as described by Vane (1957) and suspended in Tyrode solution at 37° C, gassed vigorously with oxygen.

Cat trachea

Tracheas were obtained from cats which had been used for other experiments. Two or three rings of trachea were tied together with the muscle in alignment; the cartilage was removed. The preparations were suspended in Krebs-Henseleit solution at 37° C, gassed with 5% carbon dioxide in oxygen. The inhibitory action of a prostaglandin was demonstrated by adding it to the organ bath either 30 sec before a dose of acetylcholine, or after acetylcholine had produced a sustained contraction (Main, 1964).

Spinal chick preparation

The technique is described in the preceding paper (Horton & Main, 1967a).

RESULTS

Identification of prostaglandins in chicken brain

Solvent extraction and partition

Ethanolic extracts of 115 g chicken brain contracted the isolated rabbit jejunum. On partition between ethyl acetate and water at different pHs as described under Methods (Fig. 1) the biologically active material behaved like an acidic lipid. On partition between 67% aqueous ethanol and petroleum ether (B.P. 40–60°) the biological activity was recovered from the aqueous ethanol phase. From the results of previous workers (Samuelsson, 1963; Bergström & Samuelsson, 1963) these results are compatible with the biological activity being due to prostaglandins.

Silicic acid chromatography

The next stage was chromatography on a silicic acid column to purify the extract further and also to separate prostaglandins E from F. According to Samuelsson (1963) Es and Fs are eluted with concentrations of 60% and 80% ethyl acetate in benzene, respectively. Control experiments were first carried out using 10–20 μg of pure E_1 and $F_{1\alpha}$. In these experiments both types of prostaglandins were eluted with 60% ethyl acetate and none with 80% ethyl acetate. However, by using 40% ethyl acetate in benzene E could be eluted first, while F was eluted by 60% ethyl acetate in benzene as shown in Fig. 2.

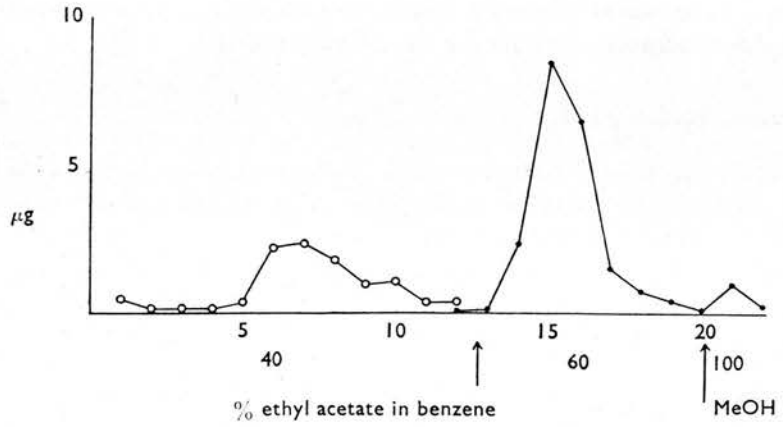

Fig. 2. Chromatography of a mixture of prostaglandins E_1 (10 μg) and $F_{1\alpha}$ (20 μg) on a 2 g column of silicic acid. Elution rate 0.8–1 ml./min. 10 ml. fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin E_1 (open circles) and $F_{1\alpha}$ (closed circles). Abscissa: upper line, fraction number; lower line, nature of eluant.

In this experiment the recoveries of E_1 and $F_{1\alpha}$ applied to the column were 90% and 98% respectively. The identity of the prostaglandins was confirmed by parallel biological assay. Furthermore in experiments in which prostaglandin E_1 was chromatographed alone, all the biological activity was eluted with 40% ethyl acetate.

The chicken brain extract obtained from the solvent partition procedure described above was chromatographed on a silicic acid column. The result is shown in Fig. 3. Apart from a small initial peak of activity (≡2·2 μg $F_{1\alpha}$), there was one peak eluted with 40% ethyl acetate in benzene corresponding to prostaglandins E (≡19 μg E_1) and another peak eluted with 60% ethyl acetate in benzene corresponding to prostaglandins F

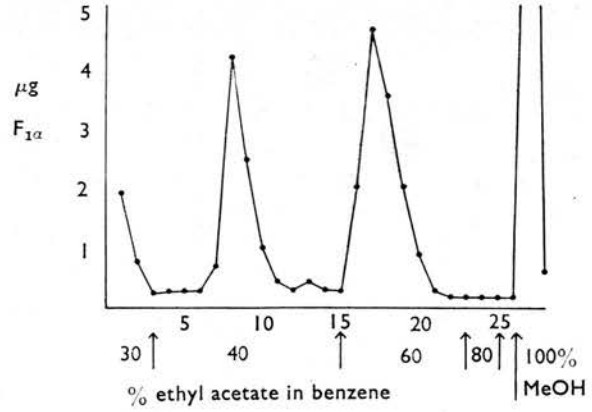

Fig. 3. Chromatography of chicken brain extract on a 2 g column of silicic acid. Elution rate 1–1.2 ml./min. 20 ml. fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin $F_{1\alpha}$. Abscissa: upper line, fraction number; lower line, nature of eluant.

(≡12·1 μg $F_{1\alpha}$). Considerable biological activity (≡16 μg $F_{1\alpha}$) was eluted with methanol, but this did not correspond to either a prostaglandin E or F.

Thin layer chromatography in the AI solvent system

The material in fractions 7–10 (pool 1) and 16–20 (pool 2) was further purified by thin layer chromatography in the AI solvent system. On chromatography of pool 1, 97% of the biological activity was recovered from zone 3 which corresponded to prostaglandins E on the marker plate (Fig. 4). On chromatography of pool 2, 90% of the activity was recovered from zone 2 of the chromatogram corresponding to prostaglandins F. The % biological activity found in the various zones of the chromatogram as assayed on the rabbit isolated jejunum is shown in Table 2.

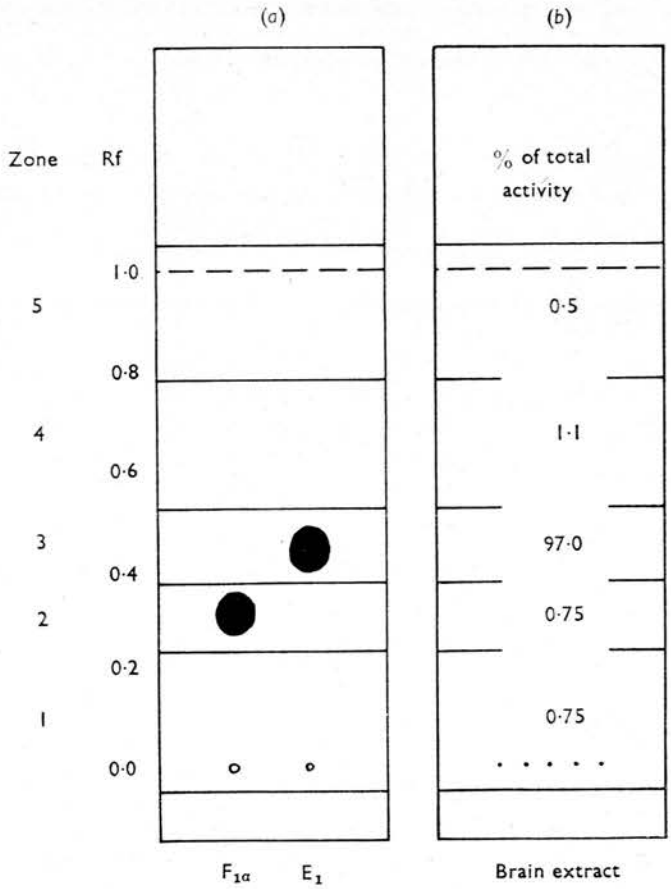

Fig. 4. Thin layer chromatography of chicken brain extract (pool 1, Fig. 3) in the AI solvent system. (a) marker plate, spotted with prostaglandins $F_{1\alpha}$ and E_1 and sprayed with phosphomolybdic acid, (b) preparative plate, spotted with brain extract, divided into zones.

TABLE 2

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN BRAIN (POOLS 1 AND 2) DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF E_1 (POOL 1) AND $F_{1\alpha}$ (POOL 2)

Zone No.	Mean Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Pool 1	Pool 2
1		Origin	0·75	7·0
2	0·32	F_α	0·75	90·0 ($\equiv$5 μg $F_{1\alpha}$)
3	0·44	E	97·0 ($\equiv$22·5 μg E_1)	<2·0
4			<1·1	<2·0
5		Front	<0·5	<2·0

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was further chromatographed on silver nitrate–impregnated plates in the AII solvent system. The results of chromatography of zone 3 (prostaglandins E from the plate run in the AI system) are shown in Table 3. Most of the biological activity as assayed on the

TABLE 3

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CHICKEN BRAIN (POOL 1, ZONE 3, PROSTAGLANDINS E FROM THE PLATE RUN IN AI, TABLE 2) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED IN PARALLEL ON THE RABBIT JEJUNUM AND GUINEA-PIG ILEUM IN TERMS OF PROSTAGLANDIN E_1

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Rabbit jejunum	Guinea-pig ileum
1		Origin	<1	<2
2	*(0·31)	E_3	1	<2
3		—	2	<1
4	0·62	E_2	92 ($\equiv$25 μg E_1)	89 ($\equiv$7 μg E_1)
5		—	2	<2
6	0·79	E_1	1	<2
7		Front	<1	<2

* Rf value calculated as described in Methods.

isolated rabbit jejunum and guinea-pig ileum was recovered from zone 4 corresponding to E_2. Traces of activity were detected in the " neutral " zones adjoining E_2 but there was no significant activity in any other zone (Fig. 5). The E_2 zone was assayed on three isolated tissues in terms of E_1 and $F_{1\alpha}$ as shown in Table 4.

The results of the parallel assays confirm that the activity is prostaglandin E-like, it could not be due to a prostaglandin F. The differences between estimates of biological activity (in terms of E_1) on these three tissues may be attributed partly to biological error in the assays and partly to differences in the relative activities of E_2 to E_1 on these preparations (Horton & Main, 1963).

The results of chromatography of the zone 2 (prostaglandins F from the plate run in the AI system) are shown in Table 5.

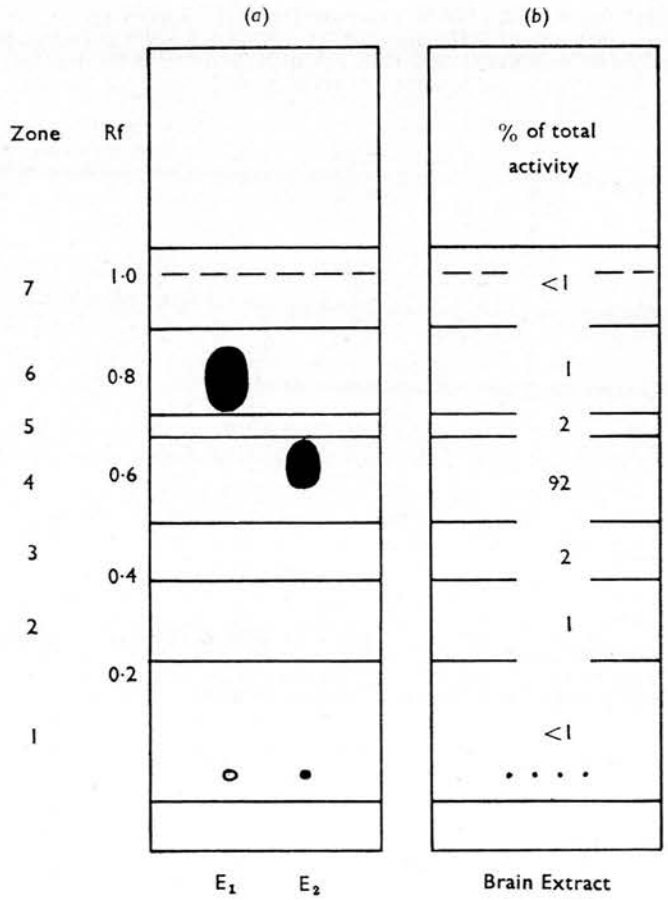

Fig. 5. Thin layer chromatography of chicken brain extract (zone 3, Fig. 4) in the AII solvent system. (a) marker plate, spotted with prostaglandin E_1 and E_2 and sprayed with phosphomolybdic acid, (b) preparative plate, spotted with brain extract divided into zones.

TABLE 4

CHICKEN BRAIN EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM ZONE 4 OF THE THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 3)

Each figure represents the result of one estimation

	Biological activity	
Preparation	μg E_1 equivalent	μg $F_{1\alpha}$ equivalent
Rabbit jejunum	25, 25	8, 5
Rat fundus	12	96
Guinea-pig ileum	7	87

TABLE 5

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A THIN LAYER PLATE (PREPARATIVE THIN LAYER CHROMATOGRAM OF CHICKEN BRAIN POOL 2, ZONE 2, FROM THE PLATE RUN IN AI SEE TABLE 2) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1		Origin	<5
2	(0·22)	$F_{3\alpha}$	<5
3		—	<5
4	(0·47)	$F_{2\alpha}$	67 (≡0·75 μg $F_{1\alpha}$)
5		—	<5
6	0·61	$F_{1\alpha}$	7
7		—	<5
8		Front	<5

Of the total activity 67% were recovered from zone 4, corresponding to $F_{2\alpha}$. A small amount of activity was also present in zone 6, which corresponded to $F_{1\alpha}$.

The $F_{2\alpha}$ zone was assayed on two isolated tissues in terms of $F_{1\alpha}$ as shown in Table 6. The results are compatible with the activity being due to prostaglandin $F_{2\alpha}$. The activity could not be due to any of the prostaglandins E.

TABLE 6

CHICKEN BRAIN EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM ZONE 4 OF THE THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 5)

Each figure represents the result of one estimation.

Preparation	Biological activity	
	μg $F_{1\alpha}$ equivalent	μg E_1 equivalent
Rabbit jejunum	0·75, 1·0	3·8, 3·0
Rat fundus	0·9	0·11

Concentration of prostaglandins in chicken brain

Although these experiments were primarily designed to identify prostaglandins, some indication of the concentration of prostaglandins E_2 and $F_{2\alpha}$ in chicken brain were obtained from the results of silicic acid chromatography (Fig. 3). Pool 1 (prostaglandins E) contained biological activity on the rabbit jejunum equivalent to 19 μg E_1. The amount of E_2 recovered from the column was therefore about 13 μg (relative activity of E_2 to E_1=1·5, Table 1). The concentration of E_2 in chicken brain was therefore of the order of 100 ng/g wet weight. Pool 2 (prostaglandins F) contained biological activity equivalent to 1 μg $F_{2\alpha}$ (relative activity of $F_{2\alpha}$ to $F_{1\alpha}$=12, Horton & Main, 1965c, Table 1). The concentration of $F_{2\alpha}$ in chicken brain was therefore of the order of 10 ng/g wet weight of tissue.

Identification of prostaglandins in chicken spinal cord

Silicic acid chromatography

Chicken spinal cord (35.7 g) was extracted with ethanol and partitioned as described under Methods. The resultant extract which contained biologically active material as assayed on the rabbit isolated jejunum was chromatographed on a silicic acid column. There were peaks of biological activity corresponding to prostaglandins E (pool 1)

(≡2.2 μg E_1) and prostaglandins F (pool 2) (≡4.5 μg $F_{1\alpha}$). A third peak of activity was eluted with methanol.

Thin layer chromatography in the AI solvent system

Pool 1 and pool 2 were chromatographed separately on thin layer plates in the AI solvent system. The results are shown in Table 7.

TABLE 7

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN SPINAL CORD DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN E_1 (POOL 1) AND PROSTAGLANDIN $F_{1\alpha}$ (POOL 2)

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Pool 1	Pool 2
1		Origin	4	11
2	0·3	$F\alpha$	4	64 (≡3 μg $F_{1\alpha}$)
3	0·4	E	88 (≡20 μg E_1)	15
4		—	<2	<5
5		Front	<2	<5

Most of the activity in pool 1 was recovered from zone 3 corresponding to prostaglandins E and most of the activity in pool 2 was recovered from zone 2 corresponding to prostaglandins F, though there was a small amount in the adjacent zones.

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was further chromatographed on silver nitrate-impregnated plates in the AII solvent system. The results of the two chromatograms are shown in Table 8.

Most of the biological activity of pool 1 zone 3 (prostaglandins E from the plate run in the AI solvent system) was recovered from zone 4 of the silver nitrate-impregnated plate corresponding to prostaglandin E_2. There was no significant activity in the zones corresponding to E_3 (zone 2) or E_1 (zone 6).

TABLE 8

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF PREPARATIVE THIN LAYER CHROMATOGRAMS OF CHICKEN SPINAL CORD (POOL 1, ZONE 3, AND POOL 2, ZONE 2, FROM THE PLATES RUN IN AI, TABLE 7) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN E_1 (POOL 1) AND PROSTAGLANDIN $F_{1\alpha}$ (POOL 2)

Zone No.	% of total biological activity recovered from plate					
	Pool 1 zone 3			Pool 2 zone 2		
	Rf			Rf		
1		Origin	<4		Origin	18
2	(0·34)	E_3	<4	(0·21)	$F_{3\alpha}$	<9
3		—	<8		—	<9
4	(0·67)	E_2	62 (≡4 μg E_1)	(0·45)	$F_{2\alpha}$	46 (≡0·5 μg $F_{1\alpha}$)
5		—	15		—	<9
6	0·77	E_1	<4	0·58	$F_{2\alpha}$	<9
7		Front	<4		Front	

Most of the biological activity of pool 2 zone 2 (prostaglandins F from the plate run in the AI solvent system) was recovered from zone 4 of the silver nitrate-impregnated plate corresponding to prostaglandin $F_{2\alpha}$. Activity was also present at the origin but not in the zones corresponding to $F_{1\alpha}$ or $F_{3\alpha}$.

The activity in the E_2 and $F_{2\alpha}$ zones was assayed in parallel on different isolated tissues and the results are shown in Table 9. The results are compatible with the activity in these zones being due to E_2 and $F_{2\alpha}$ respectively.

TABLE 9

CHICKEN SPINAL CORD EXTRACT. PARALLEL BIOLOGICAL ASSAYS OF THE MATERIAL ELUTED FROM THE E_2 AND $F_{2\alpha}$ ZONES OF THE CHROMATOGRAMS DEVELOPED IN THE AII SOLVENT SYSTEM (TABLE 8)

	Biological activity		Ratio of activity
Preparation	E_2 zone (μg E_1 equiv.)	$F_{2\alpha}$ zone (μg $F_{1\alpha}$ equiv.)	$F_1/E_{1\alpha}$
Rabbit jejunum	4·0	0·5	6·7
Rat fundus	2·0	0·6	0·9
Guinea-pig ileum	1·6	Not tested	<0·1

Concentration of prostaglandins in chicken spinal cord

By assaying the biological activity eluted from the silicic acid column, chicken spinal cord is estimated to contain of the order of 400 ng E_2/g and 10 ng $F_{2\alpha}$/g tissue wet weight.

Identification of prostaglandins in cat brain

Silicic acid chromatography

Cat forebrains obtained by decerebration were extracted with ethanol and partitioned as described under Methods. The resultant extract was chromatographed on a silicic acid column. The result is illustrated in Fig. 6. There were peaks of biological activity corresponding to prostaglandins E and prostaglandins F.

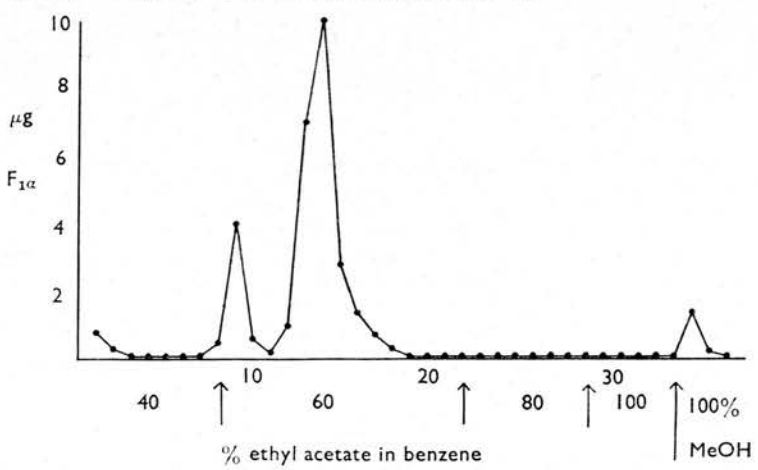

Fig. 6. Chromatography of an extract of cat brain on a 2 g column of silicic acid. Elution rate 0.8–1 ml./min. 10 ml fractions collected. Ordinate: biological activity assayed on the rabbit jejunum in terms of prostaglandin $F_{1\alpha}$. Abscissa: upper line, fraction number; lower line, nature of eluant.

Thin layer chromatography in the AI solvent system

Pool 1 (fraction 9) was chromatographed on a thin layer plate in the AI solvent system. Most of the activity was present in zone 3 corresponding to prostaglandins E. Pool 2 (fractions 13–18) was chromatographed on a thin layer plate in the AI solvent system (Table 10). At least 96% of the activity was present in zone 2 corresponding to prostaglandins F.

TABLE 10

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 2) DEVELOPED IN THE AI SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1		Origin	3
2	0·31	F_{α}	96 ($\equiv$10 μg $F_{1\alpha}$)
3	0·42	E	1
4			<0·2
5		Front	<0·2

TABLE 11

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN THE ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 1, ZONE 3 FROM THE PLATE RUN IN THE AI SOLVENT SYSTEM) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED IN PARALLEL ON THE RABBIT ISOLATED JEJUNUM AND RAT FUNDUS PREPARATIONS IN TERMS OF PROSTAGLANDIN E_1

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate	
			Rabbit jejunum	Rat fundus
1		Origin	6	9
2	(0·3)	E_3	44 ($\equiv$0·35 μg E_1)	<9
3			<6	<9
4	0·61	E_2	<6	9
5			6	<12
6	0·75	E_1	25 ($\equiv$0·2 μg E_1)	44 ($\equiv$0·38 μg E_1)
7		Front	<6	<9

TABLE 12

PERCENTAGE BIOLOGICAL ACTIVITY FOUND IN ZONES OF A PREPARATIVE THIN LAYER CHROMATOGRAM OF CAT BRAIN (POOL 2, ZONE 2 FROM CHROMATOGRAM SHOWN IN TABLE 10) DEVELOPED IN THE AII SOLVENT SYSTEM AND ASSAYED ON THE RABBIT JEJUNUM IN TERMS OF PROSTAGLANDIN $F_{1\alpha}$

Biological activity was found in all zones of the plates even those beyond the solvent front. Figures in the Table are obtained by subtracting the value of this non-specific activity from the total activity in each zone

Zone No.	Rf	Position of pure prostaglandins	% of total biological activity recovered from plate
1			0
2	(0·21)	$F_{3\alpha}$	0
3			17
4	(0·45)	$F_{2\alpha}$	69
5			9
6	0·59	$F_{1\alpha}$	5
7			0
8	Front		0

Thin layer chromatography in the AII solvent system

The material from the active zones corresponding to prostaglandins E and F was chromatographed on silver nitrate impregnated plates in the AII solvent system. The biological activity in each zone was assayed and the results are shown in Tables 11 and 12.

When assayed on the rabbit jejunum, significant activity of the prostaglandins E containing extract was found in zones 2 and 6 corresponding to E_3 and E_1 respectively. No significant activity was present in zone 4 (E_2) or the zones adjacent to it. When assayed on the rat fundus, activity was present only in zone 6, corresponding to E_1 (Table 11).

Most of the activity of the prostaglandins F containing extract corresponded to $F_{2\alpha}$ (zone 4) but a small amount of activity was present in zone 6 corresponding to $F_{1\alpha}$. The " neutral " zones 3 and 5 also contained activity suggesting that, on the preparative plate, the brain $F_{2\alpha}$ spot had extended to the adjacent zones. However, the possibility that a small quantity of $F_{1\alpha}$ was present cannot be excluded.

Parallel biological assays

The material from zone 6 (prostaglandin E_1) of the AII plate of the prostaglandin E extract was assayed on three isolated smooth muscle preparations. The results shown in Table 13, are compatible with the biological activity in zone 6 being due to prostaglandin E_1.

TABLE 13

CAT BRAIN EXTRACT (POOL 1). PARALLEL BIOLOGICAL ASSAYS CF THE MATERIAL ELUTED FROM ZONE 6 OF A THIN LAYER PLATE DEVELOPED IN THE AII SOLVENT SYSTEM (SEE TABLE 11), IN TERMS OF E_1 AND $F_{1\alpha}$

	Biological activity	
Preparation	μg E_1 equivalent	μg $F_{1\alpha}$ equivalent
Rabbit jejunum	0·2	
Rat fundus	0·38	0·38
Cat trachea	0·2	8

The material from zone 4 (prostaglandin $F_{2\alpha}$) of the AII plate of the prostaglandin F containing extract was assayed on the rabbit jejunum and rat fundus in terms of prostaglandins $F_{1\alpha}$ and E_1. The results 0·85 and 1·75 μg $F_{1\alpha}$ and 2.1 and 0.14 μg E_1 respectively were in agreement with its identification as a prostaglandin F.

An aliquot of the active material in zone 2 (prostaglandins F) from the thin layer chromatogram shown in Table 10 was assayed on six isolated smooth muscle preparations using $F_{1\alpha}$ and E_1 as standards. The results are shown in Table 14. The activity in terms of

TABLE 14

CAT BRAIN EXTRACT (POOL 2). PARALLEL BIOLOGICAL ASSAYS OF AN ALIQUOT OF TH E MATERIAL ELUTED FROM ZONE 2 OF THE THIN LAYER PLATE DEVELOPED IN THE A I SOLVENT SYSTEM (TABLE 10) EXPRESSED IN TERMS OF $F_{1\alpha}$ AND E_1

	Biological activity		Ratio of activity
Preparation	$F_{1\alpha}$ (μg)	E_1 (μg)	$F_{1\alpha}/E_1$
Rabbit jejunum	3·8	9·4	2·5
Guinea-pig ileum	3·1	0·1	0·04
Rat fundus	3·0	0·3	0·1
Cat trachea	3·0	0·3	0·1
Jird colon	1·7	0·9	0·5
Rat uterus	1·3	7·5	6·0

$F_{1\alpha}$ ranged from 1.3 μg on the rat uterus to 3.8 μg on the rabbit jejunum, thus the greatest index of discrimination between cat brain extract and $F_{1\alpha}$ was about 3. In contrast, the activity in terms of E_1 ranged from 0.1 μg on the guinea-pig ileum to 9.4 μg on the rabbit jejunum, an index of discrimination of 94. These results are compatible with the activity being due to a prostaglandin F, and confirm that it could not be due to a prostaglandin E.

A further aliquot of this material was compared with pure $F_{2\alpha}$ on the arterial blood pressure and crossed extensor reflex of the spinal chick. The intravenous injection of 0.1 μg prostaglandin $F_{2\alpha}$ caused a rise in blood pressure and potentiated the crossed extensor reflex. An amount of brain extract, equivalent to 0.1 μg $F_{2\alpha}$ as assayed on the rabbit jejunum, caused a similar rise in blood pressure and potentiation of the crossed extensor reflex as shown in Fig. 7.

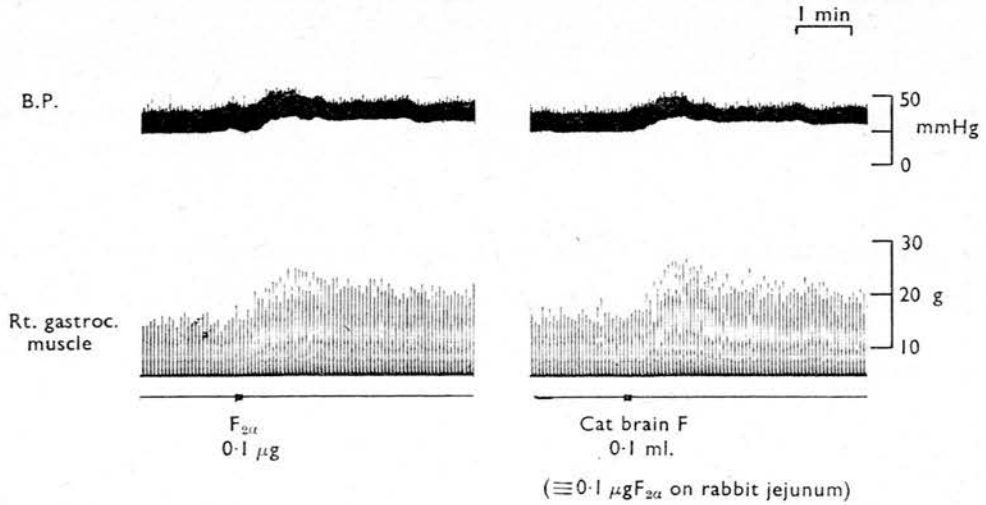

Fig. 7. Spinal chick (decapitated weight 38 g). Blood pressure and crossed extensor reflex. Upper trace: arterial blood pressure. Lower trace: gastrocnemius muscle tension, twitches were elicited by electrical stimulation of the contralateral sciatic nerve. Responses to prostaglandin $F_{2\alpha}$ (0.1 μg) and cat brain extracted (pool 2, zone 2 from AI plate, 0.1 ml.≡0.1 μg $F_{2\alpha}$ on rabbit jejunum) injected intravenously. There was an interval of 25 min between injections.

Concentration of prostaglandins in cat forebrain

Brain tissue, 17 g, removed at decerebration from one cat was extracted and chromatographed, with similar results to those obtained with pooled cat brains. From the silicic acid column peaks of biological activity corresponding to 0.09 μg prostaglandins E_1 and 1.6 μg prostaglandins $F_{1\alpha}$ were eluted.

On the basis of this experiment cat brain was estimated to contain of the order of 10 ng $F_{2\alpha}$/g and 5 ng E_1/g wet weight of tissue.

DISCUSSION

Brain extracts contain acidic lipid-soluble substances which stimulate smooth muscle (Ambache & Reynolds, 1960, 1961; Kirschner & Vogt, 1961; Ambache *et al.*, 1963;

Toh, 1963). One of these substances has been isolated from ox brain and identified as prostaglandin $F_{2\alpha}$ by chromatographic and mass spectrographic analysis (Samuelsson, 1964). Chemical identification, although desirable, is not always possible with the minute quantities of prostaglandins found in small samples of animal tissues. Furthermore, if the compound to be identified is already of known chemical structure and is available in pure form for comparison with the extracted material, such absolute confirmation of its identity may be unnecessary. The question thus arises as to how much and what kind of data are required before a claim to have identified a prostaglandin can be justified. The problem is complicated because there are many different prostaglandins with rather similar biological properties. In the present investigation the following criteria have been used for the identification of a prostaglandin (1) on solvent partition between water (at pH 3 and pH 8) and an organic solvent such as ether or ethylacetate, the unknown should behave like an acidic lipid; (2) on silicic acid chromatography the unknown should have a similar retention volume to one of the known prostaglandins; (3) on thin layer chromatography in the AI and AII (and possibly other) solvent systems of Gréen and Samuelsson (1964), the Rf value should correspond to a known prostaglandin; (4) on parallel biological assay there should be quantitative agreement on two or more tissues. Unequivocal evidence of a negative kind can be obtained from such an approach. Thus it may well be possible to conclude with confidence that an isolated substance is not, say, prostaglandin E_1 because of its different chromatographic behaviour and the lack of agreement between the parallel assays. On the other hand, agreement between the behaviour of the isolated substance and a particular prostaglandin in all these respects does not provide conclusive evidence. It may be permissible to conclude that the chemical and biological properties of the substance are compatible with its identification as, say, prostaglandin $F_{2\alpha}$ but there must always be some element of doubt. Nevertheless the more data of this kind that are accumulated the greater the probability that the identification is correct.

In this investigation solvent partition indicated that the substances extracted from brain and spinal cord were polar acidic lipids. On silicic acid chromatography peaks of biological activity were eluted corresponding to the retention volumes of prostaglandins E and F in control experiments. It should be noted that prostaglandins E and F were eluted with 40 and 60% ethylacetate in benzene respectively, whereas others have used 60 and 80% (Samuelsson, 1964; Coceani & Wolfe, 1965). This may have been due to a different source of silicic acid. Other more polar substances which stimulate the rabbit jejunum were eluted with methanol, but no attempt has been made to identify them. They are possibly hydroperoxides of unsaturated fatty acids.

Confirmation that prostaglandins E and F were present in the extract was obtained by thin layer chromatography in the AI solvent system of Gréen and Samuelsson (1964), which separates the E's from the F_α's and F_β's. Biological activity in the two peaks from the silicic acid column behaved like prostaglandins E and F_α respectively. In most experiments location of the prostaglandins depended upon biological assay of the eluates of appropriate zones of the thin layer plate, but in one experiment, in which cat brain extract was chromatographed in the AI solvent system and the plate was sprayed with phosphomolybdic acid, a spot appeared which corresponded exactly to F_α. Further evidence that the activity was due to prostaglandins E and F was obtained from the results

of parallel biological assay on smooth muscle preparations. Since there is no way of distinguishing between the different prostaglandins E and F by parallel biological assay, final identification was based on chromatographic behaviour on thin layer plates of silica gel impregnated with silver nitrate developed in the AII solvent system, which separates prostaglandins according to their degree of unsaturation.

From the combination of chromatographic and biological evidence it is concluded that cat forebrain contains prostaglandin $F_{2\alpha}$ and a prostaglandin E, tentatively identified as E_1. Evidence for the presence of E_3 was equivocal (Table 10). There may also be some $F_{1\alpha}$ present but no E_2, or $F_{3\alpha}$ could be detected. Both brain and spinal cord of the chicken contained substances which behaved chromatographically and biologically like prostaglandins E_2 and $F_{2\alpha}$, the presence of detectable quantities of E_1, E_3 and $F_{3\alpha}$,was excluded, but there may have been some $F_{1\alpha}$ in the chicken brain.

The primary object of this investigation was to identify prostaglandins in tissues, not to estimate the amounts present. However, the concentration of $F_{2\alpha}$ in cat fore-brain and of $F_{2\alpha}$ and E_2 in chicken brain and spinal cord has been estimated from the biological activity in the E and F peaks of the silicic acid chromatograms. Since $F_{2\alpha}$ and E_2 were not normally used as prostaglandin standards in the bioassay of fractions from the silicic acid columns, the relative biological activities of the mono- and di-unsaturated F's and E's on the rabbit jejunum (Horton & Main, 1963, 1966a) have been used in the calculation. By this means $F_{2\alpha}$ was estimated to be present in cat fore-brain, chicken brain and chicken spinal cord in concentrations of about 10 ng/g wet weight of tissue. The concentration of E_1 in cat fore-brain was about 5 ng/g; in contrast, the concentration of E_2 in chicken brain and chicken spinal cord was relatively high, 100 ng/g and 400 ng/g respectively.

In calculating these figures, no account was taken of losses during the extraction procedure before silicic acid chromatography; hence, the concentrations of prostaglandins in these tissues may be higher than estimated. It is unlikely, however, that extraction losses could account for the large difference between the estimated concentration of $F_{2\alpha}$ in cat fore-brain, chicken brain and chicken spinal cord (10 ng/g) and that found by Samuelsson (1964) in whole ox brain (300 ng/g). This discrepancy could be due to species variation in the concentration of prostaglandin in the central nervous system or it could also be due to differences in the concentrations of prostaglandins in different parts of the central nervous system. For example, a high concentration of prostaglandins E in the hind brain could account for the differences between cat fore-brain and chicken whole brain. This receives some support from the finding that chicken spinal cord contains even higher concentrations of prostaglandin E_2. It is not certain whether any or all of the hind-brain was included in Samuelsson's experiments or whether a similar uneven distribution of prostaglandins E could account for the absence of prostaglandins E in his extracts. It is of interest that Ambache, Brummer, Rose & Whiting (1966) reported that rabbit cerebral hemispheres contain mainly prostaglandins $F_{2\alpha}$ with some E_2.

If prostaglandins are unevenly distributed in the central nervous system this would suggest that they have a role in the specialized function of the region where their concentration is highest. Further investigations must be carried out to determine the regional distribution of prostaglandins in the central nervous system. It is also important to discover whether, for example, such prostaglandins represent a pool with a constant rate of turnover, or a store which may be released in response to a specific stimulus.

In preceding papers (Horton, 1964; Horton & Main, 1965a, 1967a) pharmacological actions of prostaglandins on the central nervous system of the cat and the chicken have been demonstrated. The identification of prostaglandins in the central nervous system of these species raises the possibility that these actions may reflect a physiological role, though the precise nature of such a role is unknown.

One possibility is that prostaglandins act as chemical transmitters of nerve impulses and evidence related to this hypothesis will be discussed briefly.

Although prostaglandins are present in brain and spinal cord it has not yet been established whether they are located in neurones or, for example, in glial cells. Investigations into the cellular and subcellular distribution of prostaglandins in the central nervous system are required to solve this problem.

That the pharmacological actions of prostaglandins on the central nervous system may be due to a direct action on neurones is suggested by the results of Avanzino, Bradley & Wolstencroft (1966a, b). Prostaglandins E_1, E_2 and $F_{2\alpha}$ applied ionotophoretically in very small amounts, had an excitatory or inhibitory action on the frequency of firing of reticulo-spinal neurones in the decerebrate cat, though desensitization, specific for the prostaglandin applied, was frequently observed. In contrast, prostaglandin E_1 had no effect when applied iontophoretically to cortical neurones in the chloralosed cat (Krnjević, 1965) suggesting either that there are marked regional differences in sensitivity to prostaglandins, or that anaesthesia blocks the effect.

Release of prostaglandins from the central nervous system has been demonstrated (Ramwell & Shaw, 1963a and b, 1966; Ramwell, Shaw & Jessup, 1966; Ramwell, 1967). On direct electrical stimulation, transcallosal stimulation or stimulation of the contralateral superficial radial nerve, prostaglandins (and acetylcholine) were detected in superfusates of the cat somatosensory cortex. Similarly, electrical stimulation of the frog hind limbs caused increased release of prostaglandins into spinal cord perfusates. Prostaglandins (or prostaglandin-like substances) have been detected in superfusates of cat cerebellum (Coceani & Wolfe, 1965) and in fluid perfused through the cerebroventricular system of cats (Feldberg & Myers, 1965, 1966) though in these situations a direct relationship between output of prostaglandins and neuronal activity was not clearly demonstrated.

These results do not exclude the possibility that prostaglandins may be central transmitters. However, evidence has recently been presented that, on nerve stimulation, prostaglandins are released in association with the classical chemical transmitters from several tissues outside the central nervous system including the rat diaphragm (Ramwell, Shaw & Kucharski, 1965), rat adipose tissue (Shaw, 1966), cat adrenal gland (Ramwell, Shaw, Douglas & Poisner, 1966), and dog spleen (Davies, Horton & Withrington, 1967). These results suggest that prostaglandins are not transmitters but may rather be modulators of transmission. Prostaglandins are not released from the rat diaphragm preparation by direct electrical stimulation of the muscle or from the isolated phrenic nerve, but they are released from the intact preparation on nerve stimulation even after muscle twitches have been blocked by d-tubocurarine (Ramwell *et al.*, 1965). Prostaglandins are released from adipose tissue either by nerve stimulation or by adding catecholamines to the incubation medium. Moreover, the amount of prostaglandins released is sufficient to inhibit the concomitant mobilization of free fatty acids from the tissue (Shaw, 1966). These

results raise the possibility that prostaglandins may modulate transmitter action by a local negative feed-back mechanism—for example, the action of the transmitter on the post-synaptic membrane may release prostaglandins which may inhibit further release or action of the transmitter.

Prostaglandins have potent inhibitory actions on hormone-induced lipolysis in adipose tissue (Steinberg, Vaughan, Nestel & Bergström, 1963) and on the hormone-induced increase in permeability of the isolated toad bladder (Orloff, Handler & Bergström, 1965). The concept that prostaglandins are modulators of chemical transmitters at nerve endings could, therefore, be extended to include an action on true hormones throughout the body.

There is evidence that the common factor in the mechanism of action of many hormones is the activation of the adenyl cyclase system which converts adenosine triphosphate (ATP) to 3,5-adenosine mono-phosphate (3,5-AMP) which in turn activates other enzyme systems to produce, for example, lipolysis or increased permeability (Sutherland, Øye & Butcher, 1965). Since, in these situations, prostaglandins do not prevent the action of 3,5-AMP, the action of prostaglandins may be to prevent the activation of adenyl cyclase. Whether the miscellaneous biological actions of prostaglandins can be rationalized in terms of a fundamental action on the adenyl cyclase system cannot be decided from existing evidence. It is of interest to note, however, that the brain forms 3,5-AMP in response to catecholamines (Sutherland *et al.*, 1965).

It is concluded that prostaglandins may have a role in the central nervous system, possibly as modulators of the actions not only of chemical transmitters released from nerve endings but also of other local or true hormones released in the central nervous system or reaching it from the systemic circulation. That this modulator action is not confined to the central nervous system but may be of importance throughout the body does not diminish its significance in the central nervous system.

Human seminal plasma contains by far the highest concentration of prostaglandins in any tissue yet examined. It is known that these prostaglandins are absorbed from the vagina after coitus and circulate in the bloodstream as hormones acting on female reproductive tract smooth muscle. It is tempting to speculate that the function of prostaglandins in human reproduction may also involve a hormonal action on the brain, possibly on those centres which control sexual behaviour.

SUMMARY

1. The object of the investigation was to identify the prostaglandins present in the central nervous system of the cat and the chicken.

2. On the basis of their behaviour on solvent partition, silicic acid column chromatography, thin layer chromatography and on biological preparations prostaglandins $F_{2\alpha}$ and E_1 have been identified in cat brain (supracollicular) and prostaglandins E_2 and $F_{2\alpha}$ in chicken brain and spinal cord.

3. The concentration of prostaglandin $F_{2\alpha}$ in the three tissues was approximately 10 ng/g whereas the concentrations of prostaglandin E_2 in the chicken brain and spinal cord were about 100 ng/g and 400 ng/g respectively.

4. The physiological significance of the prostaglandins in the central nervous system is discussed in the light of the known pharmacological actions.

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Prostaglandins and the Central Nervous System

by

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A link between prostaglandins and the central nervous system has been established by three kinds of experimental evidence. These are:- (1) prostaglandins are natural constituents of the brain and spinal cord, (Samuelsson, 1964; Coceani & Wolfe, 1965; Ambache, Brummer, Rose & Whiting, 1966; Horton & Main, 1966a, 1967a,c&d); (2) prostaglandins are released from the central nervous system spontaneously and in response to electrical or chemical stimulation (Ramwell & Shaw, 1963, 1966; Ramwell, Shaw & Jessup, 1966; Coceani & Wolfe, 1965; Feldberg & Myers, 1966) and (3) prostaglandins on injection have powerful central nervous actions (Horton, 1964; Horton & Main, 1965a&b, 1967b,c&d; Avanzino, Bradley & Wolstencroft, 1966). This paper describes experiments which extend these three lines of investigation.

Occurrence of prostaglandins in the central nervous system

Prostaglandins have now been identified in brain tissue from seven species. In every animal $F_{2\alpha}$ has been found, but the presence of $F_{1\alpha}$, E_2 and E_1 is more variable. It may well be that the concentrations of these prostaglandins in some species are too low to permit their detection in the amounts of tissue extracted. For example, there is strongly suggestive evidence for the presence of a prostaglandin E in mouse brain but more definite identification could not be made with the amounts of brain used (Holmes & Horton, 1968b). Different regions of the

central nervous system contain prostaglandin E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ and their concentrations do not vary greatly from one region to another (Holmes & Horton, 1967). Now each of those prostaglandins has its own independent biosynthetic and metabolic pathway (Bergström & Samuelsson, 1965); they are not known to be precursors or metabolites of each other. If the hypothesis that prostaglandins are central nervous transmitters is true, then it may be predicted that, in the dog, four types of "prostanergic" neurone, each releasing a different prostaglandin, are widely and fairly evenly distributed throughout the central nervous system.

If prostaglandins are chemical transmitters at central synapses, it should be possible to demonstrate their presence in the nerve ending fraction of brain homogenates. Such evidence has been obtained using rat cerebral cortex (Kataoka, Ramwell & Jessup, 1967). In our experiments on rabbit brain between 80 and 90% of the brain prostaglandins were recovered from the high-speed supernatant. The amounts in the particulate fraction were small and so far positive identification of prostaglandins in the nerve ending fraction of rabbit whole brain has not been possible (Hopkin, Horton & Whittaker, 1967), but our results are not incompatible with the occurrence of prostaglandins in synaptosomes.

Release of prostaglandins from the central nervous system

We have perfused the cerebral ventricular system of chloralosed dogs from the lateral ventricle of the cisterna magna. We have

identified four prostaglandins, E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the perfusates by their behaviour on solvent partition, chromatography and biological assay. This result suggests that the prostaglandin-like substances in perfusates of the cat described by Feldberg & Myers (1966) are likely to be prostaglandins.

Pharmacological Actions of the Prostaglandins on the Brain and Spinal Cord

Prostaglandin $F_{2\alpha}$ injected intravenously in young chicks causes contraction of extensor muscles which is abolished by denervation of the muscle. There is a simultaneous potentiation of the crossed extensor reflex, which outlasts the muscular contracture (Horton & Main, 1965a&b, 1967b,c&d). A similar response has been observed in the decerebrate cat with both prostaglandin $F_{2\alpha}$ and E_1 and this also occurs after spinal transection at the C2 level. On the basis of this and other evidence it is concluded that prostaglandin $F_{2\alpha}$ and prostaglandin E_1 have actions on spinal neurones in the chick and cat respectively.

Electrophysiological techniques have now been used to show that prostaglandins E_1, $F_{1\alpha}$ and $F_{2\alpha}$ have potent and long-lasting effects on monosynaptic pathways in the cat's spinal cord (Duda, Horton & McPherson, 1968). For example, prostaglandin E_1 in six cats caused marked reduction in size of evoked ventral root

potentials, which had a latent period corresponding to that of a monosynaptic pathway. The effects lasted over 3 hours. Such a prolonged duration of action is reminiscent of the effect of prostaglandin E on injection into the cerebral ventricles of cats. Such an injection causes catatonia and stupor which lasts for several hours.

In the unanaethetized chick prostaglandin E_1 injected intravenously causes sedation with loss of the righting reflex (Horton, 1964). This "sedative" action of prostaglandin E_1 has been investigated further in mice using tests known to be sensitive to substances of the sedative-tranquillizer group (Holmes & Horton, 1968b).

On subcutaneous or intravenous injection in mice prostaglandin E_1 (1mg/kg) caused diminished spontaneous activity lasting about 30 minutes, during which ptosis and the passing of several liquid faeces were observed. These effects were not seen with prostaglandin $F_{2\alpha}$ (1mg/kg).

In most of these tests on mice where prostaglandin E_1 was active, it was more potent than chlorpromazine. In many respects the effects of E_1 and reserpine were similar, although the latter showed little anticonvulsant activity. The effects of E_1 were quicker in onset but shorter lasting than those of reserpine.

Both prostaglandin E_1 (0.5 - 1 mg/kg) and prostaglandin $F_{2\alpha}$

(1mg/kg) increased hexobarbitone "sleeping" time in mice. At the 1mg/kg dose level prostaglandin E_1 antagonized convulsions due to leptazol (100mg/kg) and maximal electro-shock, only slightly reduced strychnine (1mg/kg) convulsions but did not affect picrotoxin (10mg/kg) convulsions. On the other hand, prostaglandin $F_{2\alpha}$ (1 and 2 mg/kg) potentiated the action of threshold convulsant doses of leptazol. Prostaglandin E_1 (1mg/kg) produced some motor inco-ordination as tested on the rotarod. It had some activity in antagonizing the writhing or stretching induced in mice by intra-peritoneal acetylcholine, though no analgesic activity when tested by pressure applied to the tail. Prostaglandin E_1 (1mg/kg) did not antagonize the circling movements or Straub tail phenomenon induced in mice by morphine.

The ptosis produced by prostaglandin E_1 was prevented by pre-treating the mice with imipramine (20mg/kg). In this respect the effect was similar to that of reserpine. However, iproniazid (150mg/kg) failed to prevent E_1 - induced ptosis.

These preliminary results in mice confirm and extend the observations made with chicks, namely that prostaglandins in the E series have a powerful "sedative-tranquillizer" action. The potentiation of leptazol convulsions by $F_{2\alpha}$ may be due to its known action on motor pathways in the spinal cord.

In view of the small doses required to produce these central

effects, it is of interest that in both the cat and the chick little specific activity can be detected in the brain or spinal cord following intravascular injection of tritiated prostaglandin E_1 in cats and chicks, (Holmes & Horton, 1968a). Even on injection into the cerebral ventricles little of the prostaglandin E_1 injected could be recovered from brain tissue removed at the time of maximal pharmacological response. Hansson & Samuelsson, (1965) using mice, had also reported that very little tritiated prostaglandin E_1 reached the brain of mice following subcutaneous injection.

Concluding Remarks

It is now well established that prostaglandins are normal constituents of brain and spinal cord and that they are released from these organs both spontaneously and in response to nerve stimulation. The occurrence of prostaglandins in the non-cholinergic nerve ending fraction of rat cortex is suggestive evidence that these substances may be central transmitters. If so, the regional distribution in dog brain shows that such prostanergic neurones must be of wide distribution and possible of four types. When it is considered that only traces of an injected dose reach the central nervous system, the central nervous actions of prostaglandins must be considered of quite considerable potency.

In general it seems that prostaglandin E_1 in three species has

a sedative-tranquillizer action provided that the brain is intact though stimulant actions on motor pathways are revealed in the cat and the chick when the brain has been destroyed. The action of $F_{2\alpha}$ appears to be chiefly a facilitatory one on motor pathways. The only hint of a sedative action is its potentiation of hexobarbitone sleeping time in mice. Thus $F_{2\alpha}$ potentiated the crossed extensor reflex in the chick (whether the brain is intact or not), it often potentiates monosynaptic reflexes in the cat, and it potentiates the convulsant action of leptazol.

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26

THE IDENTIFICATION OF FOUR PROSTAGLANDINS IN DOG BRAIN AND THEIR REGIONAL DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM

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SUMMARY

1. Experiments were carried out to determine whether the prostaglandins, which have been identified as natural constituents of the brain, are widely distributed or concentrated in special regions of the central nervous system.

2. Prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ have been identified in dog brain using solvent partition, column and thin layer chromatographic and bioassay techniques.

3. Each of these prostaglandins is distributed throughout all regions of the central nervous system investigated (cortex, hippocampus, caudate nucleus, hypothalamus, cerebellum, medulla and pons, cortical white matter and spinal cord).

4. This distribution suggests that the role of prostaglandins in the central nervous system is not confined to any specific region.

INTRODUCTION

Prostaglandins have been isolated from central nervous tissue of ox, cat, chicken and rabbit (Samuelsson, 1964; Coceani & Wolfe, 1965; Ambache, Brummer, Rose & Whiting, 1966; Horton & Main, 1967*a*). It is not known whether the prostaglandins are concentrated in certain regions of the brain suggesting a special role for a specific prostaglandin in the region where it is concentrated, or whether they are more widely distributed. Studies of the regional distribution in the central nervous system of other naturally occurring substances have helped to elucidate their possible physiological significance (Feldberg & Vogt, 1948; Amin, Crawford & Gaddum, 1954; Vogt, 1954; Adam, 1961).

The present work concerns the identification and distribution of four

prostaglandins in ethanol extracts of central nervous tissue from the dog. The significance of this distribution, which is rather uniform throughout the central nervous system, is discussed. A preliminary report of some of this work has already been made (Holmes & Horton, 1967).

METHODS

Removal of tissue samples. A series of nineteen dogs (seventeen greyhounds and two labradors) ranging in weight from 20 to 32 kg was used. The dogs were anaesthetized with pentobarbitone sodium (40 mg/kg) injected intraperitoneally. The trachea and femoral vein were cannulated and a catheter was passed up the femoral artery into the aorta. The dog was exsanguinated via the arterial cather. During exsanguination the skin and muscle were reflected from the upper part of the cranium and the skull was trephined bilaterally. The trephine holes were extended by nibbling away the adjoining skull, the dura mater was opened and when exsanguination was complete the brain was divided from the spinal cord and removed as quickly as possible. The required areas of central nervous tissue were dissected out, weighed, chopped and placed in four volumes (w/v) of ice-cold 96% aqueous ethanol as quickly as possible. The time from death until the tissue was placed in ethanol varied from 2 to 10 min; to ensure that a longer period did not elapse only four areas were taken from any one dog. When small quantities of tissue, such as the hypothalamus, were taken the tissue from two dogs was combined.

Extraction procedure. The dissected tissue was macerated for 30 min in 96% ethanol taking care to avoid heating above 45° C. The macerate was then allowed to stand for 2 hr at room temperature with occasional stirring. The pH was then adjusted to 3 with concentrated hydrochloric acid and the macerate was left to stand for a further 2 hr. It was then filtered under reduced pressure, the residue being resuspended in a further four volumes (w/v) of 96% ethanol and re-filtered. The combined ethanol filtrates were taken to dryness, at 45° C using a rotary evaporator, and the residue was subject to a solvent partition system similar to that described by Horton & Main (1967*a*) which is summarized in Fig. 1. An emulsified area often occurred at the interface in these partitions and this was always taken with the phase containing the prostaglandins until the last partition when the interface was cleared by centrifugation.

Column chromatography. Silicic acid (Bio-Rad Laboratories, minus 325 mesh) columns were prepared in the following manner: Silicic acid (10 g for 18 mm i.d. columns, 3 g for 10 mm i.d. columns) was activated by heating for 1 hr at 110° C. It was then suspended in light petroleum, placed in a glass column and washed with more light petroleum to remove impurities. The column was then washed with 30% ethyl acetate in benzene. If the extract to be chromatographed was from more than 10 g tissue, 18 mm i.d. columns were used, if from less than 10 g, 10 mm i.d. columns were used. The columns were eluted under reduced pressure at a rate of approximately 1 cm fall/min.

The residue from the 67% ethanol phase of the solvent partition system shown in Fig. 1 was taken up in 1 ml. ethyl acetate and 2 ml. benzene and loaded on to the column with a Pasteur pipette. This was followed by two 0·5 ml. ethyl acetate washes and finally by 2 ml. benzene. The column was run in 30% ethyl acetate in benzene which was used to rinse the flask containing the sample before being passed through the column. Unless this rather complex loading procedure was followed it was found that considerable biological activity was not transferred from the flask to the column. Ethyl acetate (30%) was run through the column until the elute was colourless. The column was then run in ascending concentrations of ethyl acetate in benzene and was finally eluted with 100% methanol. The eluate from a column was monitored for biological activity on the rat fundus strip and/or rabbit jejunum throughout.

Thin layer chromatography. Glass plates (20 × 5 cm) were covered with a 0·25 mm layer of Silica Gel G (Merck) containing 2% silver nitrate. The plates were allowed to dry and were activated by heating to 110° C for 30 min. The activated plates were stored in a desiccator over self-indicating silica gel. Pure prostaglandins and tissue extracts were dissolved in methanol and applied to the thin layer plates with an Agla micrometer syringe.

The thin layer plates were developed in Gréen & Samuelsson's (1964) AII system which separated prostaglandin E_1 from E_2 from E_3 and $F_{1\alpha}$ from $F_{2\alpha}$ from $F_{3\alpha}$ and were normally run to 12 cm. Marker plates loaded with pure prostaglandins were always run at the same time as preparative plates and were visualized by spraying with 10% phosphomolybdic acid in ethanol followed by heating to 110° C for 15 min. As stocks of pure prostaglandins

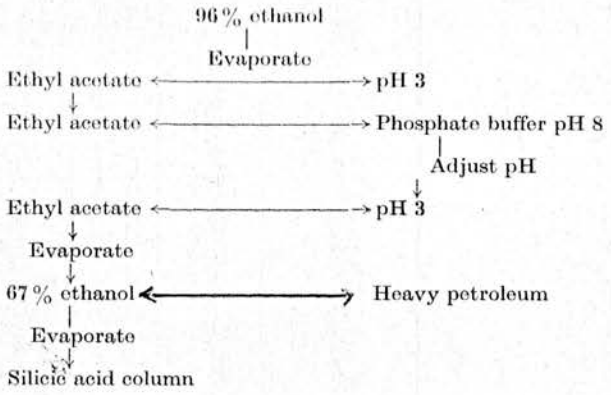

Fig. 1. Outline of extraction procedure.

were relatively small only marker plates loaded with prostaglandin E_1 or $F_{1\alpha}$ were normally run and the R_F values for prostaglandins E_2, E_3, $F_{2\alpha}$ and $F_{3\alpha}$ were determined by extrapolation from the literature (Gréen & Samuelsson, 1964). Preparative plates were divided into zones corresponding to the positions of prostaglandins E_1, E_2 and E_3 or $F_{1\alpha}$, $F_{2\alpha}$ and $F_{3\alpha}$. A blank zone near the solvent front, of similar size to the prostaglandin zones, was always taken so that correction could be made for any biological activity exhibited by the silica gel in subsequent bioassay. The zones were scraped off the thin layer plates and extracted by shaking with 5 ml. methanol, the mixture being centrifuged and the supernatant removed. The residue was resuspended in 2·5 ml. methanol and re-centrifuged. The combined methanol supernatants were evaporated to dryness and the residue taken up in 0·1 N-HCl. This took any silver present out of solution as insoluble silver chloride and the prostaglandins were extracted from the aqueous phase by a two stage solvent partition with an equal volume of ethyl acetate. The combined organic phases were evaporated to dryness and the residue was taken up in a small volume of either water or Tyrode solution and stored at −20° C until they were assayed.

Recovery experiments. Recovery experiments were carried out at the end of the study when a supply of tritiated prostaglandin E_1 became available. Tritiated prostaglandin E_1 (10 μg) was added to the ethanol macerate of cerebral cortical tissue and its recovery was determined at various stages of the extraction procedure using a Packard Tricarb liquid scintillation counter. At the end of silicic acid column chromatography a recovery of 40–45% was obtained from the appropriate eluate and most of the loss occurred in the pH 8 buffer—ethyl acetate solvent partition system. Similar recoveries were obtained in earlier studies using unlabelled prostaglandin E_1 when activity was determined by bioassay.

If 5 μg of tritiated prostaglandin E_1 was run in the AII solvent in thin layer chromatography a recovery of about 80% was obtained from the appropriate zone after removal of silver. However, if 0·5 μg was similarly treated the recovery fell to about 65%.

Biological assays, smooth muscle preparations in vitro. Segments of various organs were suspended in a 4 or 10 ml. organ-bath and longitudinal contractions were recorded isotonically with a frontal-writing lever on a smoked drum. A dose cycle of 3–5 min with a contact time of 45 sec–2·5 min was employed for all preparations. All estimates of content were made by bracketing with pure prostaglandins.

Rat fundus. Longitudinal strips from the fundus of rats weighing 150–300 g were prepared as described by Vane (1957) and suspended in Tyrode solution at 37° C vigorously gassed with oxygen.

Rabbit jejunum. Proximal jejunum from rabbits weighing 0·75–3 kg was suspended in Tyrode solution at 37° C gassed with air.

Guinea-pig ileum. Terminal ileum from guinea-pigs weighing 200–400 g was suspended in Tyrode solution at 37° C gassed with air.

Rat uterus. A uterine horn from dioestrous rats weighing 150–300 g was opened longitudinally along the line of the mesenteric blood vessels and suspended in de Jalon solution at 22° C gassed with air.

RESULTS

Identification of prostaglandins in whole dog brain. Whole dog brain (95 g) was extracted and partitioned as described under Methods. The extract was then chromatographed on a silicic acid column, the eluates being assayed for biological activity on the rat fundus and rabbit jejunum (Fig. 2). Four peaks of activity were eluted. The material in the first peak eluted with 30% ethyl acetate in benzene, and in the last peak, eluted with 100% methanol, was discarded. The second and third peaks of biological activity corresponded to the elution times of prostaglandins E and prostaglandins F respectively.

The material in the pooled fractions of the second peak were chromatographed on a thin layer of $AgNO_3$ impregnated silica gel and developed in the A II solvent system simultaneously with a prostaglandin E_1 marker plate. When the preparative plate was zoned and eluted (Fig. 3) biological activity was found in the zones corresponding to prostaglandins E_1 and E_2. The material in the E_1 zone behaved in a similar manner to pure prostaglandin E_1 when assayed on the rat fundus and guinea-pig ileum; 480 ng E_1 on the rat fundus and 570 ng E_1 on the guinea-pig ileum. The zone corresponding to prostaglandin E_2 gave a content of 950 ng E_2 when assayed on the rat fundus and 890 ng E_2 on the guinea-pig ileum.

When the fractions corresponding to the third peak of activity (80% ethyl acetate) from the silicic acid column were similarly treated it was found that the zones on the preparative plate corresponding to the R_F values of prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ both contained biological activity.

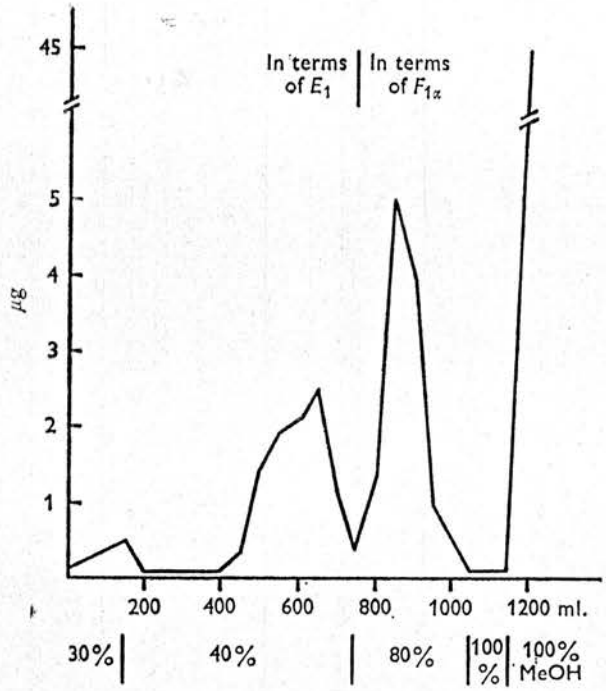

Fig. 2. Silicic acid column chromatography of the extract of 95 g dog brain. 10 g silicic acid column. Ordinate: biological activity in μg in terms of prostaglandin E_1 on the rat fundus strip and $F_{1\alpha}$ on the rabbit jejunum. Abscissa: upperline, ml. eluate; lower line, composition of eluant (% ethyl acetate in benzene).

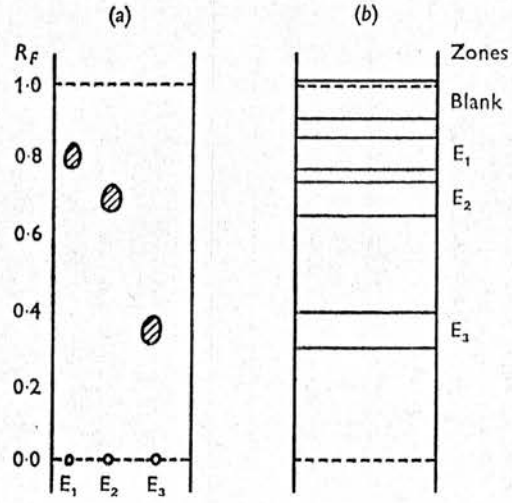

Fig. 3. Thin layer chromatography of dog brain extract (second peak, Fig. 2) in the AII solvent system. (*a*) Marker plate sprayed with 10% phosphomolybdic acid; (*b*) preparative plate divided into zones.

Estimates of the $F_{1\alpha}$ content were 500 ng (rabbit jejunum) and 590 ng (rat uterus) and of the $F_{2\alpha}$ content 390 ng (rabbit jejunum) and 480 ng (rat uterus).

No activity was detectable in the prostaglandin E_3 or $F_{3\alpha}$ zones when assayed on any of the biological preparations employed nor was there significant biological activity in other zones.

Although this experiment was carried out primarily to obtain a qualitative identification of the prostaglandins, it is apparent that the four prostaglandins are found in similar concentrations in dog brain (E_1, 35 ng/g; E_2, 62 ng/g; $F_{1\alpha}$, 63 ng/g; $F_{2\alpha}$, 56 ng/g; all uncorrected for recoveries).

Distribution of four prostaglandins in dog central nervous system. Using similar techniques to those employed for the identification of prostaglandins in whole dog brain it was found that prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ all occurred in each area of the central nervous system examined. All four prostaglandins occurred in similar concentrations throughout the central nervous system. In each experiment various areas were compared with cerebral cortical grey matter from the same dog or pair of dogs and the results are expressed in terms of total prostaglandin E's and total F's eluted from silicic acid columns (Table 1). The presence of each of the four prostaglandins found in whole dog brain was confirmed in each area studied by further purification on thin layer chromatography in the AII system and bioassay.

In order to check that the anaesthetic (pentobarbitone sodium) employed did not affect the prostaglandin levels within the brain, one dog was anaesthetized with a completely different kind of anaesthetic, tribromoethanol (Avertin-Winthrop Laboratories). It was found that its cortex contained 184 ng/g prostaglandin E_1 equivalent of prostaglandin E and 204 ng/g $F_{1\alpha}$ equivalent of prostaglandins F; the cortex of a dog anaesthetized with pentobarbitone sodium at the same time contained 185 ng/g prostaglandin E_1 equivalent and 164 ng/g $F_{1\alpha}$ equivalent respectively.

DISCUSSION

The identification of a specific prostaglandin in extracts of dog central nervous tissue was based upon its behaving in a similar manner to the pure prostaglandin in the solvent partition system, silicic acid column and thin layer chromatography and parallel biological assay.

In the solvent partition system described in the methods section the 67 % ethanol would contain the polar acidic lipids including prostaglandins from the original extract.

When the 67 % ethanol residue was subjected to silicic acid column

TABLE 1. Distribution of prostoglandins in the central nervous system of the dog. Total prostaglandins E expressed in terms of E_1 and total prostaglandins F in terms of $F_{1\alpha}$. All values are uncorrected for recovery and are expressed in terms of ng/g tissue

Area...	Cortex		Hippo-campus		Caudate nucleus		Hypo-thalamus		Cerebellum		Medulla +pons		Cortical white matter		Spinal cord	
Expt.	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F
1	102	94	—	—	—	—	—	—	75	180	—	—	—	—	—	—
2	116	190	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	96	58	—	—	—	—	—	—	—	—	—	—	—	—	78	416
4	22	94	—	—	—	—	24	240	—	—	—	—	—	—	—	—
5	22·5	100	26·5	192	—	—	—	—	—	—	—	—	—	—	—	—
6	20·5	124	—	—	—	—	—	—	—	—	21	132	—	—	—	—
7	63	100	—	—	—	—	47	206	—	—	—	—	—	—	—	—
8	86	126	140	180	—	—	—	—	—	—	—	—	—	—	—	—
9	68	100	—	—	—	—	—	—	—	—	2·5	80	—	—	—	—
10	85	238	—	—	—	—	—	—	—	—	—	—	—	—	65	100
11	116	389	19	214	34	143	—	—	—	—	—	—	—	—	—	—
12	132	132	—	—	109	154	109	154	—	—	—	—	—	—	168	56
13	185	164	—	—	—	—	—	—	138	107	39	178	27	106	—	—
14	337	151	—	—	—	—	—	—	—	—	46	154	85	187	—	—
Mean	104	147	62	195	72	149	60	200	107	144	27	136	56	146	104	191

chromatography it was found that the prostaglandins E were separated from the prostaglandins F and both were separated from other biologically active material in the extract (Fig. 2). When the column was eluted with methanol considerable non-prostaglandin biological activity came off the column. No attempt was made to identify further this activity but Horton & Main (1967*a*) reported similar activity when they passed their chick and cat central nervous tissue extracts through silicic acid columns; in the case of the dog the activity was far greater when assayed on the rabbit jejunum rather than the rat fundus.

When the zones from the thin layer plates run in the AII solvent system (Gréén & Samuelsson, 1964) were assayed in parallel on two different tissues against the appropriate pure prostaglandin and a similar potency was obtained on both tissues; it would seem reasonable to conclude that a specific prostaglandin was present in the original extract.

Although, in view of the small amounts present, no chemical characterization of prostaglandins was attempted, sufficient evidence is presented to conclude that the substances identified are prostaglandins.

The identification of prostaglandins by a combination of chromatography and bioassay has been discussed fully by Horton & Main (1967*a*).

The results of the recovery experiments carried out with tritiated prostaglandin E_1 indicate that better recoveries might be obtained if a buffer somewhat more alkaline than pH 8 was employed in the solvent partition although care must be taken not to promote the formation of prostaglandins B from the prostaglandins E.

The distribution of the four prostaglandins found in whole dog brain was found to be remarkably constant, and the only areas to show any real difference from the cortex were the medulla and pons which exhibited consistently low values for their total prostaglandins E. Also the cortical white matter possible had a low content of prostaglandins E and this fits in with the work of Coceani & Wolfe (1966) who found that white matter contained approximately one fifth the concentration of prostaglandins found in the whole cerebrum from ox brain. The variation in content of both prostaglandins E and F in the areas studied, apart from the two cases mentioned above, were almost certainly due to variation between dogs rather than areas. This is indicated when the ranges of the various estimations are studied.

Both Samuelsson (1964) and Horton & Main (1967*a*) used a further step in their identification procedure. After silicic acid column chromatography they subjected both the prostaglandins E and F peaks to thin layer chromatography in the AI system (Gréén & Samuelsson, 1964) before proceeding to the AII system. Whilst this does provide a further piece of evidence for the identificaton of a specific prostaglandin in an extract it was

found that silicic acid column chromatography efficiently separated the prostaglandins E from the F's and no improvement in performance in the AII system was observed if thin layer chromatography in the AI system was carried out first. On the basis of this, chromatography in the AI system was omitted.

Prostaglandins have been shown to be released into the perfused cerebral ventricular system of the dog (S. W. Holmes & E. W. Horton, unpublished). There is also evidence for the release of prostaglandin-like substances from the inferior and anterior horns of the lateral ventricles and the third ventricle in cats (Feldberg & Myers, 1966). Superfusates of cat somatosensory and cerebellar cortex have also been shown to contain prostaglandins (Ramwell & Shaw, 1966; Coceani & Wolfe, 1965). Their release from the frog perfused spinal cord has also been demonstrated (Ramwell, Shaw & Jessup, 1966). The level of release of prostaglandins from the cat somatosensory cortex and frog spinal cord has been shown to be affected by afferent stimuli.

When one considers the occurrence, release and profound pharmacological effects of prostaglandins in the central nervous system it would seem reasonable to postulate a transmitter function for the compounds at central synapses. Choline acetylase, noradrenaline, histamine, 5-hydroxytryptamine and dopamine among other substances concerned with chemical transmission in the nervous system are concentrated in specific regions (Feldberg & Vogt, 1948; Amin *et al.* 1954; Vogt, 1954; Bertler & Rosengren, 1959; Adam, 1961). Such concentration may be considered a criterion for a substance's possible role as a transmitter at central nervous synapses. The even distribution of all four prostaglandins found in whole dog brain then makes a transmitter function improbable. Furthermore, although these four prostaglandins are very closely related chemically, it has been shown that each has its own biosynthetic pathway (Samuelsson, 1967). This would necessitate postulating the presence of four different types of neurone, each releasing a different prostaglandin, distributed throughout the central nervous system.

It has been demonstrated that prostaglandins are released in association with known chemical transmitters in response to nerve stimulation at a variety of synapses outside the central nervous system. These sites include the rat diaphragm (Ramwell, Shaw & Kucharski, 1965) rat adipose tissue (Shaw, 1966), cat adrenal gland (Ramwell, Shaw, Douglas & Poisner, 1966) and dog spleen (Davies, Horton & Withrington, 1967). At these sites prostaglandins do not fulfil the criteria of a transmitter, although they may well be involved in the transmission mechanism as is suggested by their known pharmacological actions on the central nervous

system (Horton, 1964; Horton & Main, 1965; 1967*b*; Avanzino, Bradley & Wolstencroft, 1966).

It is concluded that, on the basis of the evidence at present available, the four prostaglandins identified in dog central nervous tissue probably fulfil some function other than that of transmitters although they may be involved in neural mechanisms. Their release in response to nerve stimulation as sites outside the central nervous system suggest that their function may be widespread, in relation to neural mechanisms, and not confined to the central nervous system.

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Prostaglandin Content of Particulate and Supernatant Fractions of Rabbit Brain Homogenates

WHOLE rabbit brains were homogenized in 0·32 molar sucrose solution, and subcellular fractions were prepared by centrifugation by the method of Gray and Whittaker[1]. Prostaglandins were extracted from the homogenates and from the various fractions by solvent partition and further purified by chromatography[2].

Extracts of rabbit brain homogenates showed prostaglandin-like activity when assayed biologically on the rat isolated fundal strip preparation. On silicic acid column chromatography, two main peaks of biological activity were detected, corresponding to the elution rate of pure prostaglandins E and F respectively. On thin-layer chromatography using plates of silica gel impregnated with silver nitrate, zones corresponding to prostaglandins E_2 and $F_{2\alpha}$ contained more than 90 per cent of the biological activity, confirming the report of Ambache, Brummer, Rose and Whiting[3]. The homogenates were estimated to contain 500 ng prostaglandin $F_{2\alpha}$ and 75 ng prostaglandin E_2/g of brain tissue. When the brain was macerated or homogenized in 96 per cent aqueous ethanol instead of sucrose, similar amounts of prostaglandins were recovered. There was no evidence in our experimental conditions for inactivation or new synthesis in the sucrose homogenates as reported by other workers[4].

In each of four experiments the final supernatant after centrifuging at 100,000*g* (4° C for 2 h) contained between 80 and 90 per cent of the total prostaglandin activity of the homogenates. Substances with chromatographic and biological properties identical to prostaglandins E_2 and $F_{2\alpha}$ were identified in the supernatant fraction, which also contained 80 per cent of the total potassium present in the whole homogenate.

The combined particulate fractions also contained the two prostaglandins. Between 5 and 10 per cent of the biological activity of the whole homogenate was in the fraction *P*2 which, according to electron microscopic evidence[1], would contain mitochondria and nerve endings. Because the amounts of prostaglandin in the *P*2 fraction were so small, further fractionation by sucrose gradient technique has not so far been feasible. The remainder of the prostaglandin-like activity of the particulate fraction was distributed between *P*1 (nuclei and cell debris), *P*3 (microsomes) and *P*4 (ribosomes and post-microsomal fractions).

The combined particulate fraction was treated in three ways in an attempt to release any bound prostaglandin. One sample was frozen and thawed ten times, another was incubated at *p*H 3 and 60° C for 1 h, and a third sample was suspended in water before extraction with ethyl acetate at *p*H 3. None yielded any additional prostaglandin.

As a control, the succinate dehydrogenase contents of the particulate and supernatant fractions were compared with those of the original homogenate. All the enzyme activity of the homogenate was recovered from the particulate fraction.

It is unlikely that the prostaglandin content of the particulate fractions was the result of contamination by supernatant, because washing with sucrose and recentrifugation did not reduce the amount recovered. But the possibility that the prostaglandin recovered from the supernatant originated from a very labile store in one of the particulate fractions cannot be excluded.

Our results differ from those of Kataoka, Ramwell and Jessup[5], who located 30–40 per cent of the prostaglandins in rat cerebral cortex in nerve ending fractions. The results from both laboratories point to a wide distribution of prostaglandins throughout subcellular compartments.

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Prostaglandins are released when the sympathetic nerves supplying the rat epididymal fat pad *in vitro* are stimulated (Shaw, 1966). It is not yet known whether this is a general phenomenon associated with adrenergic nerve stimulation but the experiments described in this paper show that prostaglandin E_2 is released when the splenic nerve is stimulated. A preliminary account of this work has already been published (Davies, Horton & Withrington, 1967).

In the investigation reported here we collected venous blood samples from the perfused dog's spleen in order to detect any prostaglandin release when the splenic nerve was stimulated. A large increase in prostaglandin E_2 output was found.

METHODS

Blood-perfused dog spleen. An isolated spleen was perfused with blood from the femoral artery of a second (donor) dog using the technique described in the following paper (Davies & Withrington, 1968). The splenic venous blood was returned to the femoral vein of the donor except during the collection of a sample, when it was diverted out of a side arm. During the collection of a sample, blood from a reservoir was pumped into the donor. Electrodes were placed on the splenic nerve and the spleen was bathed in liquid paraffin at 37° C. The nerve was stimulated with 50 V pulses of 0.5 msec duration at 10/sec.

Extraction procedures. The procedures are outlined in Fig. 1. Blood was either collected in a measuring cylinder and added to 4 vol. ice cold 96% aqueous ethanol, or added to the ethanol directly from the collecting cannula. The mixture was shaken and filtered. The residue was re-extracted with 4 vol. 96% aqueous ethanol. The combined aqueous ethanol filtrates were evaporated to dryness. The dried residue was dissolved in a mixture of equal volumes of ethyl acetate and water at pH 3. After separation, the acid acqueous phase was re-extracted with 1 vol. ethyl acetate. The combined ethyl acetate phases were concentrated to smaller volume and extracted twice with an equal volume of phosphate buffer solution pH 8. The aqueous phases were pooled, acidified to pH 3 by addition of HCl, and re-extracted twice with an equal volume of ethyl acetate. The ethyl acetate phases were pooled and evaporated to dryness.

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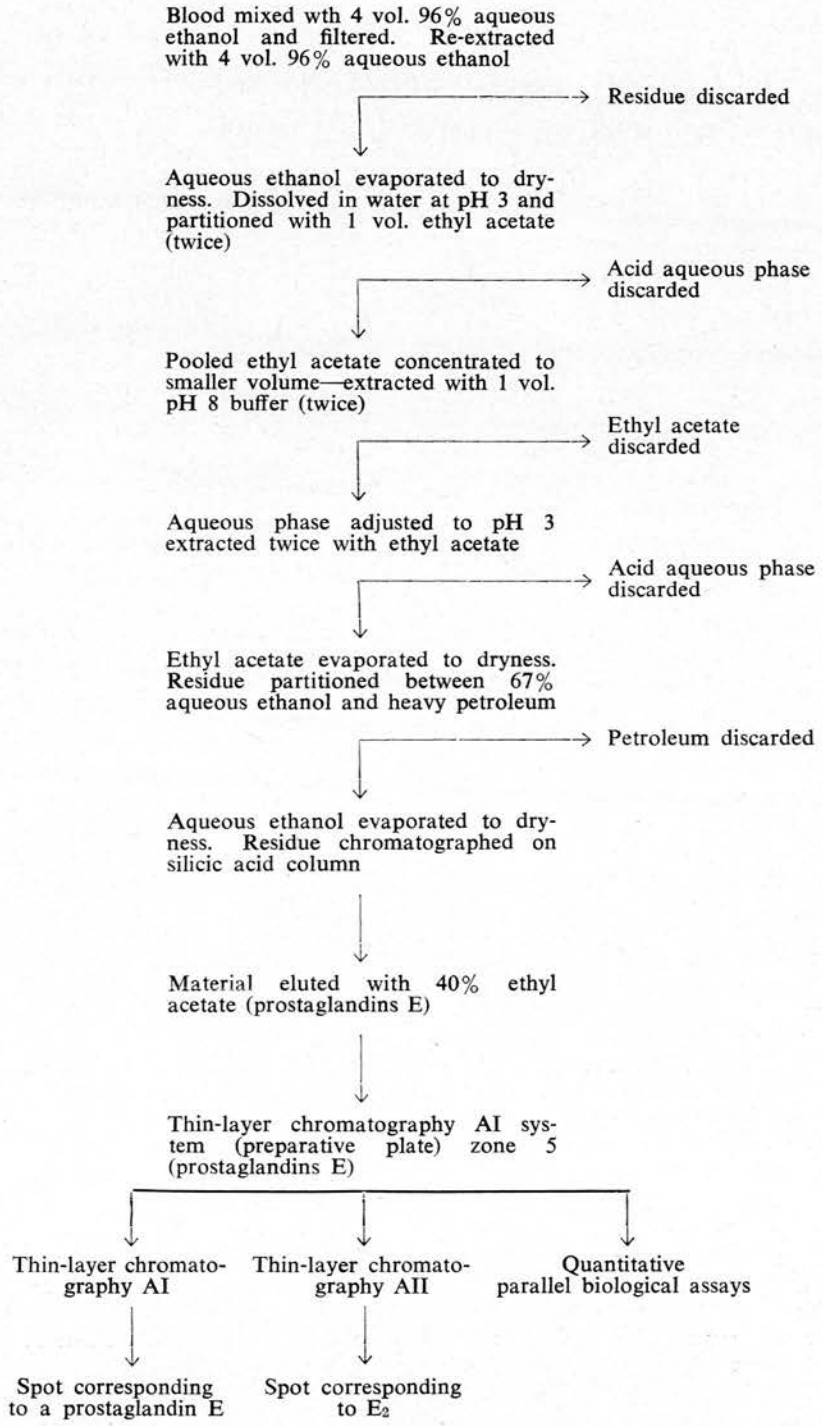

Fig. 1. Extraction and identification of prostaglandins.

The residue was partitioned between equal volumes of 67% aqueous ethanol and heavy petroleum (b.p. 60°–80° C). The aqueous ethanol was evaporated to dryness and chromatographed on silicic acid columns (1–3 g) using the procedure described by Horton & Main (1967). Thin-layer plates of dimensions 200 × 50 mm or 200 × 200 mm coated with Silica Gel G (E. Merck) 0.25 mm thick were prepared and used as previously described in detail (Horton & Main, 1967). The AI and AII solvent systems were described by Gréen & Samuelsson (1964).

Biological assays. The rat fundus was used routinely for the estimation of biological activity in the various fractions obtained during the extraction procedure. With all smooth muscle preparations contractions were recorded on a smoked drum using a frontal writing lever.

Rat fundus. A fundal strip was prepared and suspended in a 10 ml. bath containing oxygenated Tyrode solution. Doses were in contact with the tissue for 1.5–2 min and a cycle of 6–8 min was used.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 1–3 kg, was suspended in a 4 ml. bath containing Tyrode solution at 37° C and gassed with air. A dose cycle of 4–5 min with 45–90 sec contact was used.

Guinea-pig ileum. Terminal ileum from guinea-pigs weighing 150–600 g was suspended in a 4 ml. bath containing Tyrode solution at 37° 7 and gassed with air. A dose cycle of 5 min with 1 min contact was used.

Cat tracheal chain. A chain of three tracheal segments was suspended in Krebs-Henseleit solution at 37° C and gassed with 5% CO_2–95% O_2. Contractions were elicited by adding acetylcholine to the bath and the assay was performed by comparing the inhibitory action of the unknown extract with that of prostaglandin E_2 (Horton & Main, 1967).

RESULTS

Occurrence of prostaglandin-like substances in splenic venous blood

In a preliminary experiment samples of plasma were acidified with HCl and partitioned with an equal volume of ethyl acetate. After two such extractions, the combined ethyl acetate phases were evaporated to dryness. The residue was dissolved in water and assayed on the rat fundus.

The sample collected during and after electrical stimulation of the splenic nerves (300 stimuli at 10/sec) contained the equivalent of 35 ng prostaglandin E_1/ml. plasma, whereas the control samples did not contain any detectable prostaglandin. After phenoxybenzamine, administered in a dose (5 mg) sufficient to block the contraction of the spleen to nerve stimulation, the output of prostaglandin-like material in response to nerve stimulation was abolished, whereas the output of noradrenaline was increased (Table 1).

TABLE 1

PLASMA CONCENTRATION OF PROSTAGLANDIN-LIKE SUBSTANCES IN SPLENIC VENOUS BLOOD OF A DOG EXPRESSED AS PROSTAGLANDIN E_1 EQUIVALENT

	Prostaglandin E_1 (ng/ml.)	Noradrenaline (ng/stimulus)
Before phenoxybenzamine		
Venous control	<2	
Nerve stimulation (300 at 10/sec)	35	1·02
After phenoxybenzamine 5 mg		
Venous control	<6	
Nerve stimulation (300 at 10/sec)	<6	5·87

A similar increase in prostaglandin-like activity was found in four out of five dogs investigated. In a sixth dog sufficient prostaglandin E_2 for identification was extracted from the blood collected during stimulation.

Identification of prostaglandin E_2

Splenic venous blood (46 ml.) collected during and after nerve stimulation was extracted by the solvent partition procedure outlined in Fig. 1. The material was chromatographed on a silicic acid column and the eluates were assayed for biological activity on the isolated rat fundus. Several peaks of activity were found (Fig. 2), the one eluted with 40% ethyl acetate corresponding to the prostaglandins E as found in other experiments (Horton & Main, 1967 ; Holmes & Horton, 1967). The material from the " prostaglandin E " peak was purified further by thin-layer chromatography on silica gel plates using the AI solvent system of Greén & Samuelsson (1964). About one-third of the biological activity of this peak was found in the zone corresponding to the R_F value of prostaglandin E_1. The remaining two-thirds had a higher R_F value (see below).

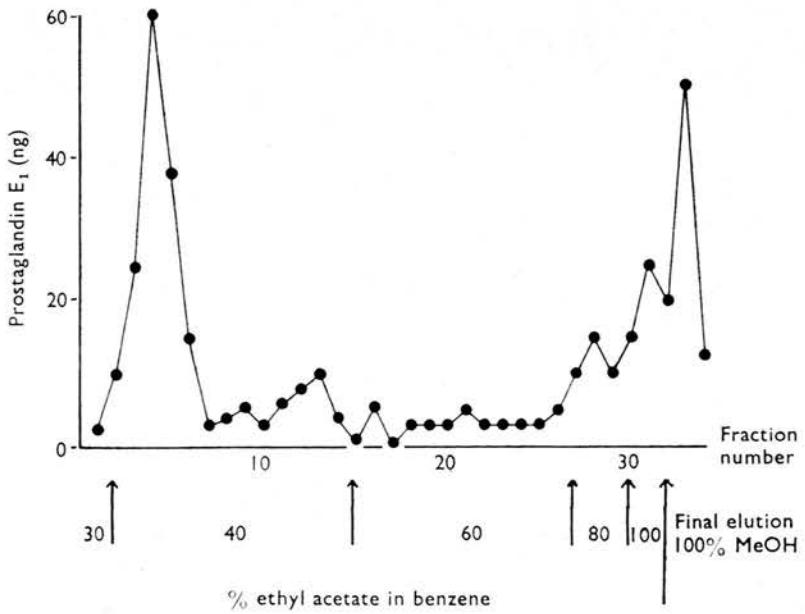

Fig. 2. Silicic acid chromatography (1 g column) of splenic blood extracts. Elution at 1 ml./min ; 20 ml. fractions collected. Ordinate: biological activity assayed on the rat fundus in terms of ng of prostaglandin E_1. Abscissa: upper line, fraction number ; lower line, % of ethyl acetate in benzene used for elution.

These results were confirmed with a larger sample (252 ml.) of blood collected from another dog during 60 sec of splenic nerve stimulation. In this experiment an activity equivalent to 20 μg of prostaglandin E_1 was eluted with 40% ethyl acetate in benzene. The material from this peak was run on a preparative thin-layer plate in the AI solvent system, the zone corresponding to the prostaglandins E was eluted and divided in three aliquots. One was re-chromatographed in the AI system. On spraying the plate with phosphomolybdic acid only one spot was seen corresponding to the R_F value (0.54) of the prostaglandins E. The second aliquot was chromatographed on a silica gel plate impregnated with $AgNO_3$ in the AII solvent system. On spraying with phosphomolybdic

acid only one spot appeared, corresponding to the position of prostaglandin E_2 (R_F 0.53). On the marker plate prostaglandins E_2 and E_1 had mean R_F values of 0.52 and 0.74 respectively.

The third aliquot from the AI preparative plate was used for parallel biological assays using prostaglandin E_2 as the standard for comparison (Table 2). Like prostaglandin E_2 the prostaglandin extracted from splenic blood contracted the rat fundus, guinea-pig ileum and rabbit jejunum but inhibited acetylcholine contractions of the cat trachea. The assay results on the four tissues also agreed well quantitatively.

TABLE 2

PARALLEL BIOLOGICAL ASSAYS OF A FINAL EXTRACT OF SPLENIC VENOUS BLOOD IN TERMS OF PROSTAGLANDIN E_2

Rat fundus	0·6 (0·4–0·8)
Guinea-pig ileum	0·6 (0·4–0·8)
Rabbit jejunum	0·7 (0·5–1·0)
Cat trachea	0·6 (0·2–1·0)

Occurrence of other lipids in extracts of splenic venous blood

In addition to prostaglandin E_2 several other biologically active lipids were found in extracts of splenic venous blood collected during and after splenic nerve stimulation. On thin-layer chromatography in the AI solvent system of the material in the " prostaglandin E " peak from the silicic acid column (Fig. 2), four substances with an R_F value greater than the prostaglandins E were detected in addition to the prostaglandin E_2. A mixture containing these four substances was biologically active on the rat fundus, but they have not been studied individually.

When the material in fractions 27–33 from the silicic acid column (Fig. 2) was run on thin-layer plates, at least two spots were present in each fraction as visualized by spraying with phosphomolybdic acid. We have no evidence as to which of these contained the biologically active principle.

In one experiment a small amount of prostaglandin F was also detected. The evidence for this was based on its chromatographic behaviour on column and thin-layer chromatography and on parallel biological assays.

Free fatty acid levels

In several experiments unesterified fatty acid levels were estimated by titrating an aliquot of the material before partitioning between petroleum and 67% ethanol. The residue was dissolved in ethanol and titrated with N/100 alcoholic KOH using thymol blue as the indicator. An experiment (Fig. 3) illustrates the increase in prostaglandin E_2 and a slight decrease in free fatty acid levels in the sample collected during and after nerve stimulation. This confirms that the nerves to adipose tissue adjacent to the spleen were not being stimulated.

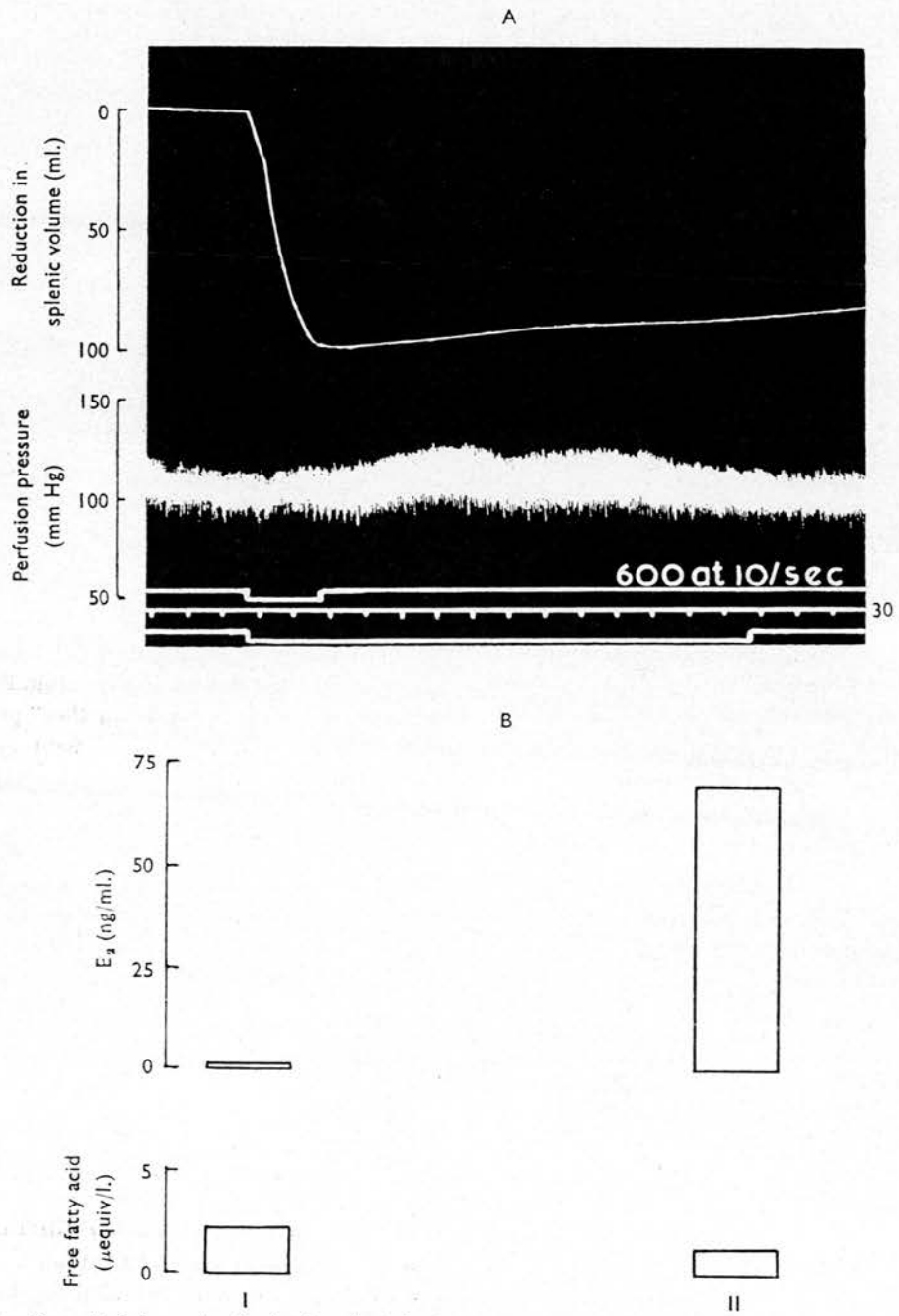

Fig. 3. Dog, 11.0 kg. A: Reduction in splenic volume in response to a train of 600 stimuli at 10/sec applied to the splenic nerve. A pooled blood sample was collected for the 7 min period during and after the stimulus, as indicated by the event marker. B: Assay of prostaglandin E_2 and free fatty acids in a control blood sample I taken 3 min before nerve stimulation and the blood sample II.

Identification of a prostaglandin E in extracts of dog spleen

Five dog spleens weighing a total of approximately 400 g were macerated in ethanol and extracted by the procedure outlined in Fig. 1. On the basis of column and thin-layer chromatography it was concluded that dog spleen contains a prostaglandin E, but there was insufficient material for identification by chromatography in the AII solvent system. On the basis of extracting another spleen weighing 24 g the concentration seemed to be of the order of 2 ng prostaglandin E_2/g tissue.

DISCUSSION

Identification of prostaglandin E_2

As discussed at length by Horton & Main (1967), the identification of prostaglandins on the basis of chromatographic behaviour and biological activity is not conclusive but the evidence is strongly suggestive. From the experiments described in this paper it can be stated that the behaviour of the substance isolated from splenic venous blood collected during and after nerve stimulation was identical to prostaglandin E_2 on three chromatographic systems and could not be distinguished from prostaglandin E_2 by quantitative parallel biological assay on four tissues, three of which contracted and one of which relaxed in response to prostaglandin E_2. It is certain that this activity was not caused by a prostaglandin F nor by prostaglandins E_1 or E_3. Nor from the data published by others was it likely to have been prostaglandin A_1, B_1, A_2 or B_2 or one of their 19-hydroxy-derivatives (Hamberg & Samuelsson, 1966).

Presence of other prostaglandin-like substances

Although the major biologically active constituent of the extracts was prostaglandin E_2, the presence of other prostaglandins cannot be excluded. There were certainly other substances with partition and chromatographic behaviour similar to the prostaglandins, but the nature of these is unknown except in one experiment in which a prostaglandin F was tentatively identified. It seems likely that the stimulus which releases prostaglandin E_2 causes a simultaneous release of several prostaglandin-like substances. This fact should be taken into account when considering the physiological implications of these observations.

Origin of the prostaglandins

The absence of any increase in free fatty acid levels when splenic nerves were stimulated excludes the possibility that the prostaglandins arise from adipose tissue adjacent to the spleen. This is not to say that fatty acids other than prostaglandins are not released from the spleen itself, but the amounts concerned are likely to be minute compared with the large output which occurs when fat is mobilized.

The absence of prostaglandins in samples collected after administration of phenoxybenzamine suggests that the prostaglandins are released in response to the action of noradrenaline at the post-synaptic site but an action of phenoxybenzamine in blocking prostaglandin release from a pre-synaptic site, though less likely, cannot be excluded.

Physiological significance of prostaglandin release

Prostaglandins are kidely distributed in animal tissues (Bergström & Samuelsson, 1965) and are known to be released in response to a variety of stimuli. For example they are released from the central nervous system on sensory nerve stimulation (Ramwell & Shaw, 1966), from the rat isolated diaphragm on stimulation of the phrenic nerve (Ramwell, Shaw & Kucharski, 1965), from the adrenal glands in response to acetylcholine (Ramwell, Shaw, Douglas & Poisner, 1965) and from adipose tissue in response to adrenergic nerve stimulation (Shaw, 1966). The experiments in this paper provide a second instance of prostaglandin release on stimulation of adrenergic nerves, although there is some evidence that the release from the diaphragm is caused by stimulation of adrenergic fibres in the phrenic nerve (Ramwell *et al.*, 1965). Our experiments provide the first demonstration that prostaglandins are released directly into the blood from an organ. It is significant that the output following nerve stimulation for 1 min was greatly in excess of the total amount of prostaglandin which could be extracted from a spleen. This suggests a rapid synthesis and agrees with the findings on cat adrenal glands (Ramwell *et al.*, 1965).

In view of the known inhibitory actions of prostaglandins E on the adenyl cyclase system which, in many instances, is thought to mediate the actions of adrenaline and noradrenaline, the possibility of a local feedback mechanism has been envisaged by various workers (see Bergström & Samuelsson, 1967). Prostaglandin E_1 antagonizes some of the vascular effects of the catecholamines (Holmes, Horton & Main, 1963), but the results in the following paper indicate that such an antagonism does not occur in the dog spleen (Davies & Withrington, 1968). Further information is needed about the mechanism of prostaglandin release and about release from other organs on sympathetic nerve stimulation, before any conclusions can be drawn as to how these findings fit into the picture of events at adrenergic synapses.

SUMMARY

1. In five dogs prostaglandin-like activity was detected in splenic venous blood samples collected during and after nerve stimulation.

2. Prostaglandin E_2 was identified in extracts of the splenic blood by its behaviour on solvent partition, chromatography in three systems, and by its biological activity on four isolated tissues.

3. Several other lipids which stimulate smooth muscle were also detected but these have not been identified.

4. Prostaglandin output was blocked by the administration of phenoxybenzamine (5 mg), whereas noradrenaline output was increased.

5. Splenic nerve stimulation did not increase free fatty acid levels in splenic venous blood, which excludes the possibility that the prostaglandins arise from adipose tissue adjacent to the spleen.

6. A prostaglandin E has been extracted from splenic tissue but the amounts present were too small to permit complete identification.

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blood to give a final concentration of 1 μg/ml. The blood was immediately mixed with 4 vol 96% aqueous ethanol and filtered. The filtrate plus washings were evaporated to dryness; the residue was dissolved in water and assayed on the rat isolated fundus.

Ethyl acetate extraction of prostaglandin from dog's brain

Recovery experiments were performed as follows : Prostaglandins E_1 was added to fresh dog's blood at 37° and incubated for 5 min. The blood was diluted with an equal volume of water and, by dropwide addition of hydrochloric acid, its p H was adjusted to 3.0 using a pH meter. The acidified diluted blood was then partitioned twice with an equal volume of ethyl acetate. The ethyl acetate was evaporated to dryness and the residue dissolved in aqueous solution for biological assay.

Incubation of prostaglandin E_1 with blood

Dog's blood and a solution of prostaglandin E_1 in 0.9% sodium chloride solution were warmed to 37° and mixed to make the appropriate concentration of prostaglandin E_1 in blood. The mixtures and control samples of blood (to which saline but no prostaglandin had been added) were incubated in a shaking water bath for periods up to 3 hr. The samples were then extracted with ethyl acetate as described above.

Recovery of tritium-labelled prostaglandin E_1

^3H-prostaglandin E_1 was added to dog's blood and extracted with ethyl acetate as described above. Specific activity was measured using a Packard Tricarb Scintillation Counter.

Electrophoresis of prostaglandin E_1 and plasma containing prostaglandin E_1

An aqueous solution of prostaglandin E_1 or dog's plasma to which prostaglandin E_1 had been added, was applied to strips of cellulose acetate. Electrophoresis was performed in a Shandon paper electrophoresis apparatus using a constant current of 0.6mA/cm. Plasma proteins were visualised by staining of the strips with 0.2% naphthalene black 10B in methanol/acetic acid (9/1). Prostaglandin was detected by eluting strips of the paper and assaying the eluated biologically on the rat fundus strip.

Results

Ethanol extraction of dog's blood containing prostaglandin E_1 or E_2.

Between 5 and 10% of the added prostaglandin E_1 or E_2 (1 µg/ml was recovered from the filtrate after ethanol extraction of dog's blood. The control blood samples to which no prostaglandin had been added contained no detectable biological activity when assayed on the rat fundus.

Ethyl acetate extraction of acidified dog's blood containing prostaglandin E_1

When the concentration of added prostaglandin E_1 was 0.1 - 1.0 µg/ml : recoveries were 55 ± 13% (10 estimations). At concentrations of 20 and 50 ng prostaglandin E_1/ml : recoveries were more variable and sometimes as low as 25%. None of the control blood samples (no added prostaglandin) contained any detectable prostaglandin (<1 ng E_1/ml blood).

Recovery of tritium-labelled prostaglandin E_1

When the dog's blood containing 135 ng/ml of tritiated prostaglandin E_1 was incubated for 5 minutes at 37°C and then extracted into ethyl acetate it was found that 80% of the added radioactivity was present in the ethyl acetate fraction.

Effect of incubation of prostaglandin E_1 with blood

Prostaglandin E_1 was incubated with blood at a concentration of 1 µg/ml for periods from 5 min. to 3 hr. The amount of prostaglandin E_1 recovered at the end of each incubation period was within the range of that recovered by extracting the blood immediately after adding the prostaglandin.

Electrophoresis of prostaglandin E_1 and plasma to which prostaglandin E_1 had been added

Pure prostaglandin E_1 and dog's plasma were subjected to electrophoresis in parallel on separate cellulose acetate strips. The electrophoretically fractionated plasma proteins were located by staining and the strips on which prostaglandin E_1 alone had been run was divided into zones corresponding to these fractions. The biological activity due to the prostaglandin eluted mainly from the zone corresponding to the α_1 globulin fraction, some was present in the α_2 globulin zone. No activity could be detected in other zones.

When dog's blood containing prostaglandin E_1, 5 µg/ml, was incubated for 30 min. or 1 hr. at 37°C : all the added prostaglandin was recovered from the α_1 and α_2 globulin zones after electrophoresis of the plasma. No biological activity was detected in other zones.

Discussion

There have been several reports recently of the release of prostaglandins from tissues (1, 4, 5, 6, 7, 8). One of these concerned the identification of prostaglandin E_2 in venous blood collected from the dog's spleen (1). When the prostaglandin had been positively identified, the next step was to devise a method for the quantitative estimation of this substance in blood. The

experiments reported here show that extraction with ethanol is not efficient for extracting added prostaglandins from blood, although it has proved an effective method with other tissues. One explanation for these poor recoveries is that the added prostaglandin becomes bound to a substance such as plasma protein which is precipitated by the ethanol. The electrophoretic evidence indicates that if such an association occurs, it must be with an α globulin.

The modified extraction procedure, which simply involves omission of the initial ethanol extraction, is similar to that used by earlier workers for the extraction of prostaglandins from seminal plasma (9, 10). The method gives good and fairly consistent recoveries provided that the concentrations of prostaglandin are sufficiently high (0.1 µg/ml or more). Whether this is within the physiological range is unknown; although in one experiment reported previously splenic venous blood contained 200 ng E_2/ml even when using the ethanol extraction procedure. If the 5 - 10% recoveries with ethanol extraction also applied to those experiments, the concentration of prostaglandin E_2 in splenic venous blood may have been as high as 1 - 2 µg/ml. It should be noted, however, that poor recoveries of added prostaglandins are not necessarily evidence that the naturally-occurring compounds will not be extracted efficiently by a given procedure. The converse is, of course, also true.

A better method capable of recovering quantitatively lower blood concentrations of prostaglandin is highly desirable. The basis for such a method may be found in the recent report of Hickler who uses a methylal - ethanol mixture in the initial extraction of plasma (11).

Using the method described here it has been shown that prostaglandin E_1 is not biologically inactivated by incubation with dog's blood <u>in vitro</u> for periods up to 3 hr. It is concluded that the removal of prostaglandins from the circulation is by a mechanism other than inactivation by enzymes in blood. This finding is in agreement with that of Ferreira and Vane using the blood-bathed superfused organ technique.

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THE EFFECT OF PROSTAGLANDIN E_1 ON RESPONSES OF SMOOTH MUSCLE TO CATECHOL AMINES, ANGIOTENSIN AND VASOPRESSIN

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THE EFFECT OF PROSTAGLANDIN E_1 ON RESPONSES OF SMOOTH MUSCLE TO CATECHOL AMINES, ANGIOTENSIN AND VASOPRESSIN

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Reduction of the pressor responses to adrenaline in the rabbit following administration of prostaglandin E_1 has been confirmed. The effect is, however, nonspecific since noradrenaline, angiotensin and vasopressin are also antagonized. Analogous responses were observed in blood flow experiments on the cat hind limb but not on the rabbit isolated auricles or the rabbit isolated duodenum. Contractions of the cat nictitating membrane produced by sympathetic preganglionic stimulation or by adrenaline were not decreased following injection of prostaglandin E_1 but the relaxation period was shorter. Contractions of the rabbit vas deferens induced *in vivo* by adrenaline were smaller after prostaglandin E_1 and this effect tended to be long-lasting.

In 1938, Euler observed that pressor responses to adrenaline in the rabbit were reduced following an intravenous injection of prostaglandin. Steinberg, Vaughan, Nestel & Bergström (1963) have recently made a similar observation using pure prostaglandin E_1. These authors have also shown that the break-down of triglyceride in adipose tissue induced by catechol amines is antagonized. In this investigation we have confirmed the action of prostaglandin E_1 on pressor responses to catechol amines and have shown that this effect is nonspecific since responses to angiotensin and vasopressin are also reduced.

METHODS

Blood pressure experiments. Blood pressure was recorded from a carotid artery using either a mercury manometer or a Statham pressure transducer. Cats were anaesthetized with pentobarbitone sodium (40 mg/kg), rabbits and rats with urethane (1.75 g/kg) injected intraperitoneally. In some experiments rats were anaesthetized with ether and pithed using a strong wire introduced through an orbit and passed down the cerebrospinal axis.

Cat hind-limb blood flow. Cats, weighing 2.5 to 4.5 kg, were anaesthetized with pentobarbitone sodium (40 mg/kg) injected intraperitoneally. The trachea, a carotid artery and both external jugular veins were cannulated. Blood pressure was recorded by a mercury manometer. Venous outflow from a hind-limb was recorded by passing blood from the femoral vein through a Palmer drop-chamber connected to a Gaddum drop-recorder, the blood being returned into a jugular vein. The artery supplying the gracilis muscle was cannulated with fine polyethylene tubing connected to a three-way tap for retrograde intra-arterial injections. Heparin (1,000 U/kg) was injected intravenously and further doses of 500 U/kg were given every 2 hr. Contractions of the hind-limb muscles produced by stimulation of

the sciatic nerve (3 to 4 V, 0.2 msec duration, 6 shocks/min) ensured a brisk venous outflow throughout the experiment.

Nictitating membrane contractions. Nictitating membrane contractions were recorded isometrically using a Grass force-displacement transducer (model FTO3). The ipselateral preganglionic cervical sympathetic nerve was stimulated and intra-arterial injections were made retrogradely through a polyethylene cannula in the ipselateral lingual artery.

Rabbit isolated auricles. The method described by Burn (1952) was used. The auricles were suspended in a 4 ml. organ-bath containing Locke solution gassed with oxygen and their spontaneous contractions were recorded isometrically using a Grass force-displacement transducer (model FTO3).

Rabbit isolated duodenum. A 3 to 4 cm segment of duodenum was suspended in a 4 ml. organ-bath containing Tyrode solution at 35 to 36° C gassed with air. Longitudinal contractions were recorded isometrically with a frontal writing lever on a smoked drum. A tension of 1 g and a lever magnification of five-times were used. The dose cycle was 5 min with 40 sec contact.

Rabbit vas deferens. Male rabbits, weighing 2 to 3 kg, were anaesthetized with urethane (1.75 g/kg) injected intraperitoneally. The trachea, an external jugular vein and a carotid artery were cannulated. The abdomen was opened and the viscera were displaced to allow access to one vas deferens. The distal end was ligated and the proximal end was cannulated with a polyethylene cannula introduced through a small incision and tied in position. The cannula was filled with Tyrode solution and connected to a Statham pressure transducer. Intraluminal pressure was then recorded from this artificial blind sac of vas deferens. The preparation showed spontaneous activity and responded by contraction to intravenous injections of adrenaline.

Drugs. These were (−)-adrenaline acid tartrate (B.D.H.); (−)-noradrenaline bitartrate (Levophed, Bayer); valyl[5]angiotensin II aspartyl-β-amide (Hypertensin, Ciba); vasopressin injection B.P. (Pitressin, Parke, Davis & Co.); and histamine acid phosphate (B.D.H.).

RESULTS

Blood pressure experiments. Following an intravenous injection of prostaglandin E_1 (10 μg/kg) into the rabbit, pressor responses to adrenaline were smaller than before (Fig. 1*a*). Although responses to successive doses of adrenaline gradually increased, complete recovery was long-delayed (15 to 30 min) and in some experiments did not occur. Similar reductions in pressor responses to both adrenaline and noradrenaline were also observed in the cat and the rat. In the experiment illustrated in Fig. 1*b* pressor responses to 0.2 μg noradrenaline in the rat anaesthetized with urethane did not return to their former magnitude during the course of the experiment after an intravenous injection of prostaglandin E_1.

This effect was not specific for catechol amines. Pressor responses both to angiotensin and to vasopressin were similarly reduced following injection of prostaglandin E_1. In an experiment on a pithed rat illustrated in Fig. 1*c*, the pressor response to 2.5 mU of vasopressin was similarly reduced.

Blood flow experiments. Vasoconstrictor responses to intra-arterial injections of adrenaline, noradrenaline or angiotensin into the hind limb of the cat were reduced following an intra-arterial injection of prostaglandin E_1 (Fig. 2). In one experiment as little as 32 ng of prostaglandin E_1 caused detectable inhibition of the vasoconstriction due to 100 ng of noradrenaline and in the same animal 500 ng of prostaglandin E_1 almost completely abolished the response to 100 ng of nor-

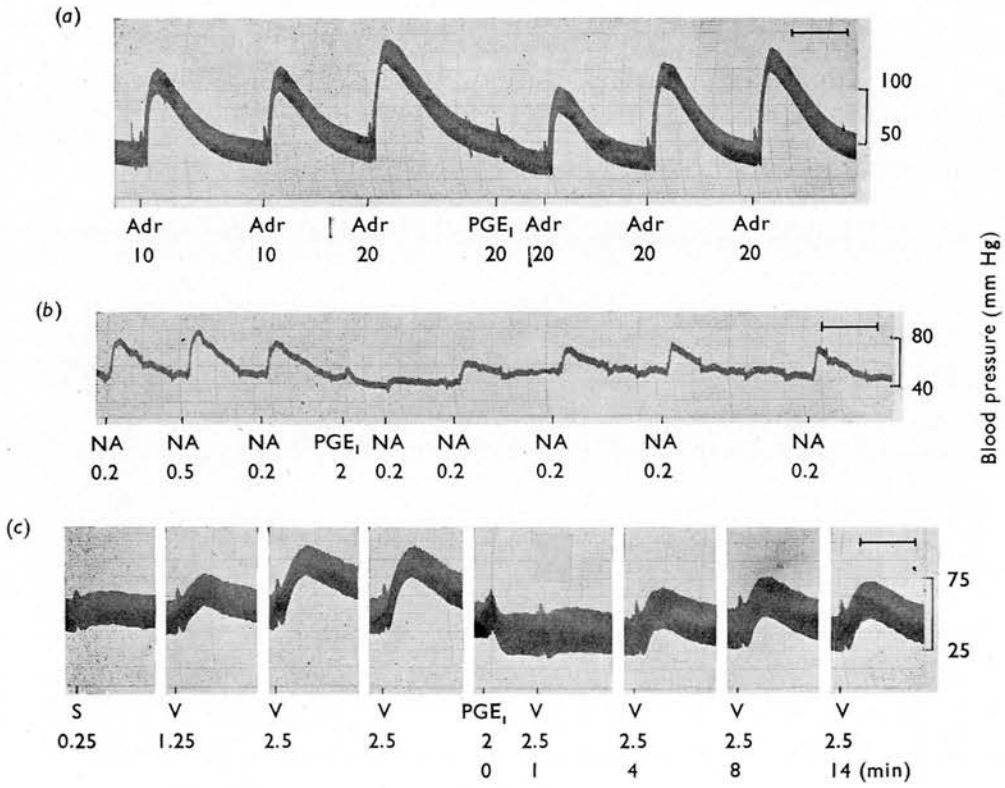

Fig. 1. Records of carotid arterial blood pressure from: (*a*) a rabbit (2 kg) anaesthetized with urethane; (*b*) a rat (290 g) anaesthetized with urethane; and (*c*) a pithed rat (200 g). Drugs were injected intravenously. Adr=adrenaline (μg); NA=noradrenaline (μg); V=vasopressin (mU); PGE_1=prostaglandin E_1 (μg); S=saline (ml.). Time calibrations are 1 min. In (*c*) the lower figures indicate times in minutes after administration of prostaglandin E_1 at which doses of vasopressin were given.

adrenaline. Similar results were obtained with adrenaline and angiotensin but responses to neither drug appeared as sensitive to prostaglandin E_1 as that to noradrenaline. The effect of prostaglandin E_1 on the vasodilatation due to isoprenaline (100 ng) was insignificant for doses of prostaglandin up to 5 μg. Inhibition of the response to the vasoconstrictor drugs was maximal immediately after the prostaglandin had been administered, with a gradual return to normal in 5 to 15 min. Similar effects were found when other vasodilator drugs, for example histamine and bradykinin, were injected in place of prostaglandin E_1.

Contractions of the cat nictitating membrane. The force of contraction of the nictitating membrane in response either to sympathetic preganglionic stimulation or to intra-arterial injection of adrenaline was not reduced after administration of prostaglandin E_1 in doses up to 20 μg/kg. However, the duration of the contraction was markedly reduced (Fig. 3).

Responses of other smooth muscle preparations. Increased force of contraction of the rabbit isolated auricles following administration of isoprenaline or adrenaline

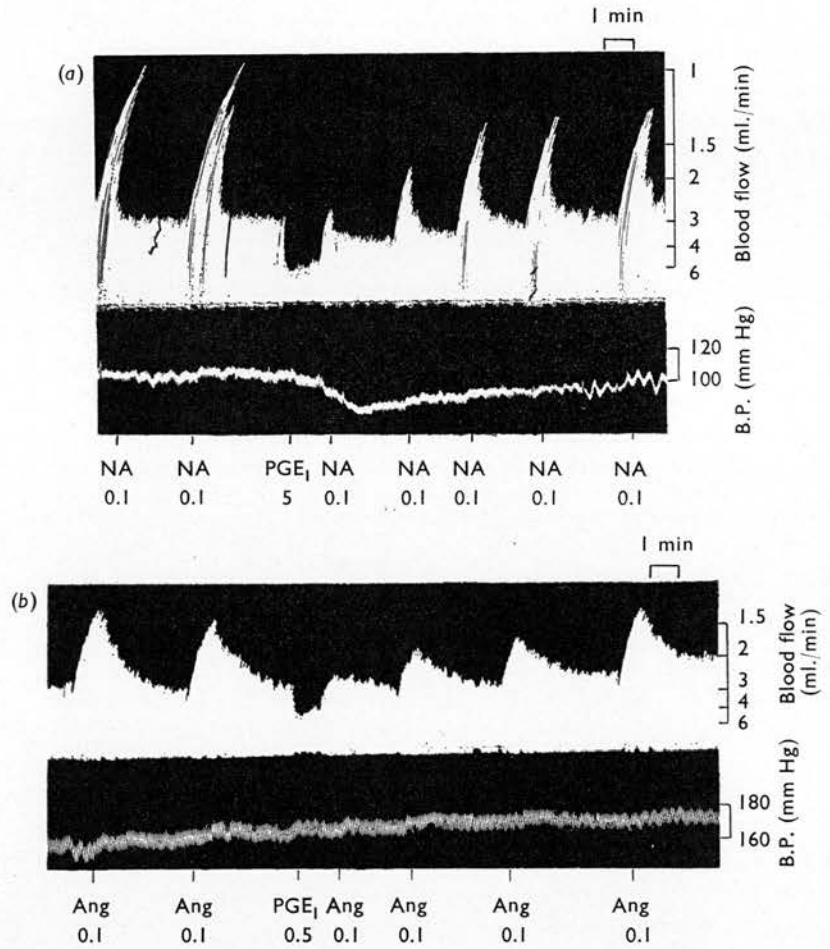

Fig. 2. Records from cats (*a*, 2.2 kg; *b*, 2.5 kg) anaesthetized with pentobarbitone sodium, 40 mg/kg injected intraperitoneally. Upper trace, hind-limb blood flow; lower trace, carotid arterial blood pressure. Drugs were injected retrogradely into the femoral artery from a cannula in the artery to the gracilis muscle. Time calibrations are 1 min. All doses in μg. NA=noradrenaline; PGE_1=prostaglandin E_1; Ang=angiotensin II.

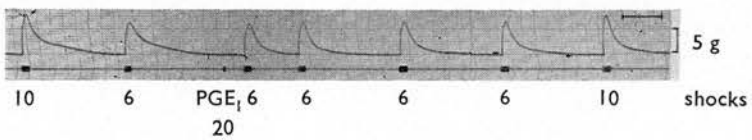

Fig. 3. Cat (2.35 kg) anaesthetized with pentobarbitone sodium, 40 mg/kg injected intraperitoneally. Isometric contractions of a nictitating membrane in response to stimulation of the ipselateral preganglionic cervical sympathetic nerve with 5 V shocks of 1 msec duration. Figures indicate number of shocks. PGE_1=prostaglandin E_1 (μg) injected retrogradely through a cannula in a lingual artery. Time calibration, 30 sec.

was not altered by prostaglandin E_1 in concentrations up to 2.5 μg/ml. Similarly, relaxations of the rabbit isolated duodenum induced by adrenaline were unaltered by prostaglandin in a dose which itself caused contraction of the muscle.

Contractions of the rabbit vas deferens induced *in vivo* by adrenaline were, however, reduced following administration of prostaglandin E_1 (Fig. 4). This effect was also observed in the rabbit vas deferens *in vitro*.

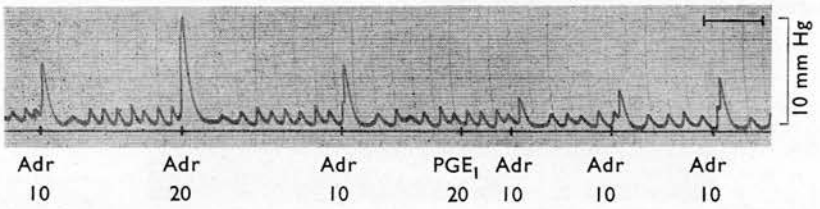

Fig. 4. Rabbit (2 kg) anaesthetized with urethane injected intraperitoneally (1.75 g/kg). Records of the intraluminal pressure of one vas deferens *in vivo*. Adr=adrenaline, PGE_1=prostaglandin E_1, each injected intravenously. Doses are in μg. Time calibration, 1 min.

DISCUSSION

Effect of prostaglandin E_1 on vascular smooth muscle. The experiments described here indicate that the antagonism of pressor responses to catechol amines reported by Euler (1938) and Steinberg *et al.* (1963) is nonspecific since responses to angiotensin and vasopressin are similarly affected. This lack of specificity has been demonstrated both on blood pressure and on blood flow. Similar antagonism of responses to catechol amines was observed when other depressor substances such as bradykinin were used instead of prostaglandin E_1. Such "physiological" antagonism might be expected to occur from algebraic summation of effects when a pressor and depressor substance are injected either simultaneously or within a short time of each other, but there is the alternative possibility, mentioned by Steinberg *et al.* (1963), that prostaglandin E_1 blocks a biochemical sequence of events initiated by vasoconstrictor substances, but has no vascular effects in the absence of these substances. From the experiments described here we conclude that, if the latter is the actual mechanism, the site of block must be somewhere on the final common path for the actions of catechol amines, angiotensin and vasopressin.

Effect of prostaglandin E_1 on other smooth muscle preparations. Prostaglandin E_1 also modified responses of other smooth muscle preparations. Contractions of the rabbit vas deferens induced by adrenaline were reduced, although resting tone and spontaneous contractions were unaffected. On the cat nictitating membrane, prostaglandin E_1 did not reduce the size of contractions in response to sympathetic nerve stimulation or to injection of adrenaline, but recovery time was shortened.

Whether these actions of prostaglandin are of any physiological significance is unknown. It is clear that prostaglandins are of much wider distribution in the body than was formerly suspected and we suggest that their presence in organs

containing smooth muscle may be associated with a function as local mediators of smooth muscle inhibition. The finding that break-down of triglyceride is also modified (Steinberg *et al.*, 1963) shows that the action of prostaglandins is not restricted to smooth muscle. It is possible that prostaglandins inhibit a biochemical pathway which is activated by different substances and which is common to several different tissues. Consequently this inhibition may lead to entirely different effects depending upon the tissue.

We are very grateful to Professor S. Bergström for a gift of pure prostaglandin E_1 and to Dr E. D. Nicolaides for a gift of synthetic bradykinin.

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THE EFFECTS OF PROSTAGLANDINS E_1 AND E_2 ON OVARIAN STEROIDOGENESIS

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Prostaglandins of the E series inhibit hormonally-induced lipolysis[1-3] and vasopressin-induced increase in water permeability of the toad bladder[4]. Both of these hormonal actions are known to be mediated by the formation of cyclic 3′, 5′- A.M.P.[5,6] Prostaglandins do not antagonise the actions of cyclic 3′, 5′- A.M.P. in these systems[4,7], but they may exert their effects by inhibiting the formation of this nucleotide.

Cyclic 3′, 5′- A.M.P. has been shown to be a mediator in the stimulant action of luteinizing hormone on ovarian steroidogenesis in vitro[8-10]. This provides another system for investigating the possible inhibitory action of prostaglandins on the formation of cyclic 3′, 5′- A.M.P.

Method

Chopped rabbit ovaries were evenly distributed among three flasks, each containing 15ml. of Krebs Ringer solution. One flask served as a control to verify the activity of the

gonadotrophins. The other two flasks contained human chorionic gonadotrophin ("Pregnyl") and pregnant mare's serum ("Gestyl"), each in a final concentration of 1 unit/ml. The prostaglandin under investigation was added to one of the flasks containing the gonadotrophins. All flasks were then incubated in a metabolic shaker at 37°C for three hours with continuous gassing with a mixture of 95% O_2 and 5% CO_2.

After incubation the contents of each flask were immediately chilled and the ovarian tissue homogenised in the incubating medium. The homogenates were titrated to pH 11.0 with sodium hydroxide, and the steroids extracted by the method of Dorrington and Kilpatrick[11], followed by the two-dimensional thin-layer chromatography as described by Armstrong et al[12]. This resulted in the isolation of the two major steroids produced by rabbit ovaries under these conditions, progesterone and 20α - hydroxypregn - 4 - en - 3 - one. These steroids were eluted with methanol (1.5ml. followed by 1.0ml.) and absorption at 240mμ measured against an eluate of a blank area of the plate, using a double-beam recording spectrophotometer.

Results

Ovarian steroidogenesis was consistently stimulated by the mixture of gonadotrophins, as shown by the increase in steroid

production by ovarian tissue incubated in the presence of gonadotrophins over that incubated in control flasks containing Krebs solution alone.

The effects of prostaglandins E_1 and E_2 on steroidogenesis in the presence of gonadotrophins are shown in Table 1 and 2. There was no evidence that either prostaglandin E_1 or E_2 had an inhibitory effect on steroidogenesis. However, in five out of six experiments prostaglandin E_2 caused a substantial increase in the production of 20α - hydroxypregn - 4 - en - 3 - one.

Discussion

The results of this preliminary investigation do not support the hypothesis that either of the two prostaglandins investigated inhibit the formation or action of cyclic 3′, 5′- A.M.P. in the rabbit ovary. The increased production of 20α - hydroxypregn - 4 - en - 3 - one. in the presence of prostaglandin E_2 may be due to a direct action of prostaglandin E_2 on steroidogenesis or may reflect a potentiation of the effect of the gonadotrophins.

Further experiments are in progress to investigate the effects of different concentrations of prostaglandins on ovarian steroidogenesis stimulated by pure ovine luteinizing hormone and cyclic 3′, 5′- A.M.P.

Acknowledgements

We are grateful to Dr. D.A. van Dorp of Unilever Research Laboratories, Vlaardingen, The Netherlands, for the prostaglandin E_1 and Dr. J.E. Pike of the Upjohn Company, Kalamazoo, Michigan, for supplies of prostaglandin E_2. The Upjohn Company also kindly supplied the 20α - hydroxypregn - 4 - en - 3 - one.

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Table 1 Steroid production (µg steroid / 500mg. Ovary / 3 hours) in the presence of gonadotrophins alone and gonadotrophins + Prostaglandin E_1 (PGE_1)

Concentration of PGE_1 (µg/ml)	Progesterone			20α-hydroxypregn-4-en-3-one		
	gonadotrophins	gonadotrophins + PGE_1	% Change due to PGE_1	gonadotrophins	gonadotrophins + PGE_1	% Change due to PGE_1
1	40.5	42.0	+ 3.7	20.9	16.2	-22.5
1	30.9	27.2	-12.0	27.2	25.3	- 7.0
1	17.5	26.4	+50.8	27.5	38.2	+38.9
10	33.6	34.2	+ 1.8	19.9	23.7	+19.1
10	28.6	28.2	- 1.4	29.4	28.9	- 1.7

Table 2 Steroid production (µg steroid / 500mg. Ovary / 3 hours) in the presence of gonadotrophins alone and gonadotrophins + Prostaglandin E_2 (PGE_2)

Concentration of PGE_2 (µg/ml)	Progesterone			20α-Hydroxypregn-4-en-3-one		
	gonadotrophins	gonadotrophins + PGE_2	% Change due to PGE_2	gonadotrophins	gonadotrophins + PGE_2	% Change due to PGE_2
1	64.1	69.6	+ 8.6	30.3	42.3	+39.6
1	55.7	55.9	+ 0.36	8.0	15.8	+97.5
1	10.9	16.8	+54.1	36.4	50.4	+38.4
1	28.6	27.4	- 4.2	29.4	21.2	-27.9
1	33.1	29.6	-10.6	9.4	12.7	+35.1
2	30.0	27.0	-10.0	17.5	25.3	+44.6

Effects of orally administered prostaglandin E_1 on gastric secretion and gastro-intestinal motility in man

by

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Summary

The effects of prostaglandin E_1 administered orally in doses of 10 - 40 µg/kg have been studied in three human subjects. There was no inhibition of pentagastrin-induced gastric secretion. Following the higher doses of prostaglandin, gastric juice contained large amounts of bile. Prostaglandin E_1 also increased intestinal motility resulting in loose faeces.

Introduction

Prostaglandins are fatty acids which have been found in a wide variety of tissues (Bergström, 1967; Pickles, 1967), including human gastric mucosa (Bennett, Murray & Wyllie, 1968). One member of this group, prostaglandin E_1, has recently been shown to inhibit the secretion of gastric juice in rats (Shaw, 1968; Shaw & Ramwell, 1968) and dogs (Robert, 1968; Robert, Nezamis & Phillips, 1967). The investigation reported here was undertaken in an attempt to extend these observations to man.

Methods

Collection of gastric juice

Three healthy male subjects aged 30, 31 and 38 and weighing 70, 60 and 80 Kg respectively, were fasted overnight for ten hours. A $\frac{1}{4}$" plastic stomach tube was passed via the nose into the stomach at approximately 8.30 a.m. The subject then lay horizontally on his left side throughout the remainder of the experiment. Blood pressure in the right brachial artery and pulse rate were recorded at 5 to 15 minute intervals.

Fasting gastric juice (sample F_1) was removed as completely as possible and further samples were withdrawn 15 and 30 minutes later (samples F_2 and F_3).

Two-fifths of the total dose of prostaglandin E_1 (or solvent control) diluted in 25 ml water was then drunk by the subject. Fifteen minutes later, residual fluid in the stomach was aspirated

and one-fifth of the total prostaglandin dose dissolved in 5 ml water was administered through the tube. Pentagastrin 6 µg/kg was then injected subcutaneously. The stomach was emptied as completely as possible at 15, 30, 45, 60 and 75 minutes after t injection. Two further doses, each amounting to one-fifth of t total dose, of prostaglandin E_1 dissolved in 5 ml water were administered via the tube after the 15 and 30 minutes samples were withdrawn. Each dose was washed in with 5 ml water. In t control experiments the same procedure was followed using the solvent control.

Prostaglandin E_1 solution and solvent control

A stock solution (1 mg/ml) of prostaglandin E_1, kindly supplied by Professor D.A. van Dorp of the Unilever Research Laboratories, Vlaardingen, was prepared by dissolving 9.5 mg in 1 ml ethanol and making up the volume to 9.5 ml with water. A solvent control was prepared by diluting 1 ml ethanol to 9.5 ml with water.

Estimation of total acidity

The volume of each sample was measured. The total acidit of each was measured by titration with $^{N}/_{20}$ sodium hydroxide solution using phenolphthalein as indicator.

Experiments to check the purity, toxicity and biological activity of the prostaglandin E_1 used

Since the prostaglandin E_1 used in this investigation had been subjected to extensive purification procedures before despatch, elaborate tests of identification or purity were considered unnecessary. An aliquot of the material was chromatographed on a plate of silica gel in Green and Samuelsson's AI solvent system (Gréen & Samuelsson, 1964). It behaved like a single substance with an Rf value equal to that of a sample of authentic prostaglandin E_1.

A second aliquot of the prostaglandin E_1 was tested for biological activity and toxic effects in mice. Intravenous injection of 5 and 10 mg/kg to three mice produced signs (sedation, ptosis and moist faeces) which have been described previously with lower doses of prostaglandin E_1 (Holmes & Horton, 1968). There were no deaths. The higher dose in mice was 250 times greater than the largest dose (40 µg/kg) administered (orally) to man in this investigation.

Using the Ghosh & Schild (Ghosh & Schild, 1958) technique, it was confirmed that this particular batch of prostaglandin E_1 perfused through the stomach of a rat in a concentration of 6 µg/ml caused up to 45% inhibition of gastric acid secretion induced by continuous intravenous infusion of pentagastrin 9 µg/kg/hour.

Finally, the prostaglandin E_1 was given to one subject to detect unexpected toxic effects. The subject who had fasted overnight was given prostaglandin E_1 (10 µg/kg) as a single dose (total 0.8 mg) dissolved in 25 ml water. The only side effect was the passing of loose faeces 2 hours after the prostaglandin administration. Thereafter defaecation was normal and no change in frequency was observed. The arterial blood pressure and heart rate were unaffected.

Effects of pentagastrin on subjects dosed with the solvent control

The gastric secretory response to pentagastrin (6 µg/kg) was detectable after 15 minutes but had passed its maximum by 45 minutes The response lasted 60 to 75 minutes. Pentagastrin caused flushing of the face, an effect which began 2 minutes after the injection and subsided competely within 15 minutes. This effect was reported by the subjects and was usually apparent to the observer. In one experiment vasodilatation of the skin was widespread over the body and an unpleasant sensation of severe heat accompanied by sweating was experienced.

Effect of prostaglandin E_1 on gastric secretion in man

In four experiments prostaglandin E_1 was given orally in doses of 10, 20, 25 and 40 µg/kg to three subjects. In none of

these experiments was there any detectable reduction in volume or acid content of the gastric juice secreted in response to pentagastrin compared with the controls (Table 1). There may even have been an increase in secretion following prostaglandin administration. The pentagastrin-induced flushing was also of similar intensity and duration in subjects who had received prostaglandin to that observed in the control experiments. There was no change in pulse rate or arterial blood pressure.

Effect of prostaglandin E_1 on the presence of bile in gastric samples

In three subjects treated with prostaglandin E_1 in doses of 20, 25 and 40 µg/kg, there were large amounts of bile in the samples, particularly those collected 30 and 45 minutes after pentagastrin. In two subjects bile was detected in the gastric juice sample collected 15 minutes after the administration of prostaglandin, that is before the pentagastrin had been injected. In none of the subjects was any bile observed in the gastric juice secreted in response to pentagastrin, when prostaglandin had not been administered.

Effect of prostaglandin E_1 on intestinal motility

In the five experiments in which prostaglandin E_1 was

administered, the passing of loose faeces was observed between 2 and 4 hours after administration. At the 40 µg/kg level the faeces were completely liquid, but defaecation was subsequently normal. From 1 hour after the prostaglandin administration until defaecation occurred, sensations of increased intestinal motility were felt. There were sometimes mild colicky pains. In the control experiments the faeces were unchanged and defaecation did not occur with any regular time relatinnship to the administration of the test solution Between experiments faeces were of normal consistency in all subject and there was no change in normal frequency of defaecation.

Prostaglandin content of the gastric juice

The prostaglandin content of fasting gastric juice (nine experiments) and juice secreted in response to pentagastrin (four experiments) was estimated.

In four experiments in which prostaglandin E_1 was administered to the subjects, part of the dose was removed from the stomach at each aspiration. The total amount of prostaglandin E_1 recovered in this way is shown in Table 1.

No prostaglandin (1.5 ng E_1 equivalent/ml) could be detected except in one fasting sample when activity equivalent to a concentration of 7 ng prostaglandin E_1/ml was found. This was not

positively identified as a prostaglandin.

Discussion

The results of this investigation show that prostaglandin E_1 administered orally in man fails to inhibit gastric secretion in doses which have effects on gastro-intestinal motility. Shaw and Ramwell reported inhibition of pentagastrin-induced gastric secretion in the rat with prostaglandin E_1 perfused through the stomach at a rate of 0.5 to 1 µg/min (Shaw, 1968; Shaw & Ramwell, 1968). The conditions of the two investigations were different and a direct comparison cannot be made. In the present study prostaglandin E_1 was present in the stomach continuously for 1 hour 15 minutes before and 45 minutes after the pentagastrin injection. In the four experiments reported here, concentrations of prostaglandin E_1 in the empty stomach were at least 13, 22, 24 and 50 µg/ml at the beginning of each 15 minute period. Our results do not exclude the possibility that human gastric secretion might have been inhibited if a higher dose of prostaglandin E_1 had been used.

Prostaglandin E_1 is released from the rat's stomach when gastric secretion is stimulated (Shaw, 1968; Shaw & Ramwell, 1968). It has been suggested that the action of this released prostaglandi

may be to inhibit further actions of the secretagogue by a negative feed-back mechanism (Shaw, 1968). We have been unable to obtain convincing evidence in man for the presence of prostaglandin in either fasting juice or in juice secreted in response to pentagastrin. It is possible that any biologically-active prostaglandins released could have been destroyed by the gastric juice. This negative finding coupled with the failure of prostaglandin E_1 to inhibit secretion does not support a role for this compound in human gastric secretion similar to that postulated for rats. Human gastric mucosa contains prostaglandin E_2 (Bennett, Murray & Wyllie, 1968). It is possible that this compound and not prostaglandin E_2 would be effective in inhibiting gastric secretion in man. However, both prostaglandins are equally active in dogs (Robert, 1968; Robert, Nezamis & Phillips, 1967).

The large quantities of bile in the aspirated gastric contents after prostaglandin must have been due to a reflux of duodenal contents; it is possible that prostaglandin E_1 relaxes the pyloric sphincter. Some evidence in support of this comes from the observation that both prostaglandin E_1 and prostaglandin E_2 relax circular muscle of the human stomach in vitro (Bennett, Murray & Wyllie, 1968). This side effect may be considered a disadvantage if oral prostaglandin E_1 were to be used therapeutically.

The purgative action of prostaglandin E_1 in man is similar to that observed in mice following intravenous or subcutaneous injection (Holmes & Horton, 1968), and may be due to a direct stimulant action on intestinal smooth muscle as observed in vitro (Horton & Main, 1963). Although the effects of prostaglandins on human intestinal smooth muscle have not been reported, prostaglandins E_1 and E_2 do contract longitudinal muscle of the human stomach in vitro. Recently, patients with thyroid carcinoma have been shown to have raised prostaglandin levels in the blood; all these patients had diarrhoea (Williams, Karim & Sandler, 1968). Our observations provide some evidence that prostaglandins may indeed by the causal agents. Since prostaglandins have been identified in menstrual fluid (Eglinton, Raphael, Smith, Hall & Pickles, 1963), they may possibly account for the increased frequency of defaecation observed at the beginning of menstruation (McCance & Pickles, 1960). They also should be borne in mind when considering the mechanism of diarrhoea associated with other disorders.

Numerous side effects have previously been reported in man following administration of prostaglandin E_1 by intravenous infusion, (Bergstrom, Duner, Euler, Pernow & Sjovall, 1959; Carlson, 1967; Bygdeman, Kwon & Wiqvist, 1967). These include cardiovascular effects, headache and a feeling of oppression in the chest. None of these symptoms nor any obvious cardiovascular change occurred in the

present investigation. Conversely, the increase in intestinal motility and diarrhoea observed in our experiments have not been reported by previous groups. This may reflect a difference in route of administration or the dose of prostaglandin which was considerably higher in the present study.

The facial flushing after pentagastrin injection was a consistent finding which may correspond to the sensation of skin heat reported by others (Logan & Connell, 1966). It is of interest that the flushing was not potentiated after prostaglandin administration, since prostaglandin E_1 is a potent vasodilator and itself caused facial flushing in ten out of thirteen cases on intravenous infusion (Bergström, Dunér, Euler, Pernow & Sjövall, 1959; Carlson, 1967; Carlson, Irion & Orö, 1968).

and on intestinal motility

The volume (ml) and acidity (mEq) refer to the total samples collected during the first hour after pentagastrin injection. N.T. = not tested. Presence of bile in the aspirated samples is indicated by ++ or +++. 0 indicates absence or only trace amounts of bile.

Control experiments (pentagastrin only)

	Subject	Vol (ml)	Acid (mEq)	Bile	Increase in Intestinal Motility
1.	EH	184	8.8	0	nil
2.	EH	175	14.5	0	nil
3.	HM	140	11.4	0	nil
4.	PW	148	14.9	0	nil
* 5.	HM	N.T.	N.T.	0	nil

Prostaglandin administration

	Subject	Dose (μgE_1/Kg) Administered	Recovered	Vol (ml)	Acid (mEq)	Bile	Increase in Intestinal Motility
1.	EH	10	3	170	10.0	0	+
2.	EH	40	11	241	15.4	+++	+++
3.	HM	20	7	314	24.8	+++	+
4.	PW	25	13	241	18.0	++	++
† 5.	EH	10	0	N.T.	N.T.	N.T.	++

* Preliminary experiment to study the side effects of pentagastrin alone

† Preliminary experiment to study the side effects of prostaglandin E_1 alone (no pentagastrin).

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DRUGS OF ANIMAL ORIGIN

BY

Professor E. W. HORTON

B.Sc., M.B., Ch.B., Ph.D.

INAUGURAL LECTURE
by the
WELLCOME PROFESSOR OF PHARMACOLOGY
at
THE SCHOOL OF PHARMACY
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MR. CHAIRMAN, Ladies and Gentlemen. First let me thank you Mr. Chairman for your introduction and for explaining the purpose of this Lecture. My own concept of an Inaugural Lecture is biased by undergraduate memories of rather grand occasions when new professors addressed impressively large and distinguished audiences on subjects which I could rarely understand. I do remember, however, on one of those occasions contemplating what I would choose as my subject in the unlikely event of my ever having to give such a Lecture. The title was to be simply "What is Truth?". That was some years ago. When faced with the reality of having to decide upon a title for to-day's lecture, I felt unqualified to speak for an hour on such a philosophical topic.

Nonetheless, it is a subject to which I shall refer, because the search for truth underlies all that University life stands for,—whether at the undergraduate or postgraduate level. I hear a good deal about teaching and research—referred to as if there were two separate parts of University education—one sometimes suspects that time and facilities for research are being offered as a kind of sugar coating around the bitter and unpalatable pill of teaching. This is a mis-conception of true University education.

I well remember the amazement, indeed dismay, that I experienced when as a Lecturer I received a circular from Senate House addressed to "Teachers of the University". What kind of University is this I thought that calls its academic staff teachers? Now I have become accustomed to the term but it still evokes a mildly unpleasant reaction.

The essence of University education is that the student does the work; after all he is supposed to be reading for a degree. It's not a question of the student absorbing as much factual knowledge as his "teachers" can cram into him. All members of a University should be studying and in that sense all are students, whether they be undergraduate, postgraduate or members of staff. All should have as their aim the discovery and further understanding of the Truth.

A comment of my former professor, Sir John Gaddum springs to mind and I should like here to pay tribute to him, because without his interest, encouragement and inspiration I should certainly not be in Pharmacology. It is indeed an honour to follow in his footsteps as Professor of Pharmacology at this school. On the occasion I refer to, Professor Gaddum was rather annoyed because I was quibbling about the priority of some quite trivial discovery. He told me "It doesn't matter *who* makes the discovery, all we're interested in is the truth". This statement might profitably be posted above the door of every University laboratory, whether the lab. be

designated "teaching" or "research". Both should have as their aim the search for truth.

But to pass on from the general to the particular case of pharmacology. What is the function of a pharmacologist in a University? What kind of truth does he look for. *One* of his functions is to study the mode of action of drugs. But this immediately raises the question "What is a drug?". The most appropriate definition I know is familiar to many of you already, and I owe it to Professor Feldberg who has defined a drug as a substance which when injected into an animal gives rise to a scientific paper.

There is, however, a potentially infinite number of drugs for a pharmacologist to study and equally a potentially infinite number of biological preparations on which to test them. It follows that some limitation must be imposed on the field of interest of any one pharmacologist. One way is to study only drugs which are of potential therapeutic or clinical interest, which in many fields implies the further limitation that the drug must be active when taken by mouth. Unfortunately the synthetic organic chemists are so productive that to-day's drug of choice in the clinic may be relegated to relative obscurity in a year or two's time.

Another limitation to one's field of interest is study of drugs which are actually derived from animal tissues. This approach has little risk of becoming outmoded. Whatever the organic chemist may synthesize or the clinician prescribe, we can be sure that our bodies (unless otherwise poisoned) will continue to produce the same drugs for the remainder of our lives. The substances will still be of interest because they are basic to the biological mechanisms of animal cells and tissues. Furthermore in studying such drugs of animal origin we may not only learn about their pharmacological mode of action but in so doing may contribute to the further understanding of the functioning of cells.

It is hardly surprising that so little is really known about how drugs act, when one realizes how little is known about cell physiology.

But the pharmacologist with a more practical or applied outlook may object. Opium was used for centuries before anyone knew anything about the physiological or pathological mechanisms of pain. Local anaesthetics were discovered without any knowledge of the mechanism of nerve conduction. The extreme view is exemplified in the statement "It doesn't matter knowing how the drug works so much as whether it does the job without causing ill effects". Certainly if a pharmacologist's primary objective is to discover drugs for the treatment of disease he cannot and should not wait until the fundamental physiological and pathological processes involved have been elucidated. Even if we understood these pro-

cesses in their entirety, it does not necessarily follow that we would be able to discover a new drug to fulfill every therapeutic need, though it should certainly be easier to understand how such a drug acted and to predict what side effects it would be likely to have.

But even though therapeutics and applied pharmacology cannot be held up while advances in more basic sciences are made, it would be foolish to ignore such advances or to pretend that they are irrelevant. If the job of the academic pharmacologist is to study the mode of action of drugs rather than to discover new ones, he must also be vitally interested in the wider aspects of biology and should contribute to such knowledge himself.

The study of drugs of animal origin in some measure may help to achieve both objectives simultaneously. It is here that pharmacology and physiology overlap. It is not inappropriate, I think, that in the department of Pharmacology in this School which also has responsibilities for Physiology and Biochemistry (not to mention Anatomy and even it is rumoured Zoology) should be interested in this field which has been called Autopharmacology.

Now how does the autopharmacologist go about his work? It is not enough simply to accumulate more and more factual data about a drug's pharmacological actions. One of a scientist's aims is to discern a pattern amongst a mass of data. The pattern may be obscured either by too many results or by an insufficiency, or most likely of all because the scientist just cannot see what is there.

The most important advances in basic medical science have resulted when a new pattern or concept has emerged and been supported by experimental evidence. Obvious examples are the concept of hormones and the concept of chemical transmission at nerve endings. Both these fields have been and are still being extended. In recent years we have had the discovery of new hormones such as calcitonin, erythropoietin and the hormones of hypothalamic origin. In the field of synaptic transmission, much progress has been made on the nature of chemical mediators in the central nervous system. It now seems fairly conclusive that acetylcholine, noradrenaline, dopamine and 5-hydroxytryptamine have such a role. Other drugs like substance P, gamma-aminobutyric acid and the prostaglandins are like actors waiting for a part to be assigned to them.

In addition to hormones which reach their target organs via the circulation, and chemical transmitters which are released by nerve endings in close proximity to their site of action, there is a miscellaneous group of substances which also interest autopharmacologists. These may be called local hormones, or perhaps better autacoids. The group includes histamine, a local mediator of gastric secretion and the triple response, and bradykinin, the most

potent vasodilator substance of mammalian origin, which probably accounts for functional hyperaemia in salivary and other glands and may be a mediator of the inflammatory response.

To some extent histamine and bradykinin like 5-hydroxytryptamine, substance P and now the prostaglandins have been substances in search of a function. Knowledge of their pharmacological actions did not lead immediately to an assignment of a physiological role, possible physiology was lagging behind. In the case of acetylcholine and the catecholamines, their pharmacological actions imitated the effects of electrical stimulation of certain nerves thus the physiological and pharmacological approaches fused leading to the idea of chemical transmission. Such a fusion has not occurred so dramatically with the local hormones.

The question may legitimately be asked whether it is not better to study a physiological mechanism, and try to identify the active principle which may be concerned in it, rather than to study the pharmacological actions of an active principle extracted from a tissue and then try to find (or even invent) a function for it. I believe that both approaches are valid. If in the course of our research we discover in tissues a new compound which has powerful and new pharmacological actions it would be wrong to ignore it simply because it did not immediately fit into any obvious physiological niche.

Some of these points may be illustrated by recent developments in a new series of compounds of animal origin, the prostaglandins, which are currently attracting a good deal of attention (Bergström & Samuelsson, 1965; Horton, 1965; Euler, 1966). I would apologise for talking in such detail about my own interests but for two reasons, first a friend gave me this advice when I said that I was a little uncertain what to talk about in this lecture, she said "talk about what you're interested in, after all they are not coming to hear you, just to see what you are like"; and the second reason is that if I talk about other drugs of animal origin there are bound to be people here who know far more about the subject. The work on prostaglandins illustrates some of the fascinations but also some of the snags of starting with a drug rather than with a mechanism.

Prostaglandin like adrenaline, the posterior lobe hormones, substance P and many other animal drugs was discovered by a biologist making an extract of a tissue, and testing its activity on a biological preparation. The discoverer of prostaglandin, Professor U. S. von Euler was searching for a substance with a particular kind of biological activity.

Von Euler's work throughout the 1930's established that prostaglandin was a hydroxy acidic lipid and thus differed chemically

from all pharmacologically-active substances then known (see Euler, 1966 for references).

It often happens in this field of autopharmacology, that a big surge forward in research interest occurs when the isolation and structural elucidation of a substance is announced. This happened for example with the adrenal corticoids, bradykinin and angiotensin. The chemical structure of prostaglandin was reported early in the 1960's by Professor Bergström and his co-workers at the Karolinska Institute. Two prostaglandins were originally described, but soon other prostaglandins were found and at least 14 of these compounds are known, all with pharmacological activity Figs. 1 and 2 (Bergström & Samuelsson, 1965).

When the first of these substances became available for investigation five years ago, little was known about the biological actions of the prostaglandins except that they acted upon various isolated smooth muscle preparations and that they caused a fall in arterial blood pressure when injected into an animal. One could hardly speculate very far about a physiological role on the basis of such limited data, especially when it is further recalled that prostaglandins at that stage were not known to occur in tissues other than the male reproductive tract.

As more information about the prostaglandins has accumulated attempts have been made to fit them into concepts with which we are already familiar namely, that of a hormone, of a chemical transmitter at nerve endings or of a local hormone. In describing the experiments which follow I should like to acknowledge the great contribution made by my co-workers, Drs. Main, Thompson and Holmes and more recently Drs. Davies and Withrington.

First are prostaglandins hormones? That is to say are they substances released by one group of cells transported via the circulation to act upon another group of cells? The evidence clearly indicates that they are, at least in humans. They are secreted in large amounts by the seminal vesicular glands of the male (Bygdeman & Samuelsson, 1966) and absorbed from the vagina of the female in amounts sufficient to modify the tone of reproductive smooth muscle (Horton, Main & Thompson, 1965; Sandberg, Ingelmann-Sundberg & Rydén, 1967); the amounts required to produce effects on the Fallopian tubes and uterus are minute—something like 10-150 ng prostaglandin E_1/kg/min (Bygdeman, Kwon & Wiquist, 1967). The approximate dose of prostaglandin administered by the male at the time of coitus is about 10 μg/kg. It has in fact been demonstrated that the amounts absorbed are sufficient to affect intraluminal pressure in the tubes (Eliasson & Posse, 1965).

Thus prostaglandins are an unusual kind of hormone produced by the male but acting upon the reproductive tract of the female. There is little doubt that this mechanism does occur in humans under physiological conditions and it is thought that it may be concerned with transport of sperm. Certainly there is some evidence that low prostaglandin levels may be associated with infertility (Hawkins & Labrum, 1961).

As work on the prostaglandins continued it became evident that they were not confined to tissues of the reproductive tract. Dr. Ambache (Ambache, Reynolds & Whiting, 1963) had shown that brain contains a smooth muscle stimulating hydroxy unsaturated fatty acid, and on the assumption that this might be a prostaglandin we began to look for central nervous actions (Horton, 1964).

Evidence that prostaglandins have actions on the central nervous system has now accumulated from work in several laboratories. Dr. Main and I made some observations on unanaesthetized chicks (Horton & Main, 1965). Fig. 3 illustrates the actions of two prostaglandins, E_1 which on intravenous injection produces a sedative-like condition and F_{2a} which caused contraction of the extensor muscles of the body. The first effect appeared likely from its nature to be a central action but the second has also been shown to be so.

In the spinal decapitated chick the extension of the limbs in response to prostaglandin F_{2a} can be recorded by measuring tension in the gastrocnemius muscle. When the muscle on one side was denervated the intravenous F_{2a} caused contraction of only the innervated side. Both sides responded to decamethonium after denervation. The action of F_{2a} is not peripheral on the muscle and there is other evidence that F_{2a} does not produce this effect by stimulating afferent neurones. It is concluded that F_{2a} acts upon neurones within the spinal cord (Horton & Main, 1967). A direct action on neurones has in fact been demonstrated by Avanzino, Bradley & Wolstencroft (1966) by applying prostaglandin to single nerve cells by micro-iontophoretic techniques.

Further evidence for an action on the spinal cord has come from experiments on reflexes. In the spinal chick F_{2a} potentiates the crossed extensor reflex and the effect of this single dose (2 μg) lasted 30 minutes or more. Similar effects with E_1 have been observed in the spinal cat.

These observations have been confirmed by Duda and McPherson at the Royal National Orthopaedic Hospital. In their experiments on cats anaesthetized with chloralose, the prostaglandins were injected close arterially to the spinal cord and their effects on the size of reflexly evoked monosynaptic potentials was

Fig 1.

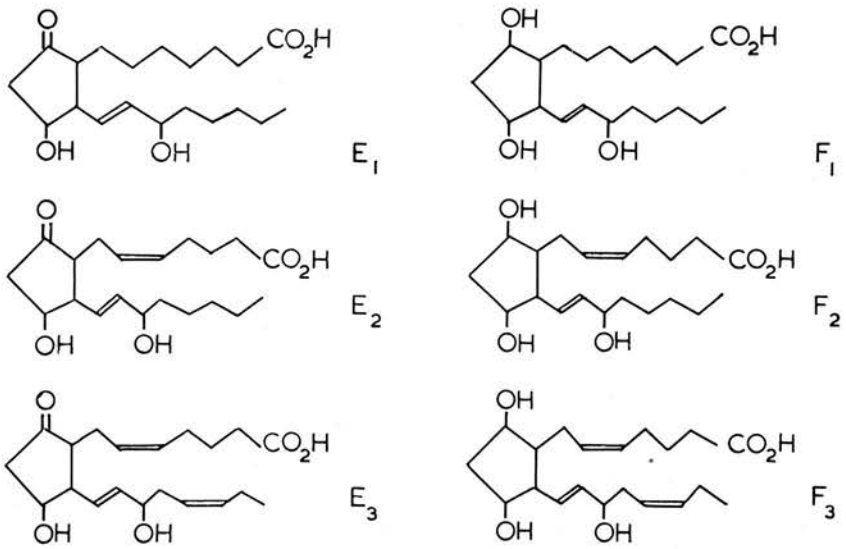
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Fig 2.

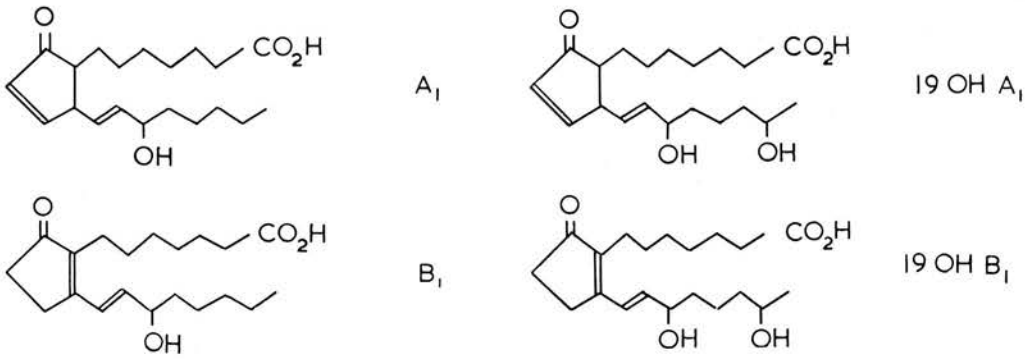
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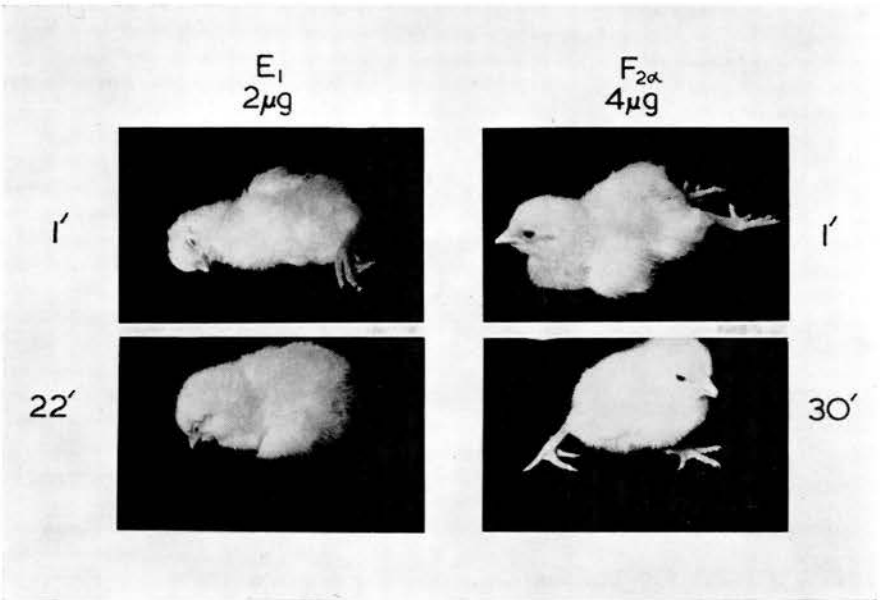
Fig 3.



Horton & Main (1965) Int. J. Neuropharmacol. 4, 65.

Fig 4.

Biosynthesis of prostaglandins

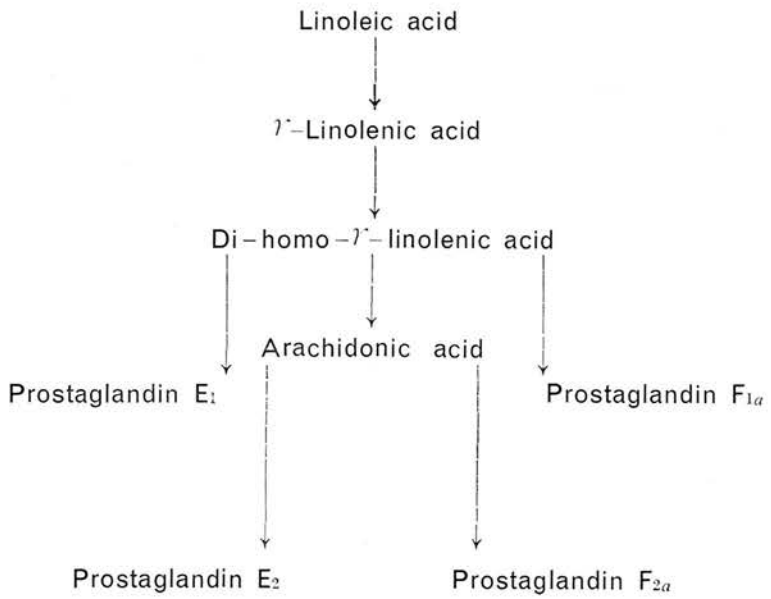


Fig 5.

METABOLISM OF PROSTAGLANDINS

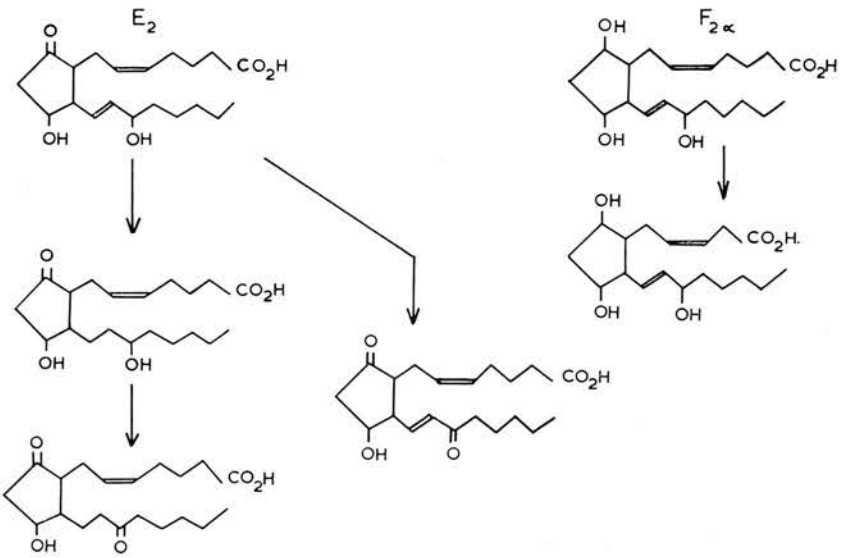
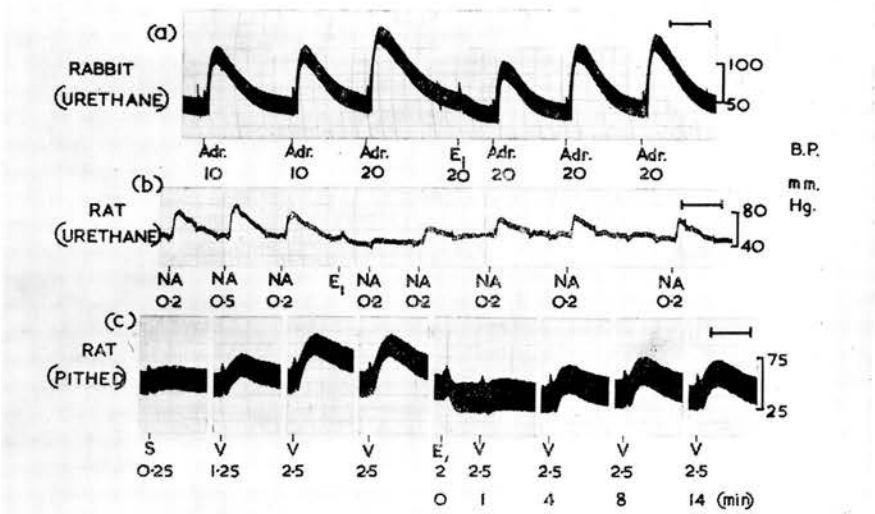


Fig 6.

EFFECT OF E_1 ON PRESSOR RESPONSES



Holmes, Horton & Main (1963) Br. J. Pharmac Chemother 21, 538.

Table 1 Occurrence of prostaglandins in brain.

	$F_{2\alpha}$	E_2	$F_{1\alpha}$	E_1
Ox	+			
Cat	+			+
Rabbit	+			
Dog	+	+	+	+
Chicken	+	+		

Table 2 Animal tissues in which prostaglandins have been identified

Semen and Vesicular gland	Human, sheep
Menstrual fluid and Endometrium Decidua, Amniotic fluid and Umbilical cord vessels..	Human
Lung	Human, monkey, ox, pig, sheep & guinea-pig
Brain	Ox, cat, dog, chicken
Spinal cord	Dog, chicken
Thymus	Calf
Iris	Sheep
Kidney	Pig
Pancreas	Ox

Table 3 Release of Prostaglandins

(see Horton, 1967 for references)

Site	*Stimulus*
Cat somato-sensory cortex	sensory nerve stimulation
Frog spinal cord	sensory nerve stimulation
Rat phrenic nerve-diaphragm	(1) phrenic nerve stimulation (2) noradrenaline
Rat epididymal fat pad	(1) adrenergic nerve stimulation (2) noradrenaline
Cat Ringer-perfused adrenals	acetylcholine
Dog blood-perfused spleen	splenic nerve stimulation
Guinea-pig Ringer-perfused lungs	phospholipase A

measured. In a dose of 50 μg $F_{1\alpha}$ produced a 2-3 fold potentiation of the reflex which lasted more than 4 hours. In contrast prostaglandin E_1 (50 μg.) inhibited the monosynaptic reflex.

I think that the potency of the prostaglandin as centrally acting substances should be emphasised. In spinal chicks doses for threshold effects on spinal reflexes were of the same order as those of strychnine. What is more, (though this may not apply to the chick) on intravenous injection very little prostaglandin E_1 ever reaches the central nervous system. In mice in which E_1 certainly has central actions, autoradiographic studies show that little or no prostaglandin reaches the brain or spinal cord (Hansson & Samuelsson, 1965). It was found chiefly in the liver, the kidneys and connective tissue.

Although prostaglandins have powerful central actions they could not be transmitters unless they are present in the central nervous system and unless they are synthesized there. It has in fact been demonstrated conclusively that prostaglandins do occur naturally in the brains of the species we have been using for the pharmacological experiments Table 1, and they have also been isolated from dog and chicken spinal cord (Horton & Main, 1966; Holmes & Horton, 1967).

Van Dorp (1966) has reported that the brain contains the enzyme responsible for synthesizing prostaglandins from the essential fatty acids as shown in Fig. 4, starting with linoleic acid, a normal constituent of the diet. You will note that the four prostaglandins which occur in dog brain have separate biosynthetic pathways.

If prostaglandins are transmitters it might be expected that they would be released in response to neuronal firing. In fact prostaglandin release occurs spontaneously into the subarachnoid space (Ramwell & Shaw, 1963; Coceani & Wolfe, 1965) and cerebral ventricles (Feldberg & Myers, 1966) from many regions of the brain, and an increase on nerve stimulation has been reported by Ramwell & Shaw (1963). There is no evidence however that the prostaglandin originates pre-synaptically rather than post-synaptically.

The usually accepted view is that a chemical transmitter must be inactivated rapidly following its release. Enzyme systems which metabolise prostaglandins by the pathways shown in Fig. 5 have been described (Anggård & Samuelsson, 1965; Samuelsson, 1965) but so far these enzymes have not been looked for in the central nervous system. It is worth noting however that these two metabolites retain some pharmacological activity on various preparations

(Anggård, 1966). Information about their central nervous actions is not yet available but would be particularly relevant.

If prostaglandins were transmitters, their concentration would be highest in areas rich in prostaglandinergic (or prostanergic) neurones. It might be expected by analogy with other transmitters that these would not be evenly distributed throughout the central nervous system. For example noradrenaline and 5-hydroxytryptamine are concentrated in the hypothalamus and dopamine in the striatum. Recent work in the department here shows that in the dog four different prostaglandins are distributed fairly evenly throughout the various regions of the central nervous system (Holmes & Horton, 1967). We feel that this is evidence against a transmitter role but more information is required. Subcellular fractionation may help to solve this problem and such investigations are in progress.

Whether or not prostaglandins are transmitters in the central nervous system, their role in other tissues is still unexplained. Their widespread distribution (Table 2) is parallelled by a wide variety of pharmacological actions (Horton, 1965; Euler, 1966) and a release from many tissues in response to stimulation of nerves or to drugs on hormones (Table 3).

If the sympathetic nerves to adispose tissue are stimulated prostaglandin E_1 is released (Shaw, 1966), similarly if the splenic nerves are stimulated prostaglandin E_2 is released into the blood stream (Davies, Horton & Withrington, 1967).

Now it is well established that the mediator at sympathetic nerve endings is noradrenaline, and there is no question of prostaglandins usurping that function. When injected to the arterial supply to adipose tissue or the spleen, prostaglandins do not mimic the effects of nerve stimulation as noradrenaline does. In fact in adipose tissue they have the opposite action and inhibit noradrenaline-induced lipolysis (Steinberg, Vaughan, Nestel, Strand & Bergström, 1964; Bergström, Carlson & Orö, 1964). This then raises the question of whether such prostaglandin release could be functionally concerned with limiting the action of the transmitter. This idea of prostaglandins as modulators has been put forward by several groups of workers to explain the antagonism between prostaglandins and catecholamines at various sites, and between prostaglandins and vasopressin on the renal tubule (Orloff & Grantham, 1967).

Fig. 6 illustrates some of these antagonist actions of prostaglandin E_1. It had been reported that E_1 was a potent adrenaline inhibitor (Steinberg, Vaughan, Nestel & Bergström, 1963) and our object was to investigate whether such antagonism was specific or

non-specific (Holmes, Horton & Main, 1963). The figure shows that it is a non-specific antagonism since effects of vasopressin were antagonized. In other tissues for example the guinea-pig uterus prostaglandins potentiate the action of vasopressin (Clegg, Hall & Pickles, 1966) and on the rabbit tracheal muscle the inhibitory action of adrenaline is potentiated (Clegg, 1966).

Thus there are interesting interactions between the prostaglandins and other drugs of animal origin, and it is possible that this is how prostaglandins function within the central nervous system by modifying the action of transmitters rather than being transmitters themselves.

It is likely that we are still far from an understanding of the mode of action and functional significance of the prostaglandins. Certain key pieces of information are needed and certain tools or methods could greatly help in solving some of the outstanding problems. We need to know for example in more detail how prostaglandins produce their effect on neurones—what is the effect on the membrane potential, ionic transport etc. We need to know where in cells prostaglandins are stored and whether this is near to the site of formation. There are many puzzling species differences in response to prostaglandins so that it is dangerous to generalize from animal to another. There is also the danger of talking about prostaglandin as if it was one substance instead of a group of at least 14 not to mention many precursors and metabolites, observations with one substance need not to extend to another. Perhaps our greatest need of all is for specific antagonists for the different prostaglandins and I have little doubt that it will not be long before such compounds are synthesized. These together with drugs which would inhibit specifically the enzyme systems concerned in the biosynthesis, release, uptake and metabolism of the prostaglandins would be of enormous help as tools in the elucidation of the mode of action and significance of these substances.

But there is more to hope for than this. Despite my apparent devotion to truth for the sake of truth, almost, it would seem, to the exclusion of searching for anything that might be useful, we must I think be ever on the look out for possible clinical applications. Experimental medicine and pathology are not far removed from physiology and pharmacology and it may well be, for example, that abnormalities in prostaglandin metabolism could account for certain disease states. The possibility must always be kept in mind. Prostaglandins may not themselves have a therapeutic application but a search for substances which interfere with the biological mechanisims which I have described should prove a rewarding field for anyone interested in breaking into fresh ground from the point

of view of applied pharmacology. Such research may even enable us to understand the mode of action of some drugs already known.

That is the position with regard to my current interests in pharmacology. I like to use drugs of animal origin (at the moment it happens to be the prostaglandins) as tools; first for investigating physiological or pathological mechanisms, secondly for the study of their mode of action and last as a means of searching for new drugs which may have pharmacological activity by acting upon some pathway in the physiological system being considered. Such drugs would at the very least be of great importance as further tools for research, but a therapeutic application is something which I would personally also welcome. Such an application would come as an unexpected and undeserved bonus because this at present is not the primary aim of my research.

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THE PROSTAGLANDINS

Prostaglandin was the name given by von Euler (1935a) to a smooth muscle stimulating lipid of unknown structure, which he discovered in human seminal plasma. Substances with similar biological activity were found in the seminal plasma of sheep and extracts of prostate and vesicular glands (Euler, 1936).

In 1957 Bergström and Sjövall (see Bergström, 1966) reported the isolation of two prostaglandins and later their full chemical structures were announced (Bergström, Ryhage, Samuelsson & Sjövall, 1963; see Bergström, 1966 for full list of references). Since then many other naturally occurring prostaglandins have been identified. Thus the word 'prostaglandin' is no longer used to refer to a single substance, but is a generic term for a family of closely related compounds, all derivatives of prostanoic acid (Bergström & Samuelsson, 1965)(Fig. 1).

A mixture of prostaglandins probably accounts for some or all of the biological activity of several previously unidentified lipids, namely darmstoff (Vogt, 1949; Vogt, Suzuki & Babilli, 1966), the menstrual stimulants (Pickles, 1957; Eglinton, Raphael, Smith, Hall & Pickles, 1963), irin (Ambache, 1957, 1959; Ambache, reynolds & Whiting, 1963), vesiglandin (Euler, 1935b) and medullin (Lee, Covino, Takman & Smith, 1965).

In 1929 Burr and Burr demonstrated that certain unsaturated long chain fatty acids, such as linoleic acid, are essential constituents of the diet (Burr & Burr, 1929). Linoleic acid is converted by a three-stage reaction (Fig. 1) to arachidonic acid in animal tissues and these products of linoleic acid metabolism can substitute for it in the diet. Until 1964 there was little indication as to the physiological role of these essential fatty acids. Then the essential fatty acid field was linked to the prostaglandin field by the important discovery that prostaglandins are synthesized in tissues from arachidonic acid and its precursor di-homo-γ-linolenic acid (Bergström, Danielsson & Samuelsson, 1964; Dorp, Beerthuis, Nugteren & Vonkeman, 1964).

In parallel with these chemical and biochemical advances it has been shown that prostaglandins have a wide variety of pharmacological actions and that they are released from various tissues in response to nerve or chemical stimulation. It seems likely that these compounds have a biochemical role which is fundamental to most if not all animal cells.

Isolation and Identification of Prostaglandins in Animal Tissues

The isolation of prostaglandins from animal tissues (Table 1) and the elucidation of their chemical structure have been reviewed (Bergström & Samuelsson, 1965; Samuelsson, 1965a, b). Table 1 refers only to those organs where a full chemical identification was made; there is good but less complete evidence based upon chromatographic behaviour and biological properties that other tissues and tissue fluids contain prostaglandins. These findings

TABLE 1

Animal tissues from which prostaglandins have been isolated and identified chemically

Source	Prostaglandin					
	E_1	E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$	$F_{3\alpha}$
Semen (human)	*	*	*	*	*	
Semen (sheep)	*	*	*	*		
Vesicular gland (sheep)	*	*	*	*		
Menstrual fluid (human)		*			*	
Endometrium (human)		*			*	
Lung (human)					*	
Lung (monkey)					*	
Lung (ox)					*	*
Lung (pig)					*	
Lung (sheep)		*			*	
Lung (guinea pig)					*	
Brain (ox)					*	
Thymus (calf)	*					
Iris (sheep)					*	
Pancreas (ox)		*			*	
Kidney (pig)		*			*	

An asterisk denotes that the prostaglandin has been isolated from that tissue. Human semen also contains prostaglandins A_1, A_2, B_1 and B_2 and the four corresponding 19-hydroxy-prostaglandins (see Table 3) and pig kidney also contains A_2 (Lee *et al.*, 1965). (Data from Bergström & Samuelsson, 1965.)

TABLE 2

Conversion (per cent) of di-homo-γ-linolenic acid into prostaglandin E_1 by particulate enzyme fractions from different organs (incubation with 25 µg. $C_{20:3}$ and 150 µg. GSH/10 mg. protein for 15 min. at 30°C.)

Organ	Sheep		Guineapig	Rat
	♂	♀	♀	♂
Genital system	70	0·37	0·04	0·38
Intestines	2·8	1·9	0·18	0·00
Lung	0·34		0·06	0·00
Thymus	0·26		—	—
Liver	0·03		0·23	0·02
Kidney	0·01		0·17	0·02
Heart	0·05		—	—
Pancreas	0·01		—	—
Brain	—		0·03	—

(Data from van Dorp, 1966.)

are described under the appropriate biological system.

The extraction of prostaglandins from tissues

Since prostaglandins are fatty acids they can be extracted by partition between a suitable organic solvent (diethyl ether or ethyl acetate) and water. At pH 4 or less, the prostaglandins are almost entirely in the non-ionized form and are more soluble in the organic phase. At pH 8 or more, they are ionized and remain in aqueous solution. This property provides a convenient method for separating prostaglandins as a group (together with other organic acids) from other lipids and most other known biologically-active substances (Samuelsson, 1964a; Horton & Main, 1967c).

By further partition between 67% aqueous ethanol and petroleum, prostaglandins can be separated from organic acids lacking hydroxyl groups for example arachidonic acid.

Various chromatographic procedures for separation of the individual prostaglandins have been described. Partition chromatography on columns of silicic acid and reversed phase chromatography have been used extensively. Gréen & Samuelsson, (1964) have described several solvent systems for thin layer chromatography, a particularly useful method in this field.

Identification of Prostaglandins

The complete structural elucidation of the different

prostaglandins has been achieved using classical and modern analytical techniques (see Bergström & Samuelsson, 1965, for references). In many instances the amounts of prostaglandin present in a tissue extract are too small to permit such extensive analysis. It may then be permissible to base the identification upon chromatographic behaviour and parallel quantitative biological assays. Such techniques are only of use if the substance to be identified has already been isolated, its structure is known and it is available in pure form. Even then only negative evidence can be conclusive, a positive identification is never certain though it may be extremely probable depending upon the amounts and kind of evidence obtained (see Horton & Main, 1967c, for a fuller discussion of this problem).

Estimation of Prostaglandins

The most sensitive preparations for the biological assay of the prostaglandins E are the rat fundus, guinea-pig ileum and guinea-pig uterus. The rat fundus, rabbit jejunum and rat uterus are the tissues most sensitive to prostaglandins F. Other preparations which have been used include the colon of the jird, hamster, rat and guinea-pig, the cat trachea (which is relaxed by prostaglandins) and human myometrium. Little information is yet available about suitable assay preparations for the prostaglandins A and B and their 19-hydroxy derivatives.

A chemical method has been devised for the estimation of the

prostaglandins E which are first oxidized to their 15-oxo derivative by an enzyme in swine lung and then dehydrated to the Δ^{8-12} form with alkali. The chromophore consisting of two oxo groups and two double bonds can be used for absorptiometric estimation (Anggård & Samuelsson, 1966).

Gas liquid chromatography offers the most likely solution to the problem of estimating prostaglandins and their various metabolites. So far the prostaglandins E have been estimated as methyl esters after dehydration to the corresponding B compound, while prostaglandins F have been estimated as trimethylsilyl derivatives also as methyl esters (Bygdeman, Hamberg & Samuelsson, 1966). Using flame ionization detectors the sensitivity is of the order of 50 ng; but it is hoped, by analogy with work in the steroid field, to increase this sensitivity many fold by preparing halogenated derivatives and using an electron capture detector.

Concentrations of Prostaglandins in Tissues

In only a relatively few instances has the concentration of prostaglandin in a tissue been reported. In most cases the workers were concerned primarily in identifying the compound. Exceptions to this are human seminal plasma (see Table 3) and ox brain where a concentration of 300 ng. prostaglandin $F_{2\alpha}$/g. tissue has been reported (Samuelsson, 1964a).

TABLE 3

Concentrations of prostaglandins in human seminal fluid and their threshold concentrations for actions on human myometrium in vitro

μg./ml. semen		Effect on myometrium	Threshold concentration (μg./ml.) Non-pregnant	Pregnant
E_1 25 E_2 23 E_3 5·5	53·5	Inhibition	0·01–0·1	0·03–0·3
$F_{1\alpha}$ 3·6 $F_{2\alpha}$ 4·4	8	Stimulation	0·3–0·5	0·01–0·03
A_1 A_2 B_1 B_2	about 50	Inhibition	0·3–1·0	
19 hydroxy A_1 19 hydroxy A_2 19 hydroxy B_1 19 hydroxy B_2	about 200	Inhibition	1·0–3·0	

(From Bygdeman, Hamberg & Samuelsson, 1966.)

TABLE 4

Actions of prostaglandins on human Fallopian tubes in vitro

	Proximal segment	Distal segments
E_1	E	I
E_2	E	I
E_3	I	I
$F_{1\alpha}$	E	E
$F_{2\alpha}$	E	E
$F_{1\beta}$	E	E
$F_{2\beta}$	I	I

E = excitatory action; I = inhibitory action. (Sandberg, Ingelman-Sundberg & Rydén, 1963, 1964, 1965.)

Metabolism of Essential Fatty Acids and Prostaglandins

The metabolic pathways from linoleic acid and linolenic acid to the prostaglandins and their metabolites are illustrated in Figs. 1 and 2. It should be emphasized that prostaglandins E and F are formed by different routes and that there is no known interconversion between a prostaglandin E and a prostaglandin F (Samuelsson, 1967). The enzyme systems for prostaglandin biosynthesis are widely distributed (Table 2) but are present in largest amounts in sheep vesicular gland homogenates.

An enzyme system in guinea-pig lung homogenates metabolizes prostaglandins E first by the reduction of the 13 double bond followed by oxidation of the 15 hydroxyl (Fig. 1) (Änggård & Samuelsson, 1964a; Änggård, Grëen & Samuelsson, 1965). Pig lung contains an enzyme, 15-hydroxy-prostaglandin dehydrogenase, which oxidizes the 15 hydroxyl without previous reduction of the double bond and which is highly specific for 15-hydroxy-prostaglandins (Änggård & Samuelsson, 1966). Prostaglandins F are metabolized with the loss of a two carbon fragment (Granström, Inger & Samuelsson, 1965). Data on the further steps in the metabolic pathways are not yet available, nor has it been reported which other tissues contain the metabolizing enzymes.

Distribution and fate of injected Prostaglandin E_1

Autoradiographic studies of the distribution of tritium-

labelled prostaglandin E_1 after intravenous injection in mice showed high concentrations of radioactivity in the liver, kidney, subcutaneous connective tissue, blood and uterine muscle during the first 30 min. after injection. After 1 hour, the highest concentrations were in the liver, bile, intestines, kidney and urinary bladder. Some radioactivity was detected in lung, pancreas and salivary glands, but little or none could be seen in the central nervous system (except the choroid plexus), adipose tissue, endocrine glands, heart, blood vessels, organs of the male reproductive tract or the reticulo-endothelial system. In the intestine the highest concentrations were in the lumen and in the connective tissue; there was none in the smooth muscle. There was also a high concentration in the thoracic duct (Hansson & Samuelsson, 1965; Samuelsson, 1966à).

On intravenous infusion of labelled E_1 in rats 50% of the radioactivity was excreted in the urine over a period of 20 hours. About one fifth of the dose was recovered either from the faeces or from a cannulated bile duct. After a subcutaneous injection of labelled E_1 in female rats, the highest concentrations of radioactivity were found in the kidneys and liver and considerably less in the lungs which nevertheless contained a higher concentration than other tissues. The maximal activity in the lungs was reached 5 to 10 min. after injection somewhat earlier than the peak in liver and kidneys. The adrenals, pituitary and heart were similar to the lungs in this respect. As in the mouse experiments little or no radioactivity could be found in brain, skeletal muscle, adipose

tissue or thymus (Samuelsson, 1964b). There is evidence that in the urine, liver and kidney the labelled compound is a more polar derivative of prostaglandin E_1.

Similarly in male and female sheep very high concentrations of tritium were found in liver and kidney following injection of labelled E_1. Relatively high concentrations were also found in the reproductive tract of both sexes, the lungs, adrenals and pituitary (Samuelsson, 1965a).

Pharmacological actions of Prostaglandins

Reproductive Tract

In 1930 Kurzrok and Lieb reported that strips of myometrium taken from fertile women relaxed in the presence of human semen but that similar strips from infertile women contracted. On the basis of these experiments they classified uteri as either receptive (fertile) or rejective (infertile) (Kurzrok & Lieb, 193 It now seems likely that the active principle concerned was a mixture of prostaglandins, since 13 of these compounds have been isolated from human seminal plasma, (Table 3), and since numerous *in vitro* studies indicate that prostaglandins act upon the smooth muscle of both human uterus and Fallopian tubes (Table 3 and 4). The nature of the response depends upon the particular prostaglan and to some extent upon the hormonal status of the preparation (Bygdeman, 1964).

It has been shown that prostaglandins also act upon female

reproductive smooth muscle *in vivo* thus accounting for the known stimulant action of seminal plasma when introduced into the uterus (Cockrill, Miller & Kurzrok, 1935; Karlson, 1959). In pregnant women intravenous infusions of prostaglandin E_1 at a rate of 0.6 µg/min. or more increased the amplitude and frequency of contractions (Bygdeman, Kwon & Wiqvist, 1967). Similarly, in Rubin's test (tubal insufflation) performed at mid-cycle, prostaglandin administered intravaginally increased the resistance to insufflation of the tubes (Eliasson & Posse, 1965). In these experiments the prostaglandins must have been absorbed from the vagina into the circulation; evidence that such absorption can occur was first obtained in rabbits (Asplund, 1947; Horton, Main & Thompson, 1965) and then in non-pregnant women using tritium-labelled prostaglandin E_1 (Sandberg, Ingelman-Sundberg & Ryden, 1967).

It now seems certain that following normal coitus prostaglandins originating from seminal plasma are absorbed from the vagina in amounts sufficient to affect smooth muscle tone in the female reproductive tract. The physiological significance of this hormonal mechanism is unknown though it may be concerned with either sperm transport or retention of the ovum within the Fallopian tube until fertilization has taken place. Certainly there is a suggestion that in some infertile males seminal

prostaglandin levels are lower than normal (Hawkins & Labrum, 1961; Bygdeman, Hamberg & Samuelsson, 1966).

The isolation and identification of prostaglandins E_2 and $F_{2\alpha}$ from human menstrual fluid and endometrium (Eglinton, Smith, Raphael, Hall & Pickles, 1963) coupled with the known action of these substances on the myometrium has raised the question of whether they may be implicated in dysmenorrhea (Pickles, Hall, Best & Smith, 1965). There is evidence that prostaglandin-like substances are relased into the circulation during menstruation and these may account for the associated increase in gastro-intestinal motility.

Prostaglandins have been identified in human amniotic fluid (Karim, 1966) and umbilical cord vessels (Karim, 1967). A possible mechanism for termination of pregnancy by their oxytocic actions can be envisaged. It may also be relevant that 'prostaglandin' constricts placental vessels (Euler, 1938).

Actions of prostaglandins on the reproductive tract smooth muscle of other species have been reported (Tables 5 and 6). In some cases the isolated tissue has proved useful for the biological estimation of prostaglandins, and some preparations have been used for the study of the mode of action of prostaglandins on smooth muscle and their interaction with other hormones and inorganic ions. For example in addition to a direct stimulant action on the guinea-pig isolated uterus the prostaglandins E enhance the contractions

TABLE 5

Actions of prostaglandin E_1 on female reproductive tract smooth muscle of various species

	In vitro (ng./ml.)	*In vivo* (μg./kg.)	Reference
Rat uterus	E (10–100)	—	Bergström, Eliasson, Euler & Sjovall, 1959; Horton & Main, 1963; Sullivan, 1966
Guinea-pig uterus	E (0·5–70)	E(1)	Bergström *et al.*, 1959; Sullivan, 1966; Berti & Naimzada, 1965
Rabbit uterus	E (400)	I (2)	Bergström *et al.*, 1959; Horton *et al.*, 1965
Rabbit cervix	—	I (2)	Horton *et al.*, 1965
Rabbit oviduct	I (50)	I (0·03)	Horton & Main, 1963; Horton *et al.*, 1965
Sheep oviduct	E or I (2·5–800)	E or I (0·4–2·5)	Horton *et al.*, 1965

E = excitatory action; I = inhibitory action. Figures in parentheses refer to approximate threshold concentrations (ng./ml.) or doses (μg./kg.)

TABLE 6

Biological activities of different prostaglandins and analogues in stimulating rat and guinea-pig uterus <u>in vitro</u>, relative to prostaglandin E_1 (=100)

	Rat	Guinea pig
E_1	100	100
E_2	100	—
E_3	30	—
Dihydro E_1	—	30
15-oxo E_1	—	3
15-oxo-dihydro E_1	—	6
nor E_1	100	1
$F_{1\alpha}$	100	3
$F_{1\beta}$	4	0·1
$F_{2\alpha}$	700	—
Di-homo-γ-linolenic acid	6	—
Arachidonic acid	3	—

(Data from Horton & Main, 1966; Änggård, 1966; Pickles, 1967.)

produced by other oxytocic substances added subsequently or by electrical stimulation (Clegg, Hall & Pickles, 1966; Sullivan, 1966). This enhancement does not occur with prostaglandins F nor on the rat uterus (Pickles, Hall, Clegg & Sullivan, 1966) and the enhancement unlike the direct stimulation is unaffected by changes in calcium and magnesium ion concentrations. It has also been shown that unlike human myometrium the hormonal status of the uterus of rat and guinea-pig does not affect their sensitivity to prostaglandins (Sullivan, 1966). The oviduct of the rabbit is inhibited by prostaglandin E_1 (Horton et al., 1965); furthermore contractions of this preparation in response to adrenaline are inhibited. Similar inhibition of adrenaline-induced smooth muscle contractions has been reported with rabbit vas deferens (Holmes, Horton & Main, 1963) but contractions of guinea-pig seminal vesicles are enhanced (Eliasson & Risley, 1966).

Cardiovascular System

The effect of prostaglandins on the cardiovascular system varies with the particular prostaglandin and with the species being studied. For example, prostaglandin E_1 injected intravenously lowers the systemic arterial blood pressure in all species so far investigated whereas prostaglandin $F_{2\alpha}$ raises the blood pressure in the dog, rat and spinal chick (Horton & Main, 1967a; du Charme & Weeks, 1967) but lowers it in the cat and rabbit (Anggård & Bergström, 1963).

The fall in arterial blood pressure may be due partly to vasodilatation, since prostaglandins dilate blood vessels in skeletal muscle and skin (Änggård & Bergström, 1963; Horton, 1963; Horton & Main, 1963, 1965b), and prostaglandin E_1 also antagonizes the vasoconstrictor action of the catecholamines, vasopressin and angiotensin (Holmes et al., 1963). In the blood-perfused rabbit and cat lung preparation prostaglandin E_1 has a vasodilator potency about equal to that of adrenaline, but unlike adrenaline the effect is neither abolished nor reversed by propanolol and is unaffected by phentolamine (Hauge, Lunde & Waaler, 1967).

The pressor action of $F_{2\alpha}$ in the dog and the rat is thought to be due to a venoconstrictor action, and is dependent upon an intact sympathetic nerve supply to the veins (du Charme & Weeks, 1967). The increased venous return increases cardiac output thus accounting for the rise in arterial blood pressure. The mechanism of the pressor action of $F_{2\alpha}$ in the spinal chick is unknown but the response is not blocked by hexamethonium, phenoxybenzamine or pronethalol (Horton & Main, 1967a).

Blood pressure responses to prostaglandins cannot be accounted for satisfactorily by their actions on the heart. E_1 injected intravenously produces tachycardia in humans, dogs and cats whereas $F_{2\alpha}$ causes bradycardia in the cat but has no effect on heart rate in the rabbit. The tachycardia in dogs is abolished by ganglionic

blockade or reserpine (Carlson, 1966). Both E_1 and $F_{2\alpha}$ increase cardiac output in the dog but E_1 reduces cardiac output in man and the rat (Bergström, Duner, Euler, Pernow & Sjovall, 1959; Weeks & Wingerson, 1964).

The responses of the Langendorff perfused heart to prostaglandins has been studied in various species. The force of contraction and rate of the guinea-pig heart is increased by E_1, and effect which is even more striking if the calcium concentration of the perfusing fluid is halved (Berti, Lentati & Usardi, 1965; Mantegazza, 1965). These effects are not abolished by adrenaline β receptor blockade. The force of contraction of the rat isolated heart is increased by $F_{1\alpha}$, but less markedly by E_1, which has a powerful coronary vasodilator action (Vergroesen, de Boer & Gottenbos, 1967). There is a similar increase in the force of contraction of the perfused frog heart in response to E_1, an effect which resembles that of calcium. It is of interest that cardiac phosphorylase in vitro is activated by E_1 (Piccinini, 1965).

Prostaglandins E_2, $F_{1\alpha}$ and A_1 have potent actions in reducing blood pressure in renal hypertensive dogs (Muirhead, Daniels, Pike & Hinman, 1967) and in human hypertension (Lee, Myler, Covino, Smith & Kocot, 1967). Since prostaglandins E_2 and A_2 occur in the kidney (Lee et al., 1965), the possibility of a hormonal role in controlling blood pressure must be considered.

Adipose Tissue

Prostaglandin E_1 in concentrations of 20 ng./ml or more

inhibits the release of glycerol and free fatty acids from rat epididymal fat pads incubated in vitro, whether the release is basal or following the addition to the medium of noradrenaline, adrenaline, corticotrophin, glucagon or thyroid stimulating hormone (Steinberg, Vaughan, Nestel & Bergström, 1963). Similar results have been obtained in vitro with human subcutaneous fat (Bergström & Carlson, 1965).

In anaesthetized dogs intravenous injections and infusions of E_1 in low dosage raise, but in higher doses lower, plasma free fatty acid levels induced by the administration of adrenaline or noradrenaline (Bergström, Carlson & Oro, 1965, 1966a,b; Carlson, 1966; Steinberg, Vaughan, Nestel, Strand & Bergström, 1964). In healthy male volunteers E_1 infusions increased plasma-free fatty acids and glycerol levels (Bergström, Carlson, Ekelund & Oro, 1965a,b; Carlson, 1966), higher doses could not be tested owing to the side-effects of E_1 infusion in man.

There is evidence that the action of E_1 on lipolysis is due to changes in cyclic AMP concentration (Butcher, Pike & Sutherland, 1967; Paoletti, Lentati, Korlkiewicz, Puglisi & Solyom, 1967; Steinberg & Vaughan, 1967).

Since stimulation of the adrenergic nerves to adipose tissue results in the release of prostaglandins in amounts sufficient to inhibit the lipolytic actions of noradrenaline, the possibility that prostaglandin acts by a local negative feed-back mechanism

has been suggested (Shaw, 1966, 1967).

Nervous System

Prostaglandins have several sites of action on the central nervous system (Tables 7 and 8). Injections of E_1, E_2 and E_3 (10 to 60 µg.) into the cerebral ventricles of unanaesthetized cats are followed after a latent period of 20 min. or more by signs of sedation, stupor and catatonia (Horton, 1964). The stupor lasts for several hours and the cat shows diminished spontaneous activity and lack of interest in its surroundings for up to 48 hours after the injection. During the catatonic phase which is maximal between 1 and 4 hours after the injection, the animal can be placed in bizarre postures which are maintained unchanged for periods up to 90 min. When the cat is placed on its side, a forelimb can be flexed so that the paw rests behind the neck or upper thorax - such a position may be retained for several minutes without interference by the observer. On the other hand, the righting reflex is always present and when the cat is disturbed its movements are brisk showing no evidence of ataxia. These responses to intraventricular injection of E_1 have been observed in 14 cats (6 male and 8 female). In marked contrast, $F_{2\alpha}$ produced no detectable changes in four cats following injections into the cerebral ventricles in doses equal to or higher than those of E_1 necessary to produce catatonia in the same animals. Furthermore the prostaglandin precursors, di-homo-γ-linolenic acid

TABLE 7

Central nervous actions of prostaglandins in the cat

aesthetic	State of CNS	Route of injection	Prostaglandin	Response	Reference
	Intact	Lateral ventricle	E_1, E_2, E_3	Sedation, stupor, catatonia	Horton, 1964
			$F_{2\alpha}$	No effect	Horton & Main, 1965a
		Intravenous	E_1	Slight sedation	
	Mid-collicular decerebration	Intravenous	E_1, $F_{2\alpha}$	1. Potentiation of decerebrate regidity 2. Contraction of gastrocnemius muscle	Horton & Main, 1967a
		Iontophoretic	E_1, E_2, $F_{2\alpha}$	Excitation or inhibition of medullary reticulo-spinal neurones (see Table 9)	Avanzino, Bradley & Wolstencroft, 1966
	Spinal section at C2, brain destroyed	Intravenous	E_1	1. Contraction of gastrocnemius muscle, not abolished by acute dorsal root section 2. Potentiation of crossed extensor reflex 3. No effect on patellar reflex	Horton & Main, 1967a
arbitone	Intact	Intravenous	E_1	1. Abolition of tremor 2. Reduction in gastrocnemius muscle tension	Horton & Main, 1967b
		Lateral ventricle	E_1	No effect on tremor	
		Direct application to cerebral cortex	E_1	No effect on tremor	
lose	Intact	Intra-aortic	E_1	Inhibition of spinal mono-synaptic reflexes	Duda & McPherson, personal communication
			$F_{1\alpha}$	Potentiation of spinal mono-synaptic reflexes	
		Iontophoretic	E_1	No effect on cortical neurones	Krnjevic, 1965

TABLE 8

Central nervous actions of prostaglandins in the chick

Anaesthetic	State of CNS	Route of injection	Prostaglandin	Response
None	Intact	Intravenous	E_1, E_2, E_3	Sedation. Loss of righting reflex
			$F_{1\alpha}$, $F_{2\alpha}$	Contraction of extensor muscles
None	Spinal section at C4, decapitated	Intravenous	$F_{2\alpha}$	1. Contraction of gastrocnemius muscle 2. Potentiation of crossed extensor reflex
			E_1	Potentiation of crossed extensor reflex
Chloralose	Intact	Intravenous	$F_{2\alpha}$	1. Contraction of gastrocnemius muscle 2. Potentiation of crossed extensor reflex
			E_1	Inhibition of crossed extensor reflex
Urethane	Intact	Intravenous	$F_{2\alpha}$	1. Contraction of gastrocnemius muscle 2. Abolition of tremor
		Cerebral cortex	E_1	Abolition of tremor
			E_1	Abolition of tremor

(Data from Horton, 1964; Horton & Main, 1965a; 1966, 1967a, 1967b.)

and arachidonic acid are also inactive (Horton & Main, 1965a, 1966).

Intravenous injection of E_1 (20 µg./kg.) in unanaesthetized cats produces transient sedation. A decrease in spontaneous activity of mice lasting about 60 min. is observed following intravenous or subcutaneous injections (Horton, 1965). In the young chick which is believed to lack a blood-brain barrier prostaglandins are particularly active on the central nervous system (Table 8). E_1, E_2 and E_3 in doses of 25 µg./kg. injected intravenously cause sedation with cessation of spontaneous movements, closure of the eyes, and, in higher doses, loss of righting reflexes (Horton, 1964). In contrast, $F_{2\alpha}$ produces not sedation but extension and abduction of the legs and dorsiflexion of the neck (Fig. 1, Horton & Main, 1965a) the effect on the legs is abolished by cutting the sciatic nerve and as it can be elicited in the chick after mid-cervical cord transection it appears to be an action of $F_{2\alpha}$ directly on the spinal cord (Horton & Main, 1967a). This conclusion is supported by experiments in decerebrate and spinal cats in which both E_1 and $F_{2\alpha}$ increase gastrocnemius muscle tension on intravenous injection but not on close-arterial injection to the gastrocnemius muscle. The response in spinal cats is abolished by cutting the motor nerve supply to the gastrocnemius muscle but not by de-afferentation (dorsal root section). These observations are compatible with an action of

prostaglandins in facilitating the firing of α-motoneurones, although an additional effect on γ-motoneurones cannot be excluded. It is not known whether the action is a direct facilitation of excitatory pathways or by inhibition of inhibitory inputs like strychnine. The crossed extensor reflex in the spinal chick and cat is potentiated by both $F_{2\alpha}$ and E_1, but no significant changes have been observed on the mono-synaptic patellar reflex in the cat with E_1 (Horton & Main, 1967a). In the chloralosed cat $F_{1\alpha}$ potentiates but E_1 usually inhibits monosynaptic spinal reflexes recorded by electro-physiological techniques - the effects are very long lasting (Duda, Horton & McPherson, 1968).

In the lightly chloralosed chick or pentobarbitone-anaesthetised cat, tremor or shivering is commonly observed during recovery from anaesthesia; E_1 abolishes this tremor and reduces gastrocnemius muscle tension (Fig. 5) when injected intravenously. However, E_1 injected into the cerebral ventricles of the cat has no effect on tremor whereas on direct application to the cerebral hemispheres of the chick, E_1 abolishes tremor as it does on intravenous injection (Horton & Main, 1967b).

Evidence that these effects are due to an action of prostaglandins on central neurones has been obtained by micro-iontophoretic application to single neurones in the central nervous system. Medullary reticular neurones in the decerebrate cat have been shown to respond to E_1, E_2 and $F_{2\alpha}$ (Avanzino, Bradley & Wolstencroft, 1966). About 20-30% of neurones are excited with

E_1 and a smaller proportion are inhibited, the remainder showing no response (Table 9). Tachyphylaxis was frequently observed but it was short-lasting and there was no cross-desensitization between the three prostaglandins. The effect appeared to be fairly specific since linolenic and di-homo-γ-linolenic acids had a very small effect. There was no obvious correlation between the effects of prostaglandins and acetylcholine applied to the same neurone. Moreover different prostaglandins never had opposite effects on the same neurone.

Other effects of the prostaglandins may be mediated via the central nervous system, for example eye-opening in the anaesthetized chick with intravenous $F_{2\alpha}$, and the depressor component of the blood pressure response to $F_{2\alpha}$ in the anaesthetized chicks with intact brain (abolished by spinal transection). It has been shown that neither E_1 nor $F_{1\alpha}$ releases adrenaline from the adrenals in the cat on close-arterial injection (Horton, 1963, unpublished), nor is the adrenaline output in response to bradykinin inhibited (Horton, unpublished), yet the effects of prostaglandin E_1 on blood glucose levels are dependent upon an intact adrenal medulla in rats (Paoletti et al., 1967). Prostaglandins are released from the adrenals in response to acetycholine (Ramwell, Shaw, Douglas & Poisner, 1965).

Contractions of the cat nictitating membrane elicited by pre-ganglionic cervical nerve stimulation were unaffected by E_1

TABLE 9

Actions of prostaglandins on spontaneously firing brain stem neurones in the decerebrate cat

Response	Prostaglandin E_1	E_2	$F_{2\alpha}$
Excitatory	89	19	40
Inhibitory	9	0	15
No effect	243	50	100
Total number of neurones tested	341	69	155

TABLE 10

Identification of prostaglandins in the central nervous system

Species	Prostaglandin	Reference
Ox (brain)	$F_{2\alpha}$	Samuelsson, 1964a
Cat (supracollicular brain)	$F_{2\alpha}$, E_1	Horton & Main, 1967c
Chicken (brain)	$F_{2\alpha}$, E_2	Horton & Main, 1967c
(spinal cord)	$F_{2\alpha}$, E_2	
Rabbit (cerebral hemispheres)	$F_{2\alpha}$, E_2	Ambache, Brummer, Rose & Whiting, 1966
Dog cortex	$F_{2\alpha}$, $F_{1\alpha}$	Holmes & Horton, 1967
hypothalamus	E_2, E_1	
hippocampus		
caudate nucleus		
cerebellum		
medulla		
spinal cord		

injected either intravenously or into the common carotid artery, although relaxations of the membrane after cessation of stimulation were more rapid in the period immediately following E_1 administration (Holmes et al., 1963). Close-arterial injection of E_1 to the superior cervical ganglion did not modify the contractions due to pre-ganglionic stimulation or to intra-arterial acetylcholine (Horton & Lewis, unpublished).

Prostaglandin E_1 in concentrations from 0.1 to 50 µg./ml applied to exposed blister bases on the human forearm did not give rise to any sensation of pain or itch, whereas bradykinin (0.1 µg./ml.) and serotonin (0.01 µg./ml.) were effective in producing pain on these preparations (Horton, 1963). E_1 (100 µg./ml produced a barely detectable sensation of pain, but it was about half as potent as its precursos, di-homo-γ-linoleic acid, and arachidonic acid (Horton & Main, 1966).

Prostaglandins occur naturally in the brain (Table 10), and they occur in rather similar concentrations in different regions (Holmes & Horton, 1967). They have been detected in ventricular cerebrospinal fluid (Feldberg & Myers, 1966; Holmes & Horton, unpublished) and in perfusates of cerebral cortex, cerebellar cortex and spinal cord (Ramwell & Shaw, 1966; Coceani & Wolfe, 1965; Ramwell, Shaw & Jessup, 1966). The out-put may be increased by nerve stimulation or chemicals.

Although prostaglandins have powerful central actions and

occur in the brain and are released from nervous tissue on stimulation, it is by no means established that these substances are or even could be transmitters. It has been shown that stimulation of peripheral nerves may lead to prostaglandin release from other tissues, for example from the diaphragm (Ramwell, Shaw & Kuchariski, 1965), the spleen (Davies, Horton & Withrington, 1967) and adipose tissue (Shaw, 1966) and at these sites the transmitter is already known. It may be that prostaglandins are released in the central nervous system from post-synaptic membranes in response to stimulation by the transmitter or other substances but that they are not transmitters themselves.

Respiratory System

The bronchoconstrictor effect of vagal stimulation or of histamine is reduced after an intravenous injection of prostaglandin E_1 in guinea-pigs (0.1 µg./kg) and rabbits (1.6 µg./kg). In some preparations a decrease in bronchial resistance after E_1 can be demonstrated without first inducing bronchoconstriction with vagal stimulation or histamine (Main, 1964). In contrast to these results both E_1 (0.3 µg./kg) and $F_{2\alpha}$ (1.5 µg./kg) in the cat increase bronchial resistance as measured by the Konzett & Rossler technique (Main, 1964; Anggård & Bergström, 1963).

An inhibitory action of prostaglandins on respiratory smooth

muscle in vitro has been shown with tracheal chain preparations from several species (Table 11) (Main, 1964). On the cat tracheal chain which is the most sensitive of these, prostaglandins E_1 and E_2 are about equi-active but prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ have only 0.2 and 3% respectively of the activity of the prostaglandins E (Main, 1964; Horton & Main, 1965b).

In view of these differences in pharmacological activity it is of interest that the lungs of most species contain predominantly prostaglandin $F_{2\alpha}$ (Table 12). This led to the hypothesis that prostaglandin $F_{2\alpha}$ might be stored as a pharmacologically less active precursor of prostaglandin E_2 in lung (Horton & Main, 1965b) and in other tissues (Horton, 1965). Biosynthetic studies show however that these two prostaglandins are derived from arachidonic acid by independent pathways and that inter-conversion between the two seems unlikely to occur.

The role of prostaglandins in the lung if unknown. It is of interest that lungs remove prostaglandins from the circulation at a high rate during intravenous infusion as shown by comparing the pharmacological effects of intravenous and intra-aortic injection (Änggård & Bergström, 1963; Horton et al., 1965) and by assaying the amounts of prostaglandins in blood after passage through the pulmonary circulation (Vane, unpublished). Vane has reported that as much as 95% of an infusion of prostaglandin E_1 is taken up by the lungs possibly by reticuloendothelial cells. Whether the lungs are acting purely as filters to remove excessive amounts of prostaglandins or as the target organ for these

TABLE 11

Threshold concentrations (ng./ml) of E_1 for inhibition of isolated tracheal smooth muscle

Species	Before prostaglandin administration muscle contracted by: Acetylcholine	Barium chloride	Inherent tone
Cat	1	< 250	NT
Monkey	20	> 2500	NT
Rabbit	50	NT	NT
Guinea pig	5	< 250	5
Ferret	5	250	5
Sheep	3000	50	> 1000
Pig	250	NT	> 2000

NT indicates no initial tone, even in the presence of the stimulant drug, therefore possible inhibitory actions of E_1 could not be detected. (Data from Main, 1964.)

TABLE 12

Prostaglandins identified in lungs of various species

	$F_{2\alpha}$	$F_{3\alpha}$	E_2
Human	+		
Monkey	+		
Ox	+	+	
Pig	+		
Sheep	+		+
Guinea pig	+		

(Data from Änggård, 1965; Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962; Samuelsson, 1964, 1965.)

TABLE 13

Threshold concentrations (ng./ml) of prostaglandins which contract gastro-intestinal smooth muscle

Preparation	E_1	F_1:	F_2:	Reference
Rat fundus	0·1	—	0·1	Coceani & Wolfe, 1967
Rat duodenum	250	—	—	Horton, 1963
Rat jejunum	10–30	80–300	—	Bergström *et al.*, 1959
Hamster colon	12	50	1·5	Horton & Main, 1963, 1965b
Chicken rectal caecum	7–30	10	—	Bergstrom *et al.*, 1959
Chicken jejunum	8	300	—	
Rabbit duodenum-jejunum	3–10	3–10	0·25–1	Horton & Main, 1963, 1965b; ngÄgård & Bergström, 1963; Bergström *et al.*, 1959
Guinea-pig ileum	10	350	10–50	

contractile response of the guinea-pig ileum to E_1. Spike activity in the guinea-pig taenia coli preparation is increased by E_1.

The isolated rat fundus is particularly sensitive to both prostaglandin E_1 and $F_{2\alpha}$. Contractions are not affected by the presence of atropine, papaverine or serotonin-blocking drugs. Responses are potentiated by procaine, bretylium, dichloroisoprenaline, ascorbic acid, doubling the calcium ion concentration or increasing the potassium ion concentration. Responses to prostaglandins are reduced by isoxuprine, noradrenaline, reduction in bath temperature, reduced oxygenation, carbon monoxide, sodium azide, cyanide ions or reduction in calcium ion concentration (Coceani & Wolfe, 1966).

Threshold concentrations for prostaglandins on various gastro-intestinal smooth muscle preparations are summarized in Table 13.

Prostaglandins are released from intestinal smooth muscle (Vogt & Distelkotter, 1967) and the possibility that they contribute to non-neuronal smooth muscle activity must be considered. The suggestion that circulating prostaglandins account for increased gastro-intestinal motility during menstruation has been mentioned above.

Miscellaneous Actions

The presence of E_1 does not appear to affect the motility of spermatozoa (Horton, 1965), the beating of cilia (Horton, 1965)

or the growth of several pathogenic micro-organisms (Holmes, 1965). On the toad bladder preparation, E_1 inhibits the action of vasopressin which increases permeability to water (Orloff, Handler & Bergström, 1965). This action of E_1 is thought to be exerted on adenyl cyclase (Orloff & Grantham, 1967). E_1 also decreases permeability to water in the isolated renal tubule of the rabbit and inhibits the action of vasopressin on this tissue. E_1 increases capillary permeability (Horton, 1963) possibly by releasing histamine from mast cells, an effect which has been demonstrated in vitro, E_1 being more active in releasing histamine and heparin from mast cells than 48/80 (Paoletti, Vincenzi & Cabut, 1967). This may also account for the oedema observed in rats on continuous infusion of E_1 (Du Charme & Weeks, 1967). It is not known whether these effects of E_1 on permeability are related to the changes in skin permeability to water in essential fatty acid-deficient rats. So far all attempts to treat essential fatty acid deficiency in rats and mice by prostaglandins have proved unsuccessful (Dorp, 1965; Du Charme & Weeks, 1967).

Prostaglandin E_1 can substitute for calcium in blood coagulation (Ferri, Galatulas & Piccinini, 1965) and prostaglandins also have effects on platelet aggregation (Kloeze, 1967). Prostaglandin E_1 inhibits but prostaglandin E_2 enhances aggregation of pig and rat platelets induced by ADP in vitro. With human platelets prostaglandin E_1 inhibits aggregation (threshold

concentration 1-10 ng./ml) but E_2 is inactive.

Substances related to the Prostaglandins

Vesiglandin

Euler (1935a) found that monkey seminal vesicles contained a lipid which lowered blood pressure but differed from 'prostaglandin' in not stimulating isolated smooth muscle preparations. In the light of recent work it seems probable that vesiglandin is a prostaglandin A.

Darmstoff

In 1949 Vogt reported that the frog intestine releases a lipid which stimulates intestinal smooth muscle. In order to obtain larger quantities of this material for chemical identification Vogt prepared acid extracts of horse intestine. He isolated and identified acetylphosphotidic acid. However, on returning to the frog intestine, he found that the original darmstoff was a mixture of fatty acids, the most active of which correspond to the more recently isolated prostaglandin E and F.

Irin

It has long been known that antidromic stimulation of the trigeminal nerve produces an atropine-resistant meiosis in the rabbit. Ambache (1957, 1959) followed up this observation and discovered in rabbit iris tissue a lipid which differed from all other smooth muscle stimulants then known and which he called 'irin'.

This substance produces miosis in the rabbit and probably accounts for the observations seen on trigeminal nerve stimulation. Furthermore, since irin produces vasodilatation it could be concerned in antidromic vasodilatation observed in other regions.

Änggård & Samuelsson (1964b) more recently have identified prostaglandin $F_{2\alpha}$ and several other prostaglandin-like substances in sheep iris. It thus seems probable that irin is a mixture of prostaglandins. Ambache and his colleagues (Ambache et al., 1963) had also found irin in rabbit brain, and here again the active substances appear to be prostaglandins.

Menstrual stimulants

Human menstrual fluid was found by Pickles (1957) to contain smooth muscle stimulating lipids which may have a hormonal function at the time of menstruation. Pickles and co-workers (Eglinton et al., 1963) isolated two of these lipids which they identified as prostaglandins E_2 and $F_{2\alpha}$. There are other unidentified lipids in extracts of menstrual fluids. These remain to be identified.

Medullin

Lee and his colleagues discovered an acidic lipid in rabbit kidneys which has a potent depressor action (Lee et al., 1965). This differed from prostaglandins E_2 and $F_{2\alpha}$ which were also present in the extract because it lacked any non-vascular smooth muscle stimulating activity and because of different chromatographic behaviour. It was identified as prostaglandin A_2 but it is now

disputed whether this substance was not formed from prostaglandin E_2 as an artefact during extraction. Nevertheless its potent depressor action is of great interest.

Concluding Remarks

It is now established that prostaglandins are substances which occur in most, if not all, tissues of higher animals. Furthermore, they are released from certain tissues in response to a variety of stimuli, for example from the brain, spinal cord, spleen and diaphragm on stimulation of the appropriate nerve; from adipose tissue in response to adrenaline and from the adrenals in response to acetylcholine. Their close association with chemical transmission suggests that they may have some modulator function at the synapse or that in certain instances (for example the central nervous system) they could be transmitters themselves.

Pharmacological actions do not necessarily reflect a physiological role, and so one must be cautious in assigning any functional significance to the many and varied pharmacological actions of the prostaglandins. Two points should be emphasized at this stage. Prostaglandins have extremely potent actions on systems as different as adipose tissue, nerve cells, smooth muscle and platelets. Secondly, prostaglandins comprise of a large groups of substances and generalizations cannot be made about the significance of this group as if it were a single substance.

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The Isolation of Bradykinin: A Plasma Kinin from Ox Blood. By D. F. ELLIOTT, G. P. LEWIS and E. W. HORTON. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Bradykinin belongs to a group of peptides which stimulate smooth muscle and have vasodilator activity, and which have been given the generic name of plasma kinins (see Lewis, 1958). It is formed by the action of trypsin on plasma globulins (Andrade & Rocha e Silva, 1956; Rocha e Silva, Beraldo & Rosenfeld, 1949).

In the present investigation ox-serum proteins were fractionated by addition of ammonium sulphate, the solid precipitating between 33 and 45% of saturation being collected and salts removed by dialysis. After treatment at 37° with 0·1N-hydrochloric acid, to inactivate bradykinin-destroying enzymes, the solution was incubated with pure trypsin for 6 hr. The reaction was terminated by addition of boiling ethanol and the substances soluble in 75% ethanol were subjected to a short counter-current distribution to remove lipoid material and salt.

Further purification was carried out by elution chromatography on two successive carboxymethylcellulose columns in ammonium acetate buffer at pH 6·5 and 5. The solid remaining after evaporation of the buffer contained 10–25% of pure bradykinin.

Complete purification by paper chromatography or paper electrophoresis was hampered by the presence of magnesium in this peptide mixture; same spectrographic analysis revealed the preflence of approximately 1% by weight. It was therefore necessary to remove at least the greater part of the magnesium by performing the second carboxymethylcellulose-column fractionation in presence of ethylenediaminetetra-acetic acid. These results suggest that bradykinin has a high affinity for magnesium ions and this may have some bearing upon its mode of action.

The pure peptide was obtained by preparative paper electrophoresis. On acid hydrolysis bradykinin yielded serine, glycine, proline, phenylalanine and arginine in the molar proportions 1:1:2:2:2. The purity was confirmed by the presence of a single *N*-terminal residue, that of arginine. The biological activity of the pure polypeptide has been found to be comparable with that of histamine and acetylcholine.

The authors wish to express their thanks to Miss M. Kinsella and to Messrs R. Blakemore, A. W. Hemmings and E. Stevens for experimental assistance.

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Biological activity of pure bradykinin—a plasma kinin from ox blood. By D. F. Elliott, E. W. Horton and G. P. Lewis.
National Institute for Medical Research, London, N.W. 7

The plasma kinin bradykinin has been isolated and was found to contain the following amino acid residues: arginine, phenylalanine, proline, serine, glycine, in the proportion 2:2:2:1:1 (Elliott, Lewis & Horton, 1959).

In the present investigation the biological activity of the pure polypeptide has been compared with a crude preparation similar to those used in most other investigations. The two were compared on the following isolated smooth-muscle preparations, the guinea-pig ileum, rat uterus, rat duodenum and rabbit duodenum. In addition, comparisons were made for vasodilatation in the skin of cat hind limb, for depressor activity by intravenous injection into cats and rats, and for effect on capillary permeability by intradermal injection into the skin of guinea-pigs and rabbits. It was found that pure bradykinin was active on all these preparations and within the errors of biological variation the increase in activity from the crude material was proportional in all tests.

All these activities are therefore inherent properties of the pure polypeptide which is probably mainly responsible for the biological activity of the crude mixture of polypeptides. However, the observations do not exclude the possibility of the presence of other active peptides differing only quantitatively from bradykinin.

The active concentrations of the pure polypeptide varied between 0·2 ng/ml. required to contract the rat uterus and 400 ng/kg to cause a depressor response in cats. On a molar basis, bradykinin was approximately as active as acetylcholine as a vasodilator and about 10 times more active than histamine in increasing capillary permeability.

Bradykinin therefore ranks among the most active of biological substances, and may be responsible for all the actions of the group of polypeptides which have been classified under the name of plasma kinin.

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THE REFLEX RESPONSE TO INTRA-ARTERIAL NOCICEPTION WITH BRADYKININ

Fed. Proc. 20, 310 (Mar 61)

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The intra-arterial injection of agents algesic in man (bradykinin, acetylcholine and KC1) into somatic and splanchnic areas evokes in the chloralose cat or dog the same reflex response (viz. vocalization, hyperpnea and hypertension) as electrical stimulation of the sciatic nerve. This response is thus identical with the pseudaffective response of Woodworth and Sherrington, (1904) obtained by nociception in decerebrate preparations. The reflex nature of this response is demonstrated by the fact that unilateral dorsal root ganglionectomy (L2 to S1) prevents the response to intra-arterial injection of algesic agents on the same side, whereas unilateral sympathectomy does not. Although other receptors are stimulated, only the nociceptors evoke vocalization. The vocalization response, taken as signal of pain, can be partially or completely blocked by narcotic and non-narcotic analgesics. Nociceptors are known to be present in skin, but since the algesic agents will evoke the same response after skin removal, and after intra-arterial injection into the spleen or intestine, they are located also below the skin. These visceral nociceptors are peri-vascular and appear to be located near capillaries. Bradykinin being active in 1.2 mcg dose may be a specific algesic agent.

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Thin layer chromatography and bio-assay of prostaglandins

By E. W. Horton and C. J. Thompson. *Miles-Ames Research Laboratories, Stoke Poges, Buckinghamshire*

Several prostaglandins have been isolated from lung, prostate and semen by Bergström and his co-workers (Bergström & Sjövall, 1960; Bergström & Samuelsson, 1962; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962; Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962). The following simple method has been used to estimate prostaglandins in extracts of tissues and tissue fluids, especially semen.

Samples of human semen are mixed with pH 4·0 phthalate buffer, extracted three times with ether and the extract evaporated to dryness. The weighed residue is dissolved in a small volume of methanol–chloroform (1:1) and a solution containing approximately 2 mg applied to a 20 cm plate (silica gel 250 μ thick) and run in petroleum ether 40, ether 50, acetic acid 5, methanol 5 parts. A marker plate is run simultaneously with pure prostaglandin E_1 (R_F 0·25–0·35, which was kindly provided by Professor Bergström). The spots are located under U.V. light after spraying with fluorescein and contrasting with bromine vapour. The spot corresponding to prostaglandin E_1 is scraped off and eluted with methanol, the solution evaporated to dryness, and the residue dissolved in 1 ml. of water.

The solution is assayed in parallel on the atropinized rabbit duodenum and the hamster colon against prostaglandin E_1.

The method is sensitive (1 μg prostaglandin E_1 can be detected) and specific. Other pharmacologically active substances are distinguished by their different chromatographic behaviour and by the parallel assays.

Supplies of semen from fertility clinics were obtained through the kind co-operation of Dr D. F. Hawkins of University College Hospital Medical School.

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The action of intravaginal prostaglandin E_1 on the female reproductive tract

By E. W. Horton, I. H. M. Main and C. J. Thompson. *Miles-Ames Research Laboratories, Stoke Poges, Buckinghamshire*

In 1947 Asplund reported that crude prostaglandin extracts injected intravenously inhibit tubal peristalsis (Asplund, 1947). He further claimed that the same effect could be observed following intravaginal administration. Since high concentrations of prostaglandins are found in semen, effects following intravaginal administration are of particular interest.

Blood pressure and intraluminal pressure of one Fallopian tube were recorded with Statham transducers. Pure prostaglandin E_1 in doses of 1 μg/kg or more injected intravenously in the urethanized rabbit reduced tubal tone and peristalsis. This was accompanied by a fall in blood pressure. Prostaglandin E_1 is a potent vasodilator, but other vasodilators such as acetylcholine and bradykinin produced a similar fall in blood pressure without inhibition of the tubes.

Intravaginal administration of higher doses of prostaglandin was followed after a variable latent period by similar inhibition of tubal tone and peristalsis. This observation indicates that prostaglandin is absorbed from the vaginal mucosa into the blood, thence acting upon the Fallopian tubes. The possibility that prostaglandin might have reached the Fallopian tube from the vagina directly via the uterine lumen is excluded since the psil ateral horn was always ligated, and sometimes divided.

In other experiments contractions of the cervix were shown also to be inhibited following prostaglandin E_1 but no change in the tone of uterine horn smooth muscle could be detected.

These results provide some support for the hypothesis that prostaglandins may be important in promoting conception by reducing smooth muscle tone at those points where spasm is most likely to occur, and thus allowing easier access of the sperm to the ovum. If this proves to be a physiological mechanism, it will be an interesting example of a hormone produced by one individual and acting upon the tissues of another.

We are grateful to Professor S. Bergström for a gift of pure prostaglandin E_1.

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Congenital Cloaca in a Ram

Sir,—A 15-month-old crossbred sheep (Kerryhill ram × Border Leicester ewe) supplied for physiological investigations was found on examination to have only one external orifice in the perineal region. There was no vagina, no penis and no visible urethral orifice. When pressure was applied to the urinary bladder *post mortem*, foul-smelling faecal-contaminated urine escaped through the anus. The urethral opening was located in the ventral wall of the rectum approximately 3 cm. from the anal sphincter. Although the urine in the bladder was faeculent, there were no macroscopic signs of infection of the urinary tract. Both kidneys appeared healthy.

The sex of the animal was determined by the presence of well-developed vasa deferentia and by the absence of female genital organs. The proximal ends of the vasa opened into the upper urethra, around which there was glandular tissue. The position suggested this to be seminal vesicles and prostatic tissue. Distally, the vasa could be traced through the inguinal canals and were found to terminate as thin bands merging superficially with the surrounding connective tissue. Neither testes nor epididymes were present, and there were no signs of a scrotum. As the early history of this animal is unknown, the absence of testes may be attributable to castration. In support of this is the finding that the vasa had migrated through their respective inguinal canals. It is unlikely that this would have occurred if testes had not, at some time, been present.

On the other hand, the position of the urethral orifice, and the cloaca so formed, must represent a congenital, not an acquired, abnormality. This primitive arrangement resembles that found in lower vertebrates. Amongst mammals, only the monotremes have a true cloaca, into which the urethra opens both directly and *via* the penis which itself forms part of the ventral wall of the cloaca (Parkes, 1956). The congenital abnormality described here could be regarded as a partial reversion to the monotreme type of anatomy.

January 14th, 1964. Yours faithfully,

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I. H. M. MAIN.

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CENTRAL NERVOUS ACTIONS AND OCCURRENCE OF THE PROSTRAGLANDINS

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Estratto dal volume "Drugs of animal origin"

FERRO EDIZIONI - MILANO

CENTRAL NERVOUS ACTIONS AND OCCURRENCE OF THE PROSTAGLANDINS

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In unanaesthetized cats prostaglandins E_1, E_2 and E_3 (10 to 60 μg) injected into the cerebral ventricles produce stupor and catatonia but these effects are not observed following intravenous injection nor do they occur on injection of prostaglandin $F_{2\alpha}$ (15 to 100 μg) into the cerebral ventricles. On intravenous injection in young chicks prostaglandin E_1, E_2 and E_3 (10 to 200 μg/kg) produce sedation with loss of the righting reflex in higher doses whereas $F_{1\alpha}$ and $F_{2\alpha}$ (25-450 μg/kg) produce extension of the neck and legs without any signs of sedation (Horton, 1964; Horton & Main, 1965a; 1966a). Catatonia, stupor and sedation are difficult to quantitate whereas the extension of the legs observed after $F_{2\alpha}$ could be measured in the anaesthetized chick.

In chicks lightly anaesthetized with chloralose or urethane an intravenous injection of $F_{2\alpha}$ (2 μg/kg) was followed by an increase in tension of the gastrocnemius muscle, thus confirming that the extension of the legs seen in the unanaesthetized chick was due to contraction of the extensor muscles. The effect could not be obtained in deeply anaesthetized chicks, suggesting that it was central. Similar responses were obtained with $F_{2\alpha}$ in the decapitated chick spinalized in the midcervical region, indicating that the brain was not necessary for the mediation of this effect. The response was abolished by acute denervation of the gastrocnemius muscle showing that $F_{2\alpha}$ does not produce its effect by an action on the neuromuscular junction or the skeletal muscle.

Similar effects were observed with both $F_{2\alpha}$ (5-30 μg/kg) and E_1 (5-50 μg/kg) in the decerebrate and spinal cat. Again, the contraction of gastrocnemius muscle was abolished by cutting the sciatic nerve but not by de-afferentation. Furthermore contraction of the gastrocnemius muscle did not occur when E_1 (50 μg) was injected close-arterially to the muscle either in the innervated, acutely denervated or chronically denervated preparation. It is concluded from these results that $F_{2\alpha}$ in the chick and E_1 and $F_{2\alpha}$ in the cat produce contraction of the gastrocnemius by an action on the spinal cord possibly by facilitation of the α-motoneurones.

Potentiation of the crossed extensor reflex in the spinal chick and cat was observed following the injection of $F_{2\alpha}$ or E_1. The effect was prolonged and was observed with smaller doses than those necessary to produce contraction of the gastrocnemius muscle. The monosynaptic patellar reflex in the cat was not usually potentiated by E_1.

In addition to these effects on the spinal cord, it is evident that prostaglandins also have actions on higher centres. For example, in the lightly anaesthetized chick

(urethane) or cat (pentobarbitone, with intact brain) E_1 (3 μg/kg) injected intravenously reduced gastrocnemius muscle tension and abolished tremor or shivering which is commonly observed during recovery from these anaesthetics. This is in marked contrast to the *increase* in gastrocnemius tension described above and observed in the spinal and decerebrate cat. Some evidence that this effect is mediated via higher centres was obtained by direct application of E_1 to the cerebral hemispheres of the chick; tremor was abolished and gastrocnemius muscle tension reduced. On the other hand, tremor in the pentobarbitone-anaesthetized cat could be abolished only by intravenous injection of E_1, not by injection into the cerebral ventricles (30 μg).

In the spinal chick $F_{2\alpha}$ (2 μg/kg) had a pressor effect, but in other preparations it had depressor effect. Prostaglandin E_1 was depressor in all preparations studied. The pressor effect of $F_{2\alpha}$ in spinal chicks is not abolished by hexamethonium or phenoxybenzamine, and therefore does not seem to be mediated via sympathetic nervous pathways.

We conclude that there is good evidence that $F_{2\alpha}$ and E_1 act on the spinal cord (Horton & Main, 1965b) and that E_1 acts on higher centres in the brain. Microiontophoretic application of E_1 and $F_{2\alpha}$ to single cells has confirmed that these substances do have actions on central neurones in the cat (Avanzino, Bradley & Wolstencroft, 1966).

While these observations were being made, Samuelsson (1964) announced the isolation of prostaglandin $F_{2\alpha}$ from ox brain. We have used his separative procedures to isolate the prostaglandins in cat brain, chicken brain and chicken spinal cord.

On silicic acid chromatography peaks of biologically-active material corresponding to prostaglandins E and F were found. By a combination of thin layer chromatography on two systems and parallel biological activity, we have shown that $F_{2\alpha}$ is present in cat fore-brain (supracollicular) and that both E_2 and $F_{2\alpha}$ are present in chicken brain and spinal cord (Horton & Main, 1966b).

Ramwell & Shaw (1963) have shown that prostaglandins can be identified in the superfusates of cat cerebral cortex and frog spinal cord. This evidence together with the identification of prostaglandins in the central nervous system and their potent central nervous actions raises the question of their physiological significance. The possibility that these substances are central transmitters cannot yet be excluded. However, their widespread occurrence and variety of pharmacological actions is suggestive of a modulator role possibly affecting a biochemical pathway which is common to several different tissues.

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Acknowledgments

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Prostaglandins were kindly supplied by Professor S. Bergström, Dr. B. Samuelsson and Dr. D. A. van Dorp.

[*From the Proceedings of the Physiological Society*, 25–26 *March* 1966]
Journal of Physiology, **185**, 36–37 *P*

The identification of prostaglandins in central nervous tissues of the cat and the fowl

By E. W. Horton and I. H. M. Main. *The Department of Physiology, Medical College of St Bartholomew's Hospital, Charterhouse Square, London, E.C.* 1

Brain extracts contain several biologically active lipids, one of which has been isolated (from bovine brain) and identified as prostaglandin $F_{2\alpha}$ (Samuelsson, 1964).

We now report the identification of prostaglandins in extracts of central nervous tissues from the cat and the fowl, the species in which prostaglandins have been observed to have pharmacological actions on the brain and spinal cord (Horton, 1964; Horton & Main, 1965*a*, *b*; Avanzino, Bradley & Wolstencroft, 1966).

Cerebral tissue removed at inter-collicular decerebration under ether was used as the source of cat brain. Whole brains and spinal cords from adult fowls were dissected out after exsanguination. The tissues were homogenized and extracted by Samuelsson's (1964) procedure which briefly consists of ethanolic extraction, solvent partition and silicic acid column chromatography. When the cat brain extracts were chromatographed on silicic acid, a small peak corresponding to prostaglandin E and a large peak corresponding to prostaglandin F were obtained.

The fractions from the F peak were pooled and chromatographed on thin layer plates of silica gel G in Gréen & Samuelsson's (1964) A I solvent system which separates prostaglandin E's from prostaglandin F's. All the biological activity was recovered from the zone corresponding to prostaglandin F. The material was then chromatographed on thin layer plates of silica gel containing 4% silver nitrate using the A II solvent system of Gréen & Samuelsson, which separates prostaglandins according to their degree of unsaturation. All the active material was eluted from the zone corresponding to prostaglandin $F_{2\alpha}$. In all experiments a marker plate was run simultaneously and the rabbit isolated jejunum was used for the biological assays.

On the basis of parallel assays on six smooth muscle preparations, of the silicic acid chromatography, and of the thin layer chromatography in the A I system, it is concluded that the substance isolated from cat brain is a prostaglandin F; it could not be a prostaglandin E.

There is no reliable way of distinguishing between different prostaglandin F's by parallel biological assay (Horton & Main, 1965*c*). Thus the identification of the prostaglandin F from cat brain as $F_{2\alpha}$ is based on its

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chromatographic behaviour in the A II system, which certainly excluded the possibility that it was either prostaglandin $F_{1\alpha}$ or $F_{3\alpha}$. Like $F_{2\alpha}$, it potentiated the crossed extensor reflex and had a pressor effect on intravenous injection in the spinal chick.

The presence of a PGE peak on silicic acid chromatography was suggestive that cat brain also contains a prostaglandin E, but there was insufficient material for conclusive identification to be made.

Using similar techniques we have identified prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in both the brain and the spinal cord of the fowl.

Grants from the Medical Research Council are gratefully acknowledged. We thank Dr D. A. van Dorp for prostaglandins E_1 and $F_{1\alpha}$ and Dr B. Samuelsson for prostaglandins E_2 and $F_{2\alpha}$.

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[*From the Proceedings of the Physiological Society*, 20 *May* 1967]
Journal of Physiology, **191**, 134–135*P*

The nature and distribution of prostaglandins in the central nervous system of the dog

By S. W. Holmes and E. W. Horton. *The Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.* 1

Eighteen dogs were anaesthetized with pentobarbitone sodium and exsanguinated. The required areas of the central nervous system (no more than four from any one animal) were dissected out, weighed and macerated in ice-cold ethanol. The time from death until the samples were placed in ethanol varied from 2 to 10 min. The extraction procedure, previously described by Horton & Main (1966), separated the polar acidic lipids including the prostaglandins.

Substances which behaved chromatographically (column and thin layer) and biologically (rat fundus and rabbit jejunum) like prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were isolated from whole brain and also from each region of the central nervous system examined. There were no large differences in prostaglandin concentration between regions (Table 1). With the exception of the prostaglandin E content of the medulla and white matter, variations could more probably be attributed to variations between individual dogs than between areas.

Table 1. Distribution of prostaglandins in the central nervous system of the dog. Total prostaglandins E expressed in terms of E_1 and total prostaglandins F in terms of $F_{1\alpha}$. All values are uncorrected for recovery and are expressed in terms of ng/g tissue. The number of estimations is given in brackets followed by the ranges

Area	Prostaglandins E	Prostaglandins F
Cortex	86 (13) 20·5–185	147 (13) 58–389
Hippocampi	62 (3) 19–140	195 (3) 180–214
Caudate nuclei	72 (2) 34–109	149 (2) 143–154
Hypothalamus	60 (3) 24–109	200 (3) 154–240
Cerebellum	107 (2) 75–138	144 (2) 107–180
Medulla	21 (3) 2·5–39	130 (3) 80–178
Spinal cord	104 (3) 65–168	191 (3) 56–416
White matter	27 (1)	106 (1)
Cortex (from dog anaesthetized with bromethol)	184 (1)	204 (1)

This widespread central nervous distribution is compatible with the reports that prostaglandin-like substances are released from many regions of the brain (anterior and inferior horns of the lateral ventricles, the third ventricle and the subarachnoid space over the cerebellar and somatosensory cortex) and from the perfused spinal cord (Coceani & Wolfe, 1965; Feldberg & Myers, 1966; Ramwell & Shaw, 1966; Ramwell, Shaw & Jessup, 1966).

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On the basis of the occurrence, release and pharmacological actions of prostaglandins a role as transmitter at central nervous synapses might be postulated. The presence of four different prostaglandins, each known to have its own independent biosynthetic and metabolic pathway, would necessitate the postulation of four such transmitters. The even distribution of these four substances throughout the brain is however suggestive of some other function.

Grants from the Medical Research Council are gratefully acknowledged. We thank Dr D. A. van Dorp of Unilever Laboratories, Vlaardingen, and Dr J. E. Pike of the Upjohn Company, Kalamazoo, for supplies of prostaglandins.

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Journal of Physiology, **188**, 38–39*P*

The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation

By B. N. Davies, E. W. Horton and P. G. Withrington. *Department of Physiology, St Bartholomew's Hospital Medical College, London, E.C.* 1

Prostaglandins are widely distributed in animal tissues (Bergström & Samuelsson, 1965) and have numerous pharmacological actions (Horton, 1965). Shaw (1966) showed that prostaglandins are released from the rat epididymal fat pad *in vitro*, in response to sympathetic nerve stimulation. Whether prostaglandins are released from other organs with an adrenergic innervation is unknown.

To examine this possibility, experiments have been made on the isolated dog spleen, perfused with blood from the femoral artery of a donor dog. The splenic venous blood was returned to the femoral vein of the donor except during the collection of a sample, when it was diverted out of a side arm. During the collection of a sample, blood from a reservoir was pumped into the donor. Electrodes were placed on the splenic nerves and the spleen was bathed in liquid paraffin at 37° C.

Splenic venous blood collected during and after electrical stimulation (600 impulses at 10/sec) of the splenic nerves, contained prostaglandin E_2 in concentrations up to 200 ng/ml., whereas blood collected in the absence of stimulation contained no detectable prostaglandin (< 1 ng/ml.).

The evidence that the substance in splenic venous blood was prostaglandin E_2 is as follows. It was soluble in 96% aqueous ethanol, and it behaved like a polar acidic lipid on solvent partition. On silicic acid column chromatography it had an elution time corresponding to a prostaglandin E. On thin layer chromatography in Gréen & Samuelsson's (1964) A I solvent system, it had an R_F of 0·54 compared with prostaglandin E_1 ($= E_2$) 0·54, and $F_{1\alpha}$ 0·37, and in their A II solvent system it had an R_F of 0·53 compared with E_2 0·52 and E_1 0·74. The substance isolated by this extraction procedure was assayed against prostaglandin E_2 on the rat fundus, guinea-pig ileum and rabbit jejunum. The results were in close agreement (0·6, 0·6 and 0·7 μg E_2 respectively).

Other acidic lipids which contract the rat fundus were also present in blood following nerve stimulation. These lipids have not been identified, except in one experiment in which some prostaglandin F was detected.

Phenoxybenzamine given by close arterial injection in a dose (5 mg) sufficient to block the response of the spleen to nerve stimulation, abolished the output of prostaglandin.

The prostaglandin could originate from either the sympathetic nerves

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themselves, or from the smooth muscle of the spleen as a result of its response to stimulation. It is unlikely that it originated from adipose tissue adjacent to the spleen because nerve stimulation did not increase the output of free fatty acids.

This work was supported by Grants from the Royal Society and the Medical Research Council. Prostaglandins were kindly supplied by Dr D. A. van Dorp.

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Proceedings of the Biochemical Society

In the press

Problems in the Identification of Submicrogram Amounts of Prostaglandins

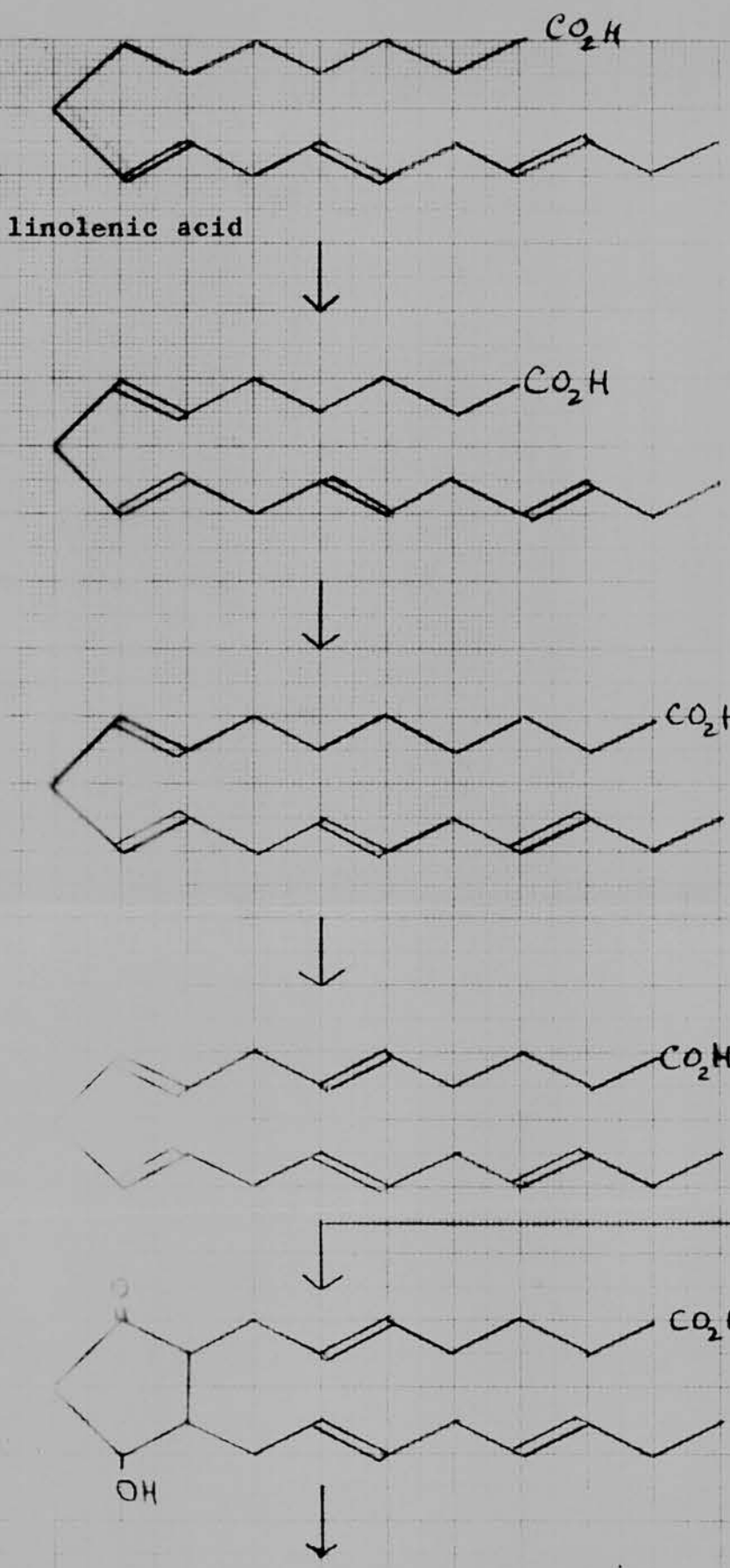
By E. W. Horton. (*Department of Pharmacology, The School of Pharmacy, University of London*)

The word 'prostaglandin' is a generic term for a family of closely-related compounds, all derivatives of prostanoic acid (Bergström and Samuelsson, 1967). Some twenty of these fatty acids have been isolated from animal tissues or tissue fluids and their full chemical structure has been elucidated. Most of these compounds are known to be directly or indirectly biosynthesized from the straight chain unsaturated fatty acids, dihomo-γ-linolenic and arachidonic acid.

Prostaglandins are not only widely distributed in animal tissues but are released from certain organs into the surrounding medium or blood either spontaneously, on nerve stimulation or in response to drugs. In such experiments the amount of prostaglandins released, for example from the brain, may be less than 0·1 μg./hr. (prostaglandin E_1 equivalent). Such minute amounts can, at present, be detected only by bioassay techniques. The question is raised as to how much evidence is needed to justify the claim to have identified a particular prostaglandin? Ideally a complete chemical analysis with structural elucidation should be performed. Even with the most favourable conditions it is unlikely that such information could be obtained from mass spectrometric analysis using a single sample containing less than 1 μg. of isolated compound.

The problem is, therefore, to identify prostaglandins in a sample containing amounts (1 μg. or less) insufficient for mass spectrometric or other chemical analysis. This can only be attempted if samples of pure prostaglandins of known structure are available for comparison. Isolation of the prostaglandin or prostaglandins can be achieved by solvent partition and a combination of column and thin-layer chromatographic procedures. Location of the prostaglandin in particular fractions or zones is dependent upon biological assay. Chromatographic evidence alone does not provide positive evidence of identification although it may be possible to conclude that an unknown substance is *not* a particular prostaglandin on the grounds of a difference in chromatographic behaviour. Further evidence of identification may be obtained from quantitative parallel biological assays on several tissues. By using suitable combinations of tissues it is easy to distinguish a prostaglandin E from a prostaglandin F, but difficult to distinguish say prostaglandin E_1 from prostaglandin E_2 which are very similar in their biological activities. It may thus be possible to conclude with confidence that an isolated substance is not, say, prostaglandin $F_{2\alpha}$ because of its different chromatographic behaviour and/or lack of agreement between the parallel assays. On the other hand, agreement between the behaviour of the isolated substance and a particular prostaglandin in all these respects does not provide conclusive evidence. It is permissible to conclude, under these circumstances, that the chromatographic and biological properties of the unknown are compatible with its identification as a certain prostaglandin. The more parallel assays between which agreement is obtained, the greater is the probability that a correct identification has been made.

Bergström, S. & Samuelsson, B. (1967). *Prostaglandins.* Proceedings of the Second Nobel Symposium held in June 1966. Stockholm: Almqvist & Wiksell.



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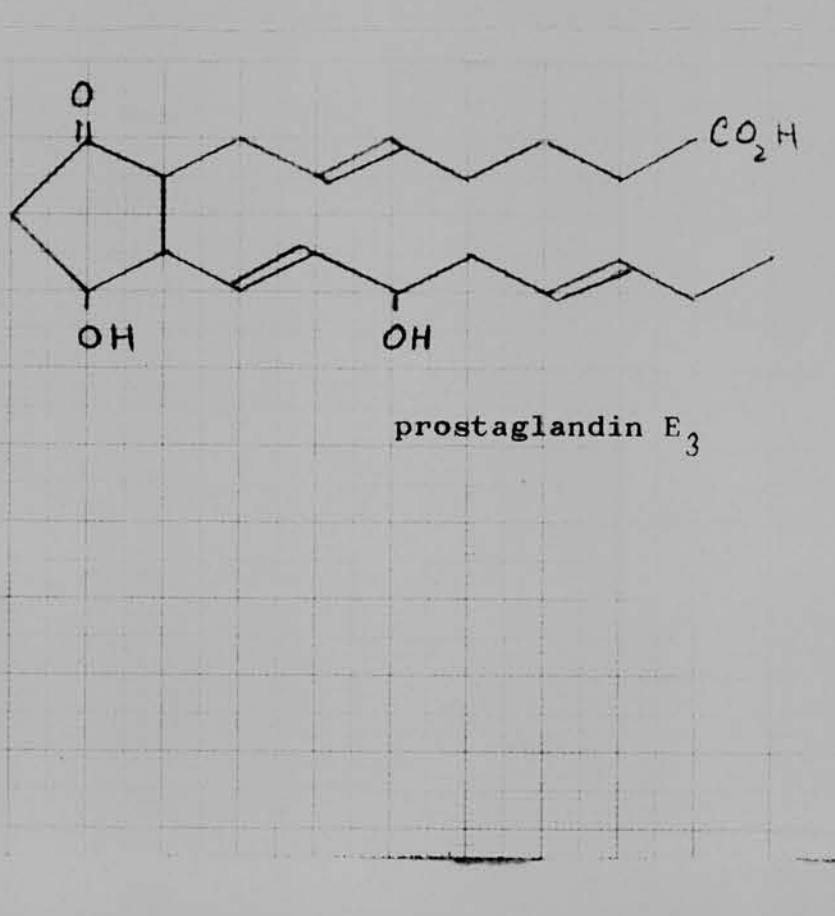

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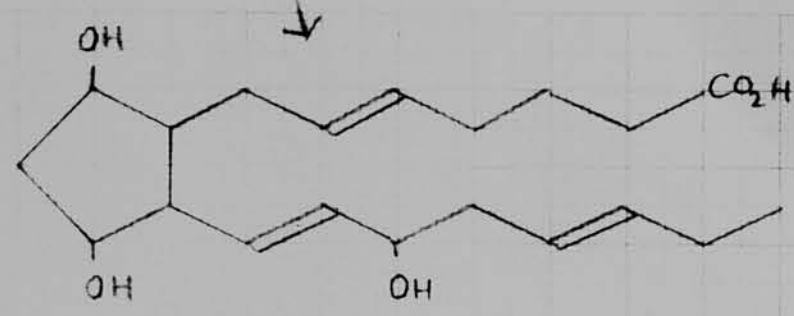

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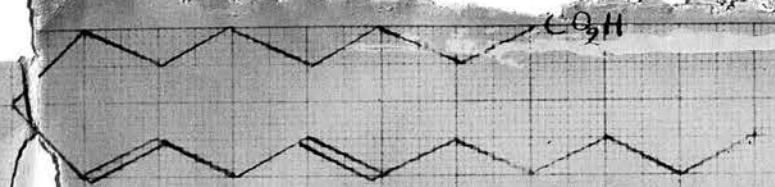

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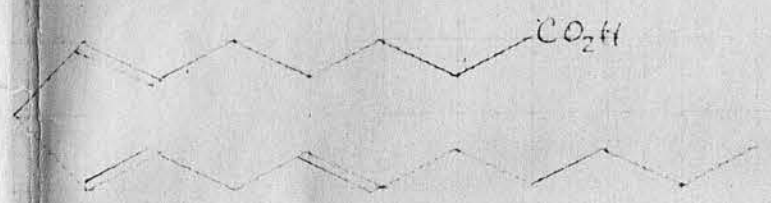

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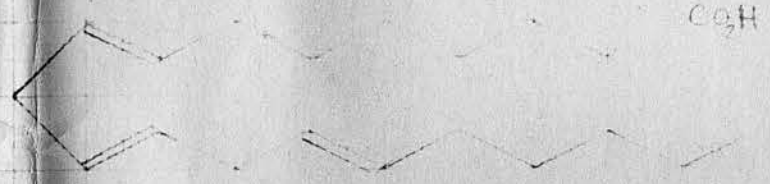

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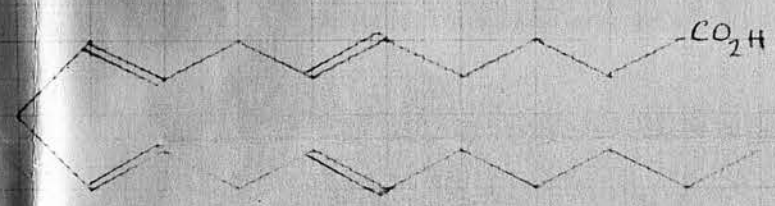
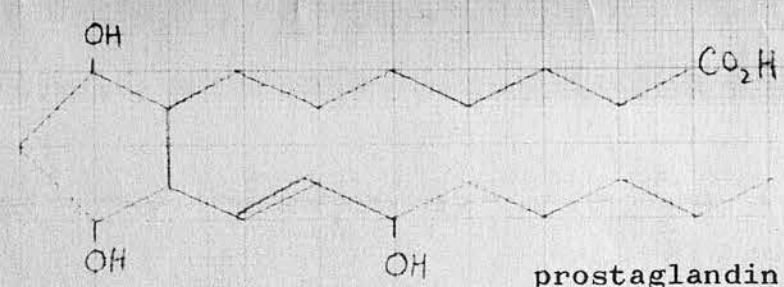
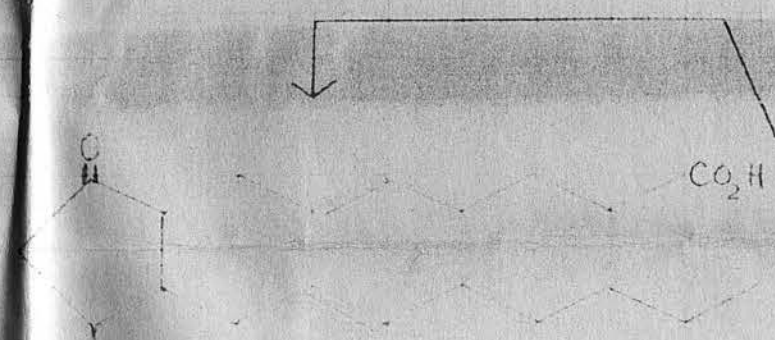


arachidonic acid

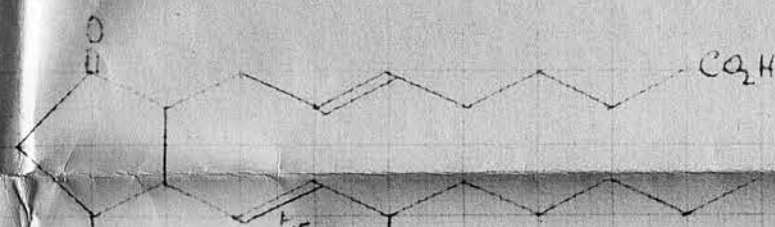


prostaglandin $F_{1\alpha}$

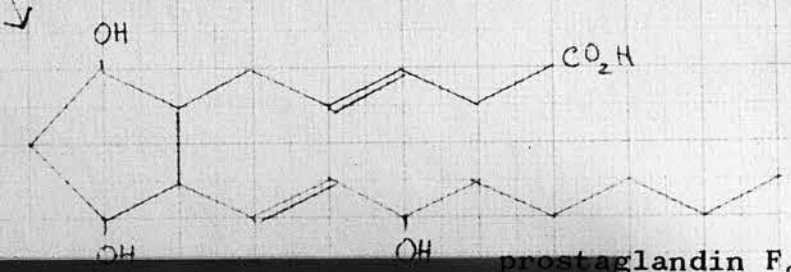


9-oxo-11 hydroxy-prost-5,14-dienoic acid

di-nor-prostaglandin $F_{1\alpha}$

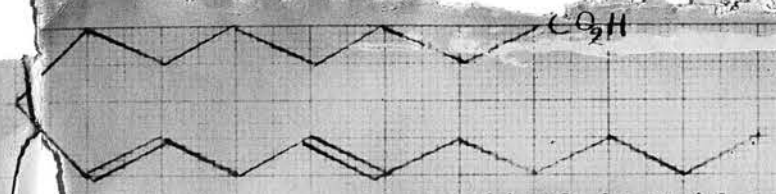


prostaglandin E_2

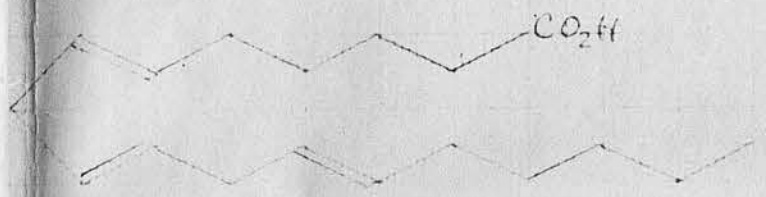


prostaglandin $F_{2\alpha}$

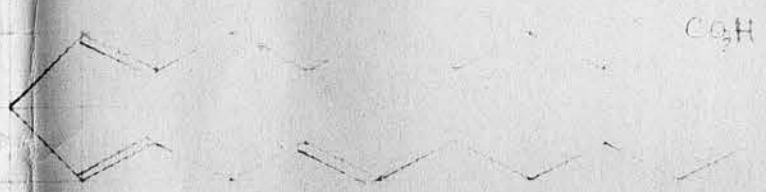
di-nor prostaglandin F_2



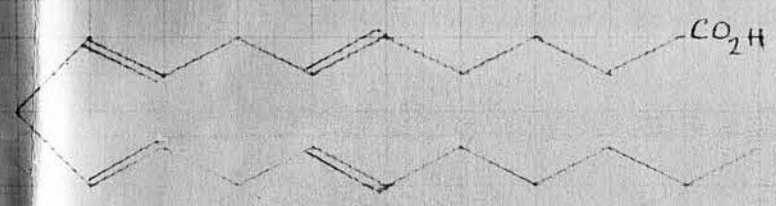
linoleic acid



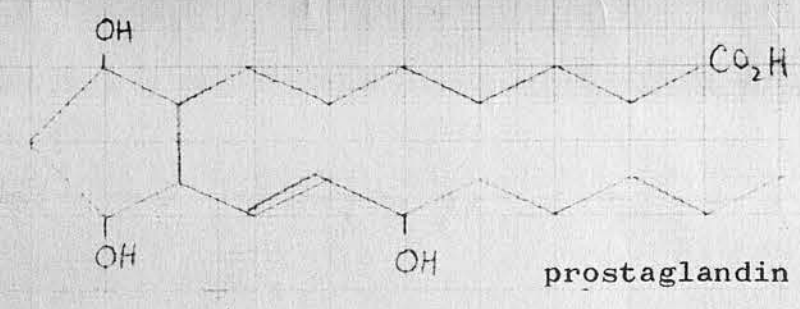
γ-linolenic acid



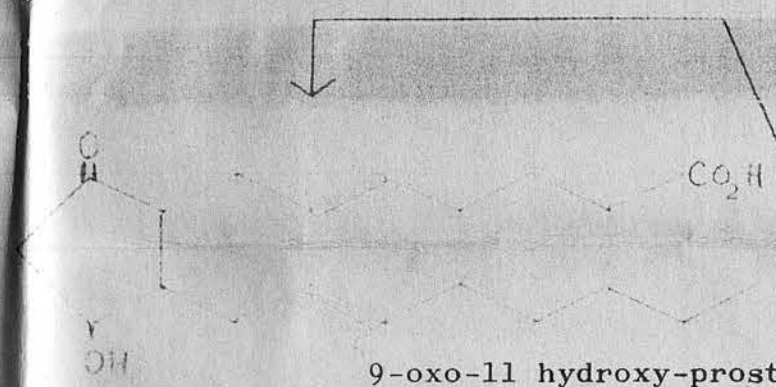
di-homo-γ-linolenic acid



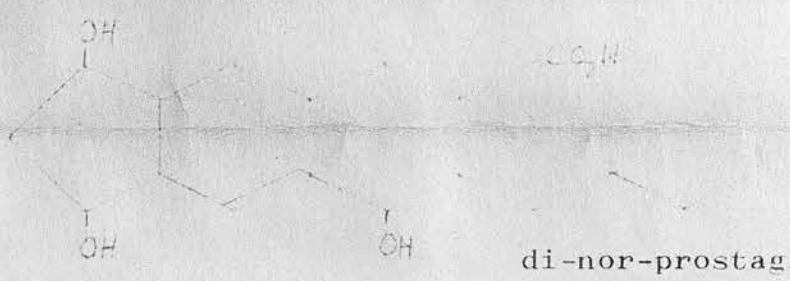
arachidonic acid



prostaglandin $F_{1\alpha}$



9-oxo-11 hydroxy-prost-5,14-dienoic acid



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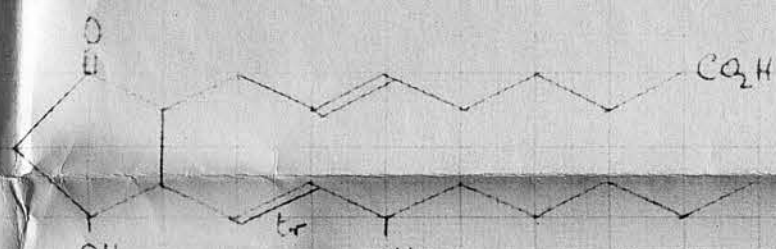
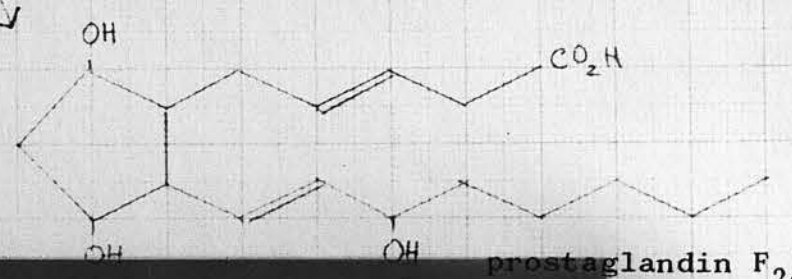
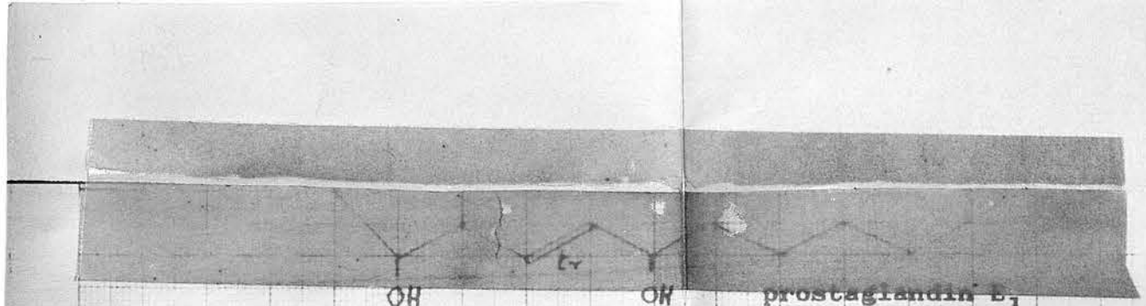


prostaglandin E_2



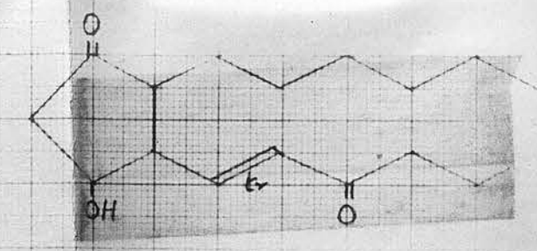
prostaglandin $F_{2\alpha}$

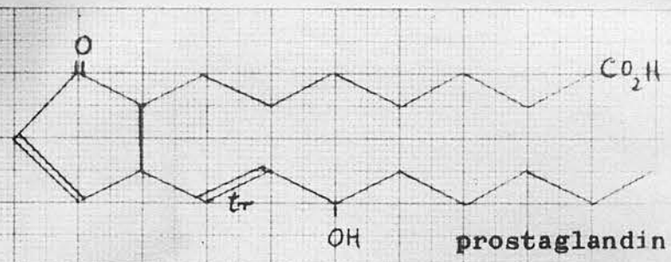
Fig 1b



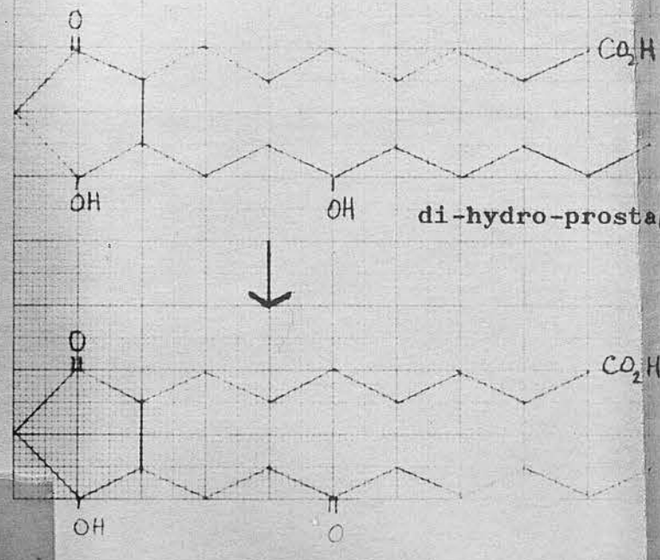
prostaglandin E_1

?





prostaglandin A_1



di-hydro-prostaglandin E_1

?

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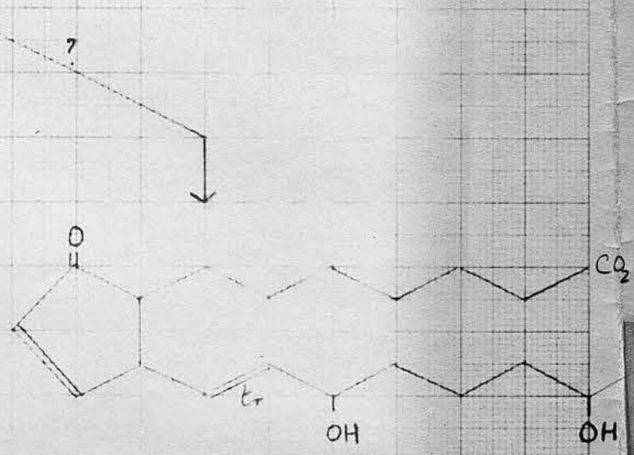

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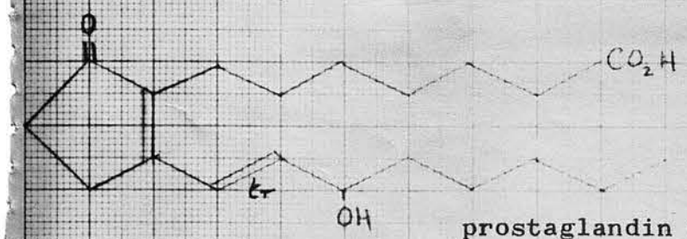
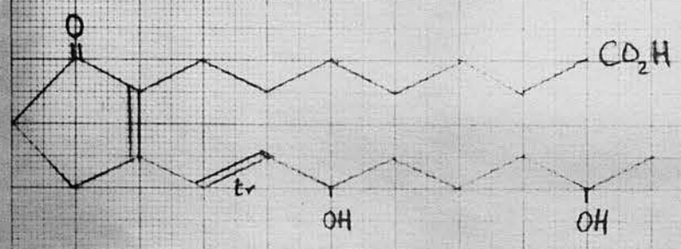


prostaglandin B_1

?



19-hydroxy-prostaglandin B_1