T R Y P T A M I N E R E C E P T O R S

I N T H E R A T S T O M A C H

S T R I P P R E P A R A T I O N

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT TE ROTTERDAM,
OP GEZAG VAN DE RECTOR MAGNIFICUS PROF.DR. P.W. KLEIN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATS VINDEN OP
WOENSDAG 20 NOVEMBER 1974
DES NAMIDDAGS TE 4.15 UUR

DOOR

ABRAHAM LODEWIJK FRANKHUIJZEN

GEBOREN OP 31 MEI 1941 TE ZUILEN

PROMOTOR	:	PROF.DR. I.L. BONTA
CO-PROMOTOR	:	PROF.DR. E.J. ARIËNS
CO-REFERENTEN:		PROF.DR. P.G. SMELIK
		DR. J. BRUINVELS

ter nagedachtenis aan mijn moeder
aan mijn vader
aan Ank

ACKNOWLEDGEMENTS

The present study was supervised by Prof.Dr. I.L. Bonta to whom I am endebted for his advice throughout this research. I am also very much endebted to Dr. J. Bruinvels. The discussions and critical corrections concerning the experiments and the manuscript, combined with those of Prof.Dr. E.J. Ariëns and Prof.Dr. P.G. Smelik, have contributed much to the final stage of this thesis.

I should also like to thank Mr. H. Tap for his assitance during part of this work and Drs. H.J.A. Schouten for advice on the statistical evaluation of the data.

Additionally I should like to express my thanks to the Audiovisual Services Department for the excellent drawings and photographs. I also wish to acknowledge the secretarial help from Mrs. M.M. Thornton-Krom and Miss A.C. Heymeriks at all the various stages of preparation of the manuscript.

CONTENTS PAGE

INTRODUCTION

5-HT (5-hydroxytryptamine, serotonin) has a widespread occurrence in numerous species of plants and animals. It is present for example in several vegetables and edible fruits, in the urticant fluid of the stinging nettle as well as in the intestine, blood platelets and nervous system of all classes of vertebrates.

Its occurrence in the central nervous system (CNS) of both vertebrates and invertebrates has attracted particular attention. In the CNS the transmission of impulses from one nerve fibre to another is accomplished by the action of chemical mediators, called neurotransmitters. The junction between two nerve fibres is called synapse. At the arrival of an action-potential the presynaptically stored transmitter is released and subsequently causes the depolarization of the postsynaptic fibre as consequence of the interaction with a specific receptor, which is a molecular system incorporated in the post-synaptic membrane. 5-HT came to be regarded as a possible neurotransmitter. However, direct evidence for its neuronal localization was not presented until 1965 when Dahlstrom and Fuxe actually demonstrated its presence within the perikarya of the brain stem raphe nuclei and its projections in discrete regions of the fore-brain.

The major portion of the 5-HT in the body of mammals is however found in the intestines where it is primarily located extraneuronally in the Entero-Chromaffin (E.C.)-system. The rat and mouse are exceptional in that quite a considerable portion is also found to be stored in mast cells.

The 5-HT of the E.C.-system is probably synthetized within the cells.

The amine is stored in the cell granules which are also believed to store polypeptide hormones like secretin, glucagon etc. Mast cell 5-HT arises both from de novo synthesis from tryptophan and uptake of the amine itself.

An enormous amount of information is now available about the distribution and effect of 5-HT. Investigation of the mechanism of action may provide information about its physiological role and the functional importance of its rather peculiar distribution. Its effects on mammalian smooth muscle of the alimentary tract have been described in some detail. 5-HT stimulates the intestines of several species including man. Its effect is quantitatively different according to the part of the intestine observed and to the species considered. The precursor of 5-HT, 5-hydroxy-tryptophan also increases intestinal motility which can be prevented by a number of anti-5-HT drugs.

The fact that 5-HT exerts a characteristic effect on smooth muscle is taken to indicate that the muscle possesses the receptors with which 5-HT can interact. Information about these receptors has been derived mostly from in vitro experiments with freshly isolated preparations of smooth muscle. From cross-tachyphylaxis experiments on the guinea-pig ileum Gaddum (1953) concluded that the same type of receptors were involved in the mediation of the effects of 5-HT and tryptamine. The results obtained from quantitative studies of antagonists for 5-HT and tryptamine, led Gaddum and Picarelli (1957) to propose the existence of actually two types of tryptamine receptors in the guinea-pig ileum, one of which being blocked by morphine was accordingly labelled as the M-receptor. The site which was inactivated by dibenzyline (phenoxybenzamine) was denoted as the D-receptor. The M-tryptamine receptor is sited in the intramural parasympathetic ganglion cell (Brownlee and Johnson, 1963). The D-tryptamine receptors are localized within the membrane of smooth muscle cells.

The sensitivity of different smooth muscles to 5-HT varies greatly. A

preparation very sensitive to 5-HT and easy in operation appeared to be the fundus strip preparation of the rat stomach (Vane, 1957). Moreover the preparation was demonstrated to obtain solely the D-type of tryptamine receptors (Offermeier and Ariëns, 1966). Besides being sensitive to 5-HT the preparation is moderately sensitive to prostaglandins and ACh and is rather insensitive to histamine, dopamine and various polypeptides like angiotensin and bradykinin. Relaxation of the organ is produced by α- and β-adrenoceptor agonists, though in the literature also α-adrenoceptors with excitatory properties are described to be present in this preparation (Ogle and Wong, 1971).
Because of the rather selective sensitivity of the fundus strip to 5-HT an explicit system is provided for drugs to be tested for antagonistic activity towards the response of 5-HT mediated by D-tryptamine receptors. However, in the literature data were presented to indicate that other than the classical D-tryptamine receptors might be involved in the response of the rat fundus preparation to tryptamine (Winter and Gessner, 1968). Such an aberant type of tryptamine receptor seems also to be involved in the constrictor response of the external carotid vessels of dogs to 5-HT and ergotamine (Saxena and de Vlaam-Schluter, 1974) and the anti-5-HT drug mianserin (Saxena et al. 1971). Moreover it has been demonstrated that 5-HT as well as tryptamine increased the release of prostaglandins (see ref. Manuscr. II). All these observations might be derogatory to the rather unique position of the rat stomach fundus preparation as a test-system for anti-5-HT activity.

Therefore the purpose of this study was

- to find out whether the same types of receptors were involved in the responses to 5-HT and tryptamine.
- to compare the effects of ergotamine and mianserin on the fundus preparation with those described for the external carotid vessels of the dog.

- to investigate the possible function of prostaglandins in the contractile responses to 5-HT and tryptamine.

REFERENCES

Brownlee, G. and E.S. Johnson, 1963, The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum, Brit.J.Pharmacol. 21, 306.

Gaddum, J.H., 1953, Tryptamine receptors, J.Physiol. 119, 363.

Dahlstrom, A. and K. Fuxe, 1965, Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta Physiol.Scand. 62, Suppl. 232, 1.

Gaddum, J.H. and Z.P. Picarelli, 1957, Two types of tryptamine receptors, Brit.J.Pharmacol. 12, 323.

Offermeier, J. and E.J. Ariëns, 1966, Serotonin I. Receptors involved in its action, Arch.int.Pharmacodyn. 164, 192.

Ogle, C.W. and C.Y. Wong, 1971, The inhibitory and excitatory properties of α-adrenoceptors in the rat stomach, Life Sci. I, 10, 153.

Saxena, P.R., P. van Houwelingen and I.L. Bonta, 1971, The effects of mianserin hydrochloride on the vascular response evoked by 5-hydroxytryptamine and related vasoactive substances, European J.Pharmacol. 13, 295.

Saxena, P.R. and G.M. de Vlaam-Schluter, 1974, Role of some biogenic substances in migraine and relevant mechanism in antimigraine action of ergotamine. Studies in an experimental model for migraine, Headache 13, 174.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Brit.J.Pharmacol. 12, 344.

Winter, J.C. and P.K. Gessner, 1968, Phenoxybenzamine antagonism of tryptamines, their indene isosteres and 5-hydroxytryptamine in

the rat stomach fundus preparation, J.Pharmacol.exp.Therap. 162, 286.

GENERAL LITERATURE

Born, G.V.R., 1970, 5-Hydroxytryptamine receptors, In: Smooth Muscle, p. 418, Edited by E. Bülbring, A.F. Brading, A.W. Jones and T. Tomita (Clowes and Sons, Ltd., London).

Erspamer, V. (Ed.), 1966, Handbook of Experimental Pharmacology, Vol. XIX, 5-Hydroxytryptamine and related indole alkylamines (Springer Verlag, New York).

Håkanson, R., 1970, New aspects of the formation and function of histamine, 5-hydroxytryptamine and dopamine in gastric mucosa, Acta Physiol.Scand., Suppl. 340.

Thompson, J.H., 1971, Serotonin and the alimentary tract, Res.Comm.Chem. Pathol.Pharmacol. 2, 687.

Welsh, J.H., 1968, Distribution of serotonin in the nervous system of various animal species, Adv.Pharmacol. 6A, 171.

EUROPEAN JOURNAL OF PHARMACOLOGY 25 (1974) 40–50. NORTH-HOLLAND PUBLISHING COMPANY

EFFECT OF MIANSERIN, A POTENT ANTI-SEROTONIN AGENT, ON THE ISOLATED RAT STOMACH FUNDUS PREPARATION*

A.L. FRANKHUYZEN** and I.L. BONTA
Department of Pharmacology, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands

Received 8 May 1973 Accepted 17 August 1973

A.L. FRANKHUYZEN and I.L. BONTA, *Effect of mianserin, a potent anti-serotonin agent, in the isolated rat stomach fundus preparation*, European J. Pharmacol. 25 (1974) 40–50.

The influence of mianserin and cyproheptadine on the responses of the rat stomach fundus in vitro to Ach (acetylcholine), 5-HT (serotonin) and tryptamine in the presence of the α-adrenolytic, piperoxan, were compared. Cyproheptadine and mianserin competitively antagonized the Ach-induced contractions, but cyproheptadine was more effective. Mianserin, a recognized antagonist of 5-HT on rat uterus and on bronchospasm of the guinea pig, did not affect 5-HT- and tryptamine-induced contractions of the isolated rat stomach fundus in concentrations up to 10^{-6} M, while cyproheptadine proved highly effective. Higher doses of mianserin contracted the organ. A closed piperazine ring seems to be essential for such action. Although no anti-5-HT action could be found with mianserin on the rat fundus, it protected 5-HT-induced contractions against inhibition by phenoxybenzamine, cyproheptadine or methysergide. The α-adrenolytic piperoxan was more effective in protecting the response to 5-HT than to Ach against inhibition by phenoxybenzamine. It is concluded that differences might exist between D-tryptamine receptors in different organs and that the receptor protection by mianserin and piperoxan towards the 5-HT-induced contractions may be related to an ability to interact at Ca^{2+}-binding sites of the smooth muscle cell membrane. However, the possibility that piperoxan might in some way interfere with an inhibitory action of mianserin towards tryptamine receptors is not ruled out. Although both the responses to 5-HT and tryptamine are affected by cyproheptadine, the 5-HT-induced contractions were more susceptible to inhibition by cyproheptadine than those of tryptamine. This possibly indicated a subsidiary action of tryptamine on other than the D-tryptamine receptors.

Anti-tryptamine
Anti-5-HT
D-Tryptamine receptors
Rat fundus strip
Mianserin
Cyproheptadine

1. Introduction

Mianserin, 2-methyl-1,2,3,4,10,14b-hexahydro-2H-pyrazino/1,2-f/morphanthridine · HCl (Van der Burg et al., 1970) was shown to be a potent antagonist of both 5-HT and histamine in a number of in vivo and in vitro pharmacological preparations. Structurally related to cyproheptadine (fig. 1), its potency was comparable to the latter in antagonizing 5-HT-induced contractions of the rat uterus, capillary permeability increases, bronchoconstriction and rat paw oedema (Vargaftig et al., 1971). It was somewhat less active than cyproheptadine against histamine-induced contractions of the isolated guinea-pig ileum and in the aerosol test, without having cholinolytic activity, whereas cyproheptadine showed anti-muscarinic ac-

* A preliminary report of some of this work has appeared in Arch. Intern. Pharmacodyn. Therap. 197 (1972) 378.

** This paper comprises part of a Doctor's thesis in Pharmacology to be submitted by A.L.F. at the Erasmus University Rotterdam.

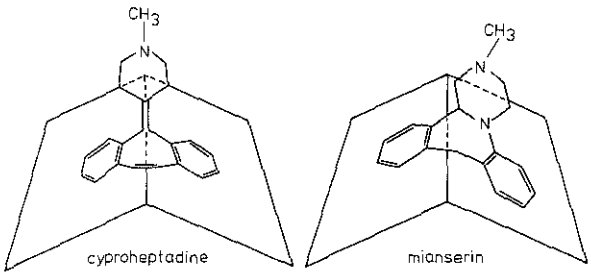


Fig. 1. Structure formula of cyproheptadine and mianserin (Van der Burg et al., 1970, by permission).

tivity. Mianserin was also shown to exhibit α-adrenolytic activity whereas cyproheptadine was devoid of it (Saxena et al., 1971).

We intended to investigate whether the antagonism by mianserin towards 5-HT was of the competitive or of the non-competitive type. For this purpose the isolated rat stomach fundus preparation was chosen, because it is more suitable than the rat uterus (Vane, 1957). On the basis of their antagonism by phenoxybenzamine and morphine respectively the 5-HT receptors are classified by Gaddum and Picarelli (1957) as belonging to the D- or M-type. Although the rat uterus as well as the rat stomach fundus both are believed to contain solely D-tryptamine receptors (Offermeier and Ariëns, 1966), the cumulative dose–response technique is more appropriate to the rat stomach fundus.

Van der Burg et al. (1970) postulated that, apart from rigidity, an intersection angle of about 120° between the planes formed by the two aromatic nuclei of mianserin and also those of cyproheptadine is a structural requirement for high anti-serotonin activity. In order to test whether this also holds for the rat fundus preparation, both compounds were compared with respect to their influence on the response to 5-HT.

We included tryptamine in the experiments to investigate whether differences existed with respect to 5-HT, possibly indicating an action of tryptamine on other than D-tryptamine receptors. For this, suggestions can be found in literature (Barlow and Khan, 1959a,b; Barlow, 1961, Winter and Gessner, 1968).

2. Materials and methods

2.1. Experimental preparation

Male albino Wistar rats weighing 200–300 g were killed by decapitation. The stomach was dissected out and strips cut according to the method described by Vane (1957). The strips were set up in an organ bath maintained at 37°C containing Tyrode solution and bubbled with a gas mixture containing 95% O_2–5% CO_2. The composition of the Tyrode solution was (mM): NaCl 136.8; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.6; NaH_2PO_2 0.4; $NaHCO_3$ 11.9; and glucose 5.6. Except in the receptor-protection experiments, piperoxan (10^{-5} M) was added to the Tyrode solution to exclude an inhibitory action of 5-HT on α-adrenoceptors as described by Offermeier and Ariëns (1966). One end of the fundus strip was attached to the lever of a Harvard heart/smooth muscle transducer loaded with 1.0 g weight. The contractions were recorded with a Harvard electronic recording module 350. Before starting the experiments the fundus strips were allowed to equilibrate for 60 min during which the bathing fluid was changed every 15 min.

2.2. Evaluation of antagonism

pA_2 and pD_2' values were calculated according to Ariëns et al. (1956) and Van Rossum and Van den Brink (1963); pA_2 values indicate the affinity of the competitive antagonist to the receptor and is denoted as the negative logarithm of the molar concentration of the antagonist which causes an elevation of the concentration of the agonist with a factor 2 in order to obtain the same response. pD_2' values indicate the affinity of the non-competitive antagonist to the receptor and is denoted as the negative logarithm of the molar concentration of the antagonist which causes a 50% reduction of the maximal effect of the agonist.

2.3. Receptor-protection experiments

The receptor-protection experiments were performed according to the method described by Furchgott (1954) as modified by Winter and Gessner (1968). For one single receptor-protection experi-

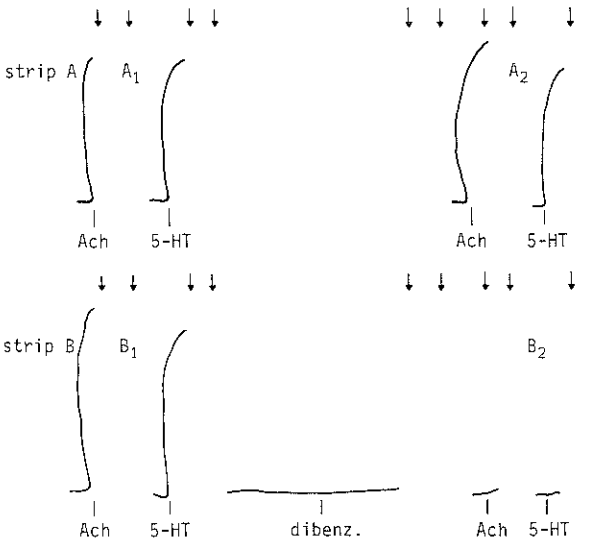


Fig. 2a. Schedule of the blocking experiments. The upper row in both figures (2a and 2b) represents the blank. The second row represents the actual blocking or receptor-protection experiment. The letter at the top of each contraction is referred to in the formulae for the calculation of the % inhibition of control or protected strips. Downward arrows indicate washing of the preparation. For the administration of drugs a 5 min time cycle was used (see under Materials and methods 2.3.). Ach = acetylcholine (10^{-4} M), 5-HT (10^{-5} M), Dibenz. = phenoxybenzamine (10^{-4} M), Mians. = mianserin (10^{-4} M).

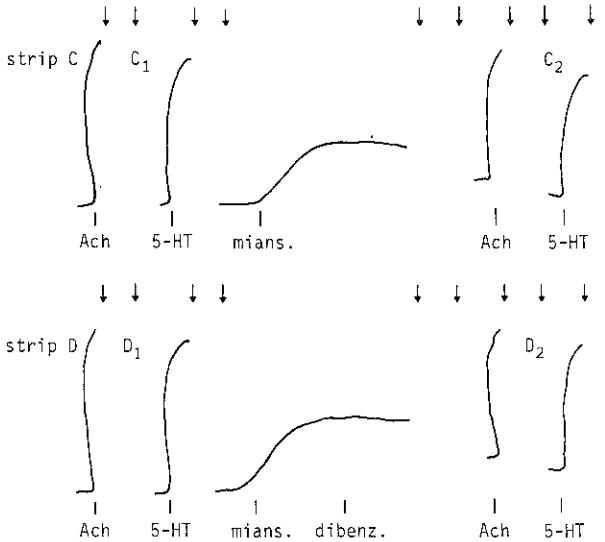


Fig. 2b. Schedule of the receptor-protection experiments. For further information see fig. 2a.

ment 4 fundus strips were used at the same time: 2 strips for the blocking experiment, the other 2 strips for the protection experiment.

2.3.1. Blocking experiment (fig. 2a)

2 fundus strips, A and B, were used at the same time; strip A served as a control, while strip B was exposed to the antagonist. At first the response of both strips to Ach (10^{-4} M) and 5-HT (10^{-5} M) was recorded. The contraction height is denoted as A_1 for strip A and B_1 for strip B. Thereafter, strip B was exposed for 5 min to the antagonist, after which time the response to Ach and 5-HT of both preparations was again recorded. The contraction height is denoted A_2 for strip A and B_2 for strip B. The response of strip B expected in the absence of the antagonist is given by:

$$B_{2exp} = B_1(A_2/A_1)$$

in which the letters stand for the contraction height of Ach or 5-HT in mm. The percentage inhibition of the response of strip B in the presence of the antagonist is then given by:

$$\%I_{contr} = 100(1-B_2/B_{2exp})$$

2.3.2. Protection experiments (fig. 2b)

2 strips, C and D, were used at the same time; strip C served as a control, strip D was used for the protection experiment. The procedure was the same as in the blocking experiments. The contraction heights of strip C (= C_1) and of strip D (= D_1) were recorded after administration of Ach (10^{-4} M) and 5-HT (10^{-5} M). Both strips were exposed to the protecting drug. The antagonist was administered after 5 min to strip D and left in contact with the tissue for another 5 min, thereafter the response to Ach and that to 5-HT was recorded denoting the contraction heights of strip C as C_2 and of strip D as D_2.

The response expected in the protection experiment is given by:

$$D_{2exp} = D_1(C_2/C_1)$$

The percentage inhibition of the protected strip D of the response to Ach or 5-HT is given by:

$$\%I_{prot} = 100\ (1 - D_2/D_{2exp})$$

and the percentage protection by:

$$\%Prot = 100\ (1 - \%I_{prot}/\%I_{contr})$$

2.4. Drugs

The following drugs were used: acetylcholine (Fluka A.G.), serotonin creatinine sulphate (Fluka A.G.), tryptamine (Fluka A.G.), cyproheptadine (Periactin, Merck Sharp and Dohme), methysergide (Deseril, Sandoz), piperoxan (May and Baker), phenoxybenzamine (Dibenzyline, Merck Sharp and Dohme), hyoscine (Scopolamine, Brocades), morphine, propranolol (Inderal, Sandoz), chlorpromazine (Largactil, S.P.E.C.I.A.), sotalol (Mead Johnson), Dexamphetamine, 6-methylaminomethyl-5,6-dihydromorphanthridine (Organon), mianserin (Organon). The solutions of the salts of the drugs were prepared freshly on the day of the experiment. Drug solutions were added to the 5 ml organ bath in a volume of 0.1 ml. All concentrations mentioned refer to the final concentration of the drug in the organ bath and are expressed in terms of molarity.

3. Results

3.1. Influence of cyproheptadine and mianserin on the response to acetylcholine, 5-HT and tryptamine

The influence of different concentrations of cyproheptadine on the log dose–response curves of Ach, 5-HT and tryptamine was investigated (fig. 3a, b, c). Cyproheptadine provided powerful antagonism against contractions induced by Ach, 5-HT as well as by tryptamine. Administration of cyproheptadine resulted in a parallel shift to the right of the log dose–response curves indicating a competitive type of antagonism. Only in the case of 5-HT was this shift accompanied by a lowering of the maximal height of

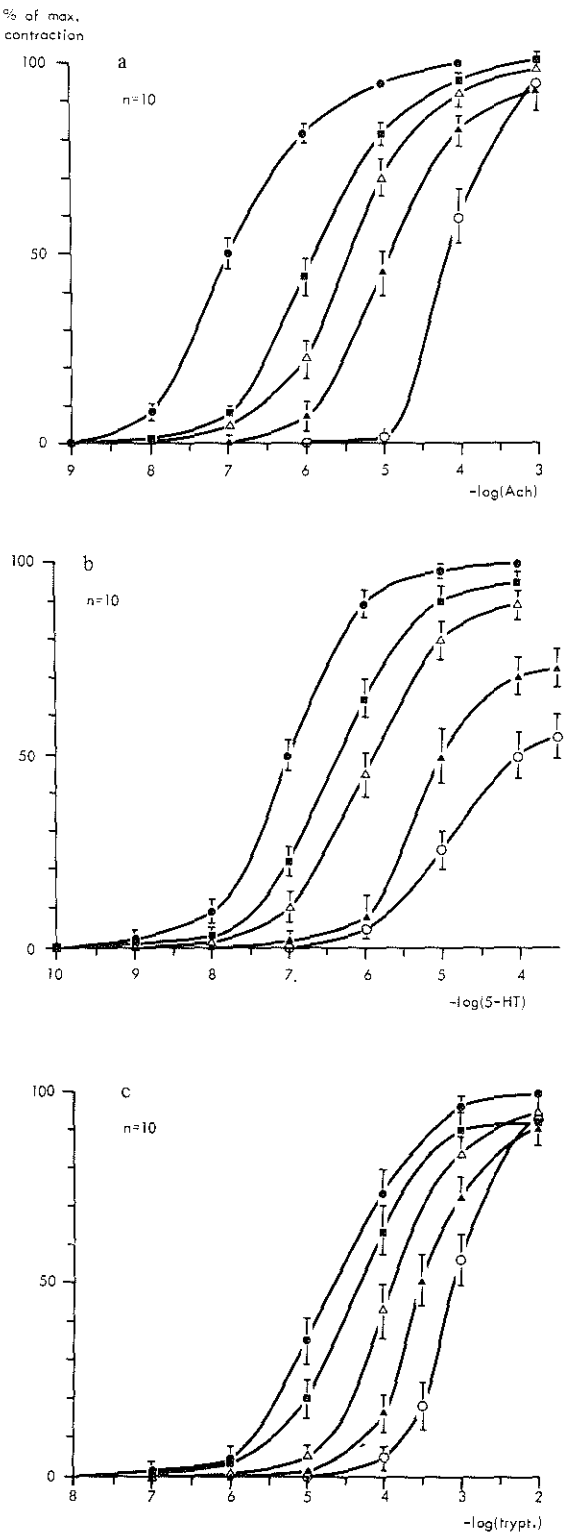
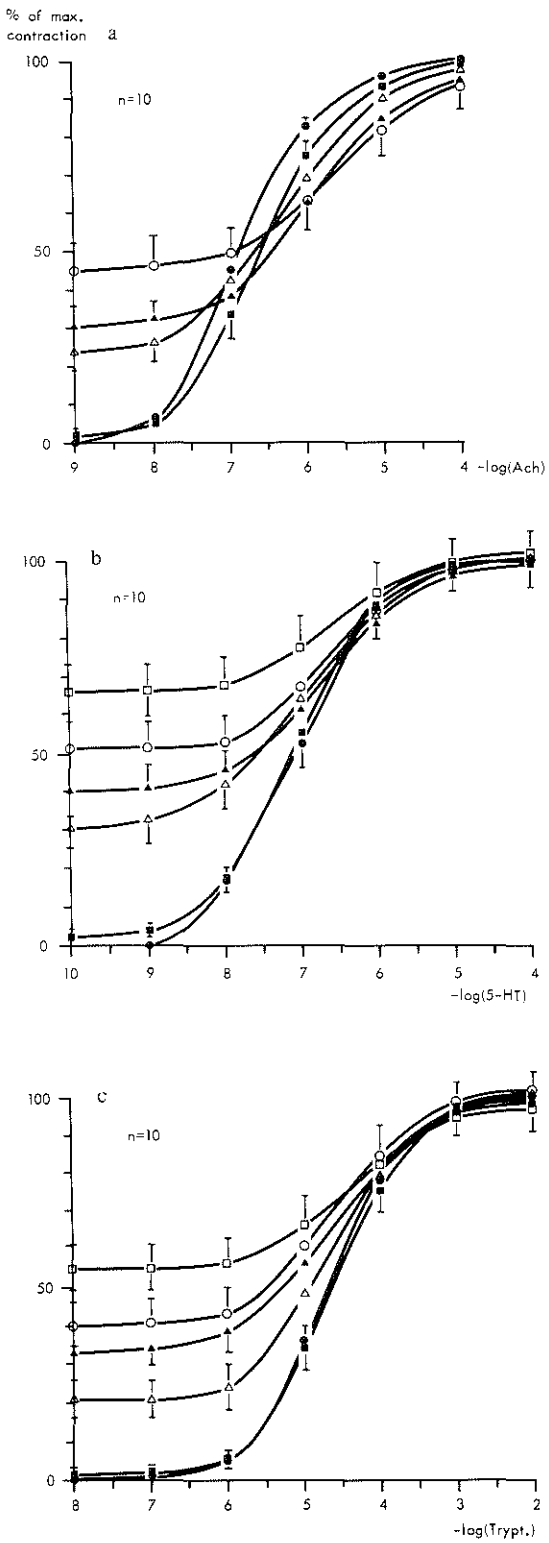


Fig. 3a, b, c. Influence of cyproheptadine on the response of rat stomach fundus strips to Ach (fig. 3a), 5-HT (fig. 3b) and tryptamine (fig. 3c) in the presence of piperoxan (10^{-5} M). ●–●, 0 M cyproheptadine; ■–■, 10^{-7} M; △–△, 3 X 10^{-7} M; ▲–▲, 10^{-6} M; ○–○, 10^{-5} M. The vertical bars in the figures indicate the S.E.M. out of 10 experiments.



the curve, possibly indicating a non-competitive character. This implies that cyproheptadine exhibited a dualism in its antagonism of 5-HT.

Compared with cyproheptadine, mianserin exerted only a weak competitive type of antagonism towards Ach (fig. 4a). In contrast to cyproheptadine, mianserin did not antagonize the response of the rat fundus to 5-HT and tryptamine up to a concentration of 10^{-6} M (fig. 4b,c). Administration of still higher doses of mianserin resulted in a contraction of the organ. This is shown in all 3 figures by the elevated cyproheptadine. From the same table it can be seen, that the tryptamine-induced contractions are less affected by cyproheptadine than those of 5-HT. The pA_2 value of cyproheptadine towards tryptamine is significantly lower than towards 5-HT ($p < 0.001$).

3.2. Contraction of the rat fundus strip by mianserin

The contraction induced by mianserin had a slow onset and reached its maximal height after about 2–5 min. After washing the preparation the tonus of the organ stayed elevated and even after 1 hr the preparation had not returned to its original base line. In fig. 5 log dose–response curves are shown of $BaCl_2$, Ach, 5-HT, tryptamine and mianserin. In the case of mianserin the single dose–response technique was used instead of the cumulative technique. Because of the relatively long time needed by mianserin to obtain its maximal contraction height, possible processes such as desensitization or auto-inhibition might develop (Guimaraes, 1972). The pD_2 value of the mianserin-induced contraction was 4.65 ± 0.15. Its maximal contraction height relative to $BaCl_2$ was $71 \pm 3\%$, which is lower than for Ach ($91 \pm 2\%$), 5-HT ($79 \pm 2\%$) and tryptamine ($81 \pm 3\%$). The piperazine ring seemed to be a structural requirement for the spasmogenic action of mianserin. The open diamine analogue of mianserin (table 2), in a concentration of 10^{-4} M, did not contract the organ.

Fig. 4a, b, c. Influence of mianserin on the response of rat stomach fundus strips to Ach (fig. 4a), 5-HT (fig. 4b) and tryptamine (fig. 4c). ●–●, 0 M mianserin; ■–■, 10^{-6} M; △–△, 10^{-5} M; ▲–▲, 2×10^{-5} M; ○–○, 3×10^{-5} M; □–□, 10^{-4} M. Log dose–response curves of Ach, 5-HT and tryptamine were recorded in the presence of piperoxan (10^{-5} M). The preincubation time of the antagonists was 1 hr. The values (expressed in % of maximal contraction) of each curve are the mean out of 10 experiments ± S.E.M.

Table 1

pA_2 and pD_2' values of cyproheptadine and mianserin towards the response of the isolated rat stomach fundus preparation to Ach, 5-HT and tryptamine. These values (± S.E.M.) are the mean of the pA_2 and pD_2' values obtained from the experiments in which different concentrations of the antagonists were used.

Antagonist \ Antagonist	Cyproheptadine		Mianserin	
	pA_2	pD_2'	pA_2	pD_2'
Ach	8,03 ± 0,10	-	6,05 ± 0,35	-
5-HT	7,50 ± 0,10	5,43 ± 0,09	no effect	
Tryptamine	6,87 ± 0,11	-	no effect	

3.3. Inhibition of mianserin-induced contraction

In an attempt to analyse the contraction of the rat fundus strip to mianserin, the following antagonists were used: atropine, scopolamine, morphine, methysergide, cyproheptadine, propranolol, phenoxybenzamine, and chlorpromazine. All antagonists were used in concentrations which completely blocked re-

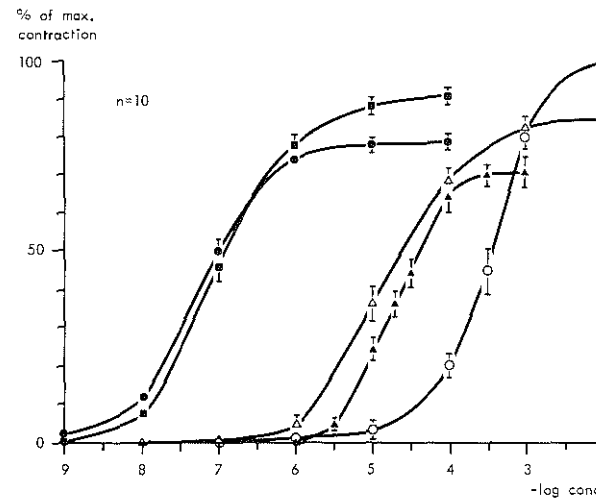
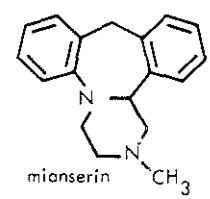
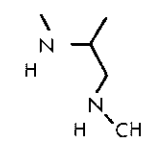


Fig. 5. Log dose–response curves of 5-HT (●–●), acetylcholine (■–■), tryptamine (△–△), mianserin (▲–▲) and $BaCl_2$ (○–○) on the isolated rat stomach fundus preparation in the presence of piperoxan 10^{-5}M. The values (expressed in % of maximal contraction relative to that of $BaCl_2$) of each curve are the mean out of 10 experiments ± S.E.M.

Table 2

% Protection of the response of rat fundus strips to Ach (10^{-4} M) and 5-HT (10^{-5} M) by mianserin, its open diamine analogue and piperoxan against inhibition by phenoxybenzamine (Dibenzyline) (10^{-4}M), cyproheptadine (10^{-6}M) and methysergide (10^{-6}M).

Agonist	Antagonist	Compound tested for receptor protection	concentr.	% Protection
5-HT	Dibenzyline	 mianserin	10^{-4}M	92 ± 7 (13)*
Ach	id.		10^{-4}M	86 ± 4 (13)
5-HT	Cyproheptadine**		10^{-6}M	110 ± 10 (10)
5-HT	Methysergide**		10^{-6}M	90 ± 10 (10)
5-HT	Dibenzyline		10^{-4}M	31 ± 8 (7)
Ach	id.	6-methylaminomethyl-5,6-dihydromorphanthridine***	10^{-4}M	1 ± 1 (7)
5-HT	id.	piperoxan	10^{-5}M	49 ± 6 (13)
Ach	id.	piperoxan	10^{-5}M	20 ± 5 (13)

* Mean values are given ± S.E.M. The number of experiments is given between parentheses.

** The procedure was slightly changed in the experiments using cyproheptadine and methysergide. The contractions were recorded in the presence of both protecting and blocking drug.

*** No spasmogenic action of the open diamine analogue of mianserin.

sponses to acetylcholine, 5-hydroxytryptamine and α- and β-adrenoceptor agonists. In these experiments only one concentration of mianserin was used (3×10^{-5} M), which gave approximately 50% of the maximal contraction height of the mianserin log dose–response curve. Only chlorpromazine (10^{-4} M) was able to inhibit the mianserin-induced contraction. However, the same concentration of chlorpromazine also inhibited the response of the rat fundus to $BaCl_2$, probably indicating an unspecific spasmolytic action of chlorpromazine. When incubating the tissue with 5-HT, dexamphetamine or tryptamine for 1 hr, cross-tachyphylaxis developed between 5-HT, dexamphetamine and tryptamine. However, normal responses to mianserin could still be obtained.

3.4. Receptor-protection experiments

Another means of investigating whether or not mianserin shows affinity towards tryptamine receptors is to determine whether mianserin is able to prevent inhibition of the response to 5-HT by a competitive antagonist. The first antagonist used was the alkylating agent, phenoxybenzamine. Administration of mianserin (10^{-4}M) 5 min prior to phenoxybenzamine (10^{-4}M) protected both the response to 5-HT and to Ach from inhibition by phenoxybenzamine (table 2). A representative record of the receptor-protection experiments with phenoxybenzamine is shown in fig. 6a.

Phenoxybenzamine is a recognized α-adrenolytic agent. Responses to 5-HT which are mediated by α-adrenoceptors are known from the literature (Innes, 1962) and also for the isolated rat fundus preparation (Abramets, 1969). Saxena et al. (1971) showed in their experiments, in which they investigated the influence of mianserin on vascular responses, that mianserin exerted α-adrenolytic activity. Because of their common affinity towards α-adrenoceptors, 2 other serotonin antagonists were used in these receptor-protection experiments, methysergide and cyproheptadine. For these 2 antagonists, no interaction with α-adrenoceptors has been described. Because both antagonists are slightly reversible, the procedure for the receptor-protection experiments was slightly changed. The responses to 5-HT were recorded in the presence of both mianserin and the respective antagonist. Mianserin, in a concentration of 10^{-6} M, prevented also the inhibition of both cyproheptadine and methysergide of the response to 5-HT. Representative records of the receptor-protection experiments with cyproheptadine and methysergide are shown in fig. 6b, c.

Using the open diamine analogue of mianserin it was possible to investigate the influence of the piperazine-ring structure on both the spasmogenic action and the receptor-protective capacity of mianserin. Administration of the open diamine analogue did not result in a contraction of the organ. The percentage protection against inhibition by phenoxybenzamine of both 5-HT and Ach was drastically diminished. No protection was found anymore with respect to the response to Ach. The percentage protection towards 5-HT was only about 1/3 of the protection exerted by mianserin (table 2).

Piperoxan was also included in the receptor-protection experiments. It was observed, that the inhibition of the response to 5-HT by phenoxybenzamine was much less than of the response to Ach when piperoxan was present in the Tyrode solution. From table 2 it can be seen, that piperoxan in a concentration of 10^{-5} M showed receptor-protective capacity towards 5-HT as well as towards Ach. The percentage protection by piperoxan towards the 5-HT-induced contractions was significantly ($p < 0.001$) higher than towards Ach.

4. Discussion

The potency of mianserin in antagonizing the response to 5-HT in a variety of in vitro and in vivo pharmacological preparations was comparable with cyproheptadine (Van der Burg et al., 1970; Vargaftig et al., 1971). On the rat stomach fundus preparation, in vitro cyproheptadine provides a potent competitive antagonism towards 5-HT and tryptamine as well as towards Ach-induced contraction. Compared with cyproheptadine ($pA_2 = 8.03 \pm 0.10$), mianserin posesses only weak competitive cholinolytic properties ($pA_2 = 6.05 \pm 0.35$). In contrast mianserin did not inhibit the responses to 5-HT and tryptamine in concentrations up to 10^{-6} M. Administration of still higher doses of mianserin resulted in a contraction of the organ. It was not possible to inhibit the contraction by mianserin with antagonists of acetylcholine, histamine, 5-HT or α- and β-adrenoceptor agonists. Inhibition of

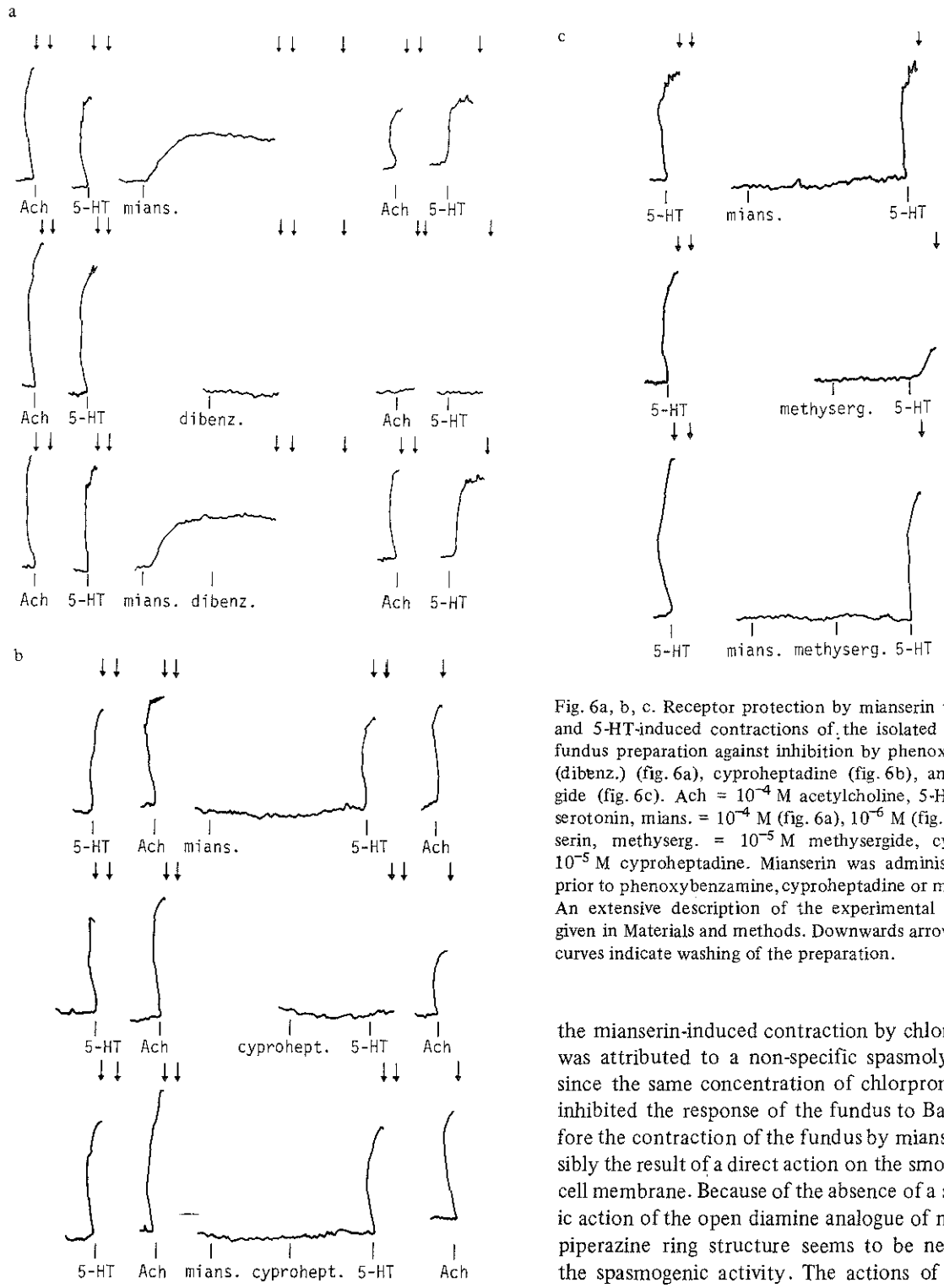


Fig. 6a, b, c. Receptor protection by mianserin towards Ach and 5-HT-induced contractions of the isolated rat stomach fundus preparation against inhibition by phenoxybenzamine (dibenz.) (fig. 6a), cyproheptadine (fig. 6b), and methysergide (fig. 6c). Ach = 10^{-4} M acetylcholine, 5-HT = 10^{-5} M serotonin, mians. = 10^{-4} M (fig. 6a), 10^{-6} M (fig. 6b, c) mianserin, methyserg. = 10^{-5} M methysergide, cyprohept. = 10^{-5} M cyproheptadine. Mianserin was administered 5 min prior to phenoxybenzamine, cyproheptadine or methysergide. An extensive description of the experimental procedure is given in Materials and methods. Downwards arrows above the curves indicate washing of the preparation.

the mianserin-induced contraction by chlorpromazine was attributed to a non-specific spasmolytic action, since the same concentration of chlorpromazine also inhibited the response of the fundus to $BaCl_2$. Therefore the contraction of the fundus by mianserin is possibly the result of a direct action on the smooth muscle cell membrane. Because of the absence of a spasmogenic action of the open diamine analogue of mianserin, a piperazine ring structure seems to be necessary for the spasmogenic activity. The actions of both 5-HT

and tryptamine on smooth muscle are mediated by two types of receptors according to Gaddum (1957): the M- and D-types, on the basis of their antagonism by morphine and phenoxybenzamine, respectively. The rat fundus contains exclusively the D-type of tryptamine receptor (Offermeier and Ariëns, 1966). Nevertheless mianserin was a potent antagonist of 5-HT in the isolated rat uterus with a pA_2 value of 8.0 ± 0.3 (Vargaftig et al., 1971), but no influence of mianserin could be found on the response of the rat fundus up to a concentration of 10^{-6} M. This suggests a difference between the receptors in the two tissues. However, the potency of cyproheptadine towards the response to 5-HT in both organs was similar: the pA_2 value of cyproheptadine on the rat uterus was 8.4 ± 0.1 (Vargaftig et al., 1971) and on the rat fundus 8.04 ± 0.08.

Barlow and Khan (1959b) pointed to the difference in sensitivity of both preparations with respect to the antagonistic activity using analogues of 5-HT on the rat fundus and uterus. They found that in general the effects on the rat uterus and stomach fundus preparation were not dissimilar, although there were substantial differences. They concluded, that it is not justified to extend the characteristics of the action at one site to another site. The difference in activity of mianserin towards 5-HT in the rat uterus and rat stomach fundus is in line with this conclusion.

Because of the difference in the effects of cyproheptadine and mianserin on the response to 5-HT of the fundus, factors other than rigidity of the antagonist molecule and an intersection angle of 120° between the planes formed by the aromatic nuclei are possibly important for high anti-serotonin activity.

Although it is not possible to ascribe an anti-serotonin action for higher concentrations of mianserin, because of its spasmogenic action, administration of mianserin prior to phenoxybenzamine, methysergide or cyproheptadine prevents to inhibition of the response to 5-HT of the rat fundus. According to Ariëns (1964) receptor protection can only be obtained with competitive antagonists. Mianserin is a competitive antagonist of Ach on the rat fundus, which is in line with its receptor protective activity towards Ach. The 5-HT receptor protection by mianserin seems to be in conflict with this view unless the affinity of mianserin towards the tryptamine receptor

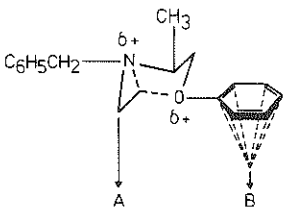


Fig. 7. Conformation of the hybrid state of dibenzyline resulting in a 'phenylethylamine' alignment at the receptor. The electrons of the phenyl group, by interacting with site B of the receptor surface secure the molecule, fixing it in the right position for alkylation of the receptor site A by the N-ethylimine ion (Belleau, 1958).

is masked by its spasmogenic action. Phenoxybenzamine exerts its irreversible antagonism, according to Belleau (1958), by interaction with two sites on the cell membrane called A and B (fig. 7). Site B is the attachment site of the phenyl group of phenoxybenzamine fixing the molecule in the right position for the alkylation of site A by the N-ethylimine ion. Mianserin might prevent alkylation of the 5-HT receptor by interference with the attachment of the phenyl group of phenoxybenzamine at site B of the cell membrane in this way protecting site A from alkylation. Phenoxybenzamine does not inhibit the mianserin-induced contraction of the rat fundus. This implies, that the interaction of mianserin with site B should be of no importance for its spasmogenic action. The observation, that the piperazine ring is important for the spasmogenic action of mianserin is in accord with this conclusion. Remarkable was the receptor protection of 5-HT by the α-adrenolytic piperoxan. For piperoxan no competitive antagonism towards 5-HT on the rat fundus is described. According to Offermeier and Ariëns (1966), it even potentiated the 5-HT-induced contractions of the rat fundus by preventing the inhibitory action of 5-HT on α-adrenoceptors. Apperently piperoxan is able to prevent the alkylation of the tryptamine receptors by phenoxybenzamine in some other way than by interaction with the tryptamine receptors. This means, that piperoxan might have prevented an inhibitory action of mianserin on tryptamine receptors in a similar manner as phenoxybenzamine.

A fundamental role is ascribed to membrane-bound Ca^{2+} in the excitation–contraction coupling. (Lüllman, 1970). A situation comparable with the one described here, i.e. receptor protection provided by mianserin and piperoxan to tryptamine receptors, is the ability of β-adrenolytics to protect events mediated by α-adrenoceptors against inactivation by irreversible α-adrenolytics (Kohli and Ling, 1967; Patil et al., 1968). This mutual interaction appeared to be related to the ability of the antagonists to interact at a specific Ca^{2+}-binding site. A definite association of 2-halogenoethylamines with a specific Ca^{2+}-binding site has been described (Moran et al., 1970). An alternative explanation would be, that the receptor protection afforded by mianserin and piperoxan towards inhibition by phenoxybenzamine of the response to 5-HT, without showing affinity towards the tryptamine receptor, might possibly be related to an interaction with membrane Ca^{2+}-binding sites.

The tryptamine- and 5-HT-induced contractions of rat fundus strips were similarly affected by cyproheptadine (fig. 3b,c). Preincubation of strips with cyproheptadine resulted in a shift to the right of both log dose–response curves. Cyproheptadine was however more then ten times as active against 5-HT-induced contractions than tryptamine. In contrast to 5-HT the shift to the right of the log dose–response curve of tryptamine was not accompanied by a decrease of the maximal contraction height. These two observations possibly indicate a subsidiary action of tryptamine on other than the classical D-tryptamine receptors.

Acknowledgements

We are indebted to Dr. W.J. Van der Burg for his useful suggestions. Mianserin and 6-methylaminomethyl-5,6-dihydromorphanthridine were kindly supplied by Dr. Van der Burg, Organon N.V.

References

Abramets, I.I., 1969, Similarity between D-serotonin receptors and α-adrenergic receptors, Byul. Eksp. Biol. Med. 67, 158.

Ariëns, E.J., 1964, Molecular Pharmacology I (Academic Press, New York).

Ariëns, E.J., J.M. Van Rossum and A.M. Simonis, 1956, A theoretical basis for molecular pharmacology, Arzneim. Forsch. 6, 282.

Barlow, R.B., 1961, Effects on amine oxidase of substances which antagonize 5-hydroxytryptamine more than tryptamine, Brit. J. Pharmacol. 16, 153.

Barlow, R.B. and I. Khan, 1959a, Actions of some analogues of tryptamine on the isolated rat uterus and the rat fundus strip preparations, Brit. J. Pharmacol. 14, 265.

Barlow, R.B. and I. Khan, 1959b, Actions of some analogues of tryptamine on the isolated rat uterus and on the isolated rat fundus strip preparations, Brit. J. Pharmacol. 14, 99.

Belleau, B., 1958, The mechanism of drug action at receptor surfaces, part I, Can. J. Biochem. Physiol. 36, 731.

Furchgott, R.T., 1954, Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors, J. Pharmacol. 111, 265.

Gaddum, J.H. and Z.P. Picarelli, 1957, Two types of tryptamine receptors, Brit. J. Pharmacol. 12, 323.

Guimaraes, S., 1972, The slopes of cumulative and non-cumulative dose–response curves for noradrenaline and isoprenaline, European J. Pharmacol. 17, 44.

Innes, I.R., 1962, An action of 5-hydroxytryptamine on adrenaline receptors, Brit. J. Pharmacol. 19, 427.

Kohli, J.D. and G.M. Ling, 1967, α-Adrenergic blocking action of propranolol, J. Pharm. Pharmacol. 19, 629.

Lüllman, H., 1970, Smooth Muscle (Edward Arnold Ltd., London) p. 151.

Moran, J.F., V.C. Swamy and D.J. Triggle, 1970, Irreversible antagonism at the adrenergic α-receptor: the role of calcium, Life Sci., Part I 9, 1303.

Offermeier, J. and E.J. Ariëns, 1966, Serotonin. I. Receptors involved in its action, Arch. Intern. Pharmacodyn. 164, 192.

Patil, P.N., A. Tye, C. May, S. Heley and S. Miyagi, 1968, Steric aspects of adrenergic drugs. XI. Interactions of dibenamine and β-adrenergic blockers, J. Pharmacol. Exptl. Therap. 163, 309.

Saxena, P.R., P. van Houwelingen and I.L. Bonta, 1971, The effects of mianserin hydrochloride on the vascular responses evoked by 5-hydroxytryptamine and related vasoactive substances, European J. Pharmacol. 13, 295.

Van der Burg, W.J., I.L. Bonta, I. Delovelle, C. Ramon, B.B. Vargaftig, 1970, A novel type of substituted piperazine with high anti-serotonin activity, J. Med. Chem. 13, 35.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Brit. J. Pharmacol. 12, 344.

Van Rossum, J.M. and F.G. Van den Brink, 1963, Cumulative dose–response curves, Arch. Intern. Pharmacodyn. 143, 299.

Vargaftig, B.B., I.L. Coignet, C.J. de Vos, H. Grijsen and I.L. Bonta, 1971, Mianserin hydrochloride: Peripheral and central effects in relation to antagonism against 5-hydroxytryptamine, European J. Pharmacol. 16, 366.

Winter, J.C. and P.K. Gessner, 1968, Phenoxybenzamine antagonism of tryptamines, their indene isosteres and 5-hydroxytryptamine in the rat stomach fundus preparations, J. Pharmacol. Exptl. Therap. 162, 286.

ERRATUM

On page (44), right column, line 11-12 should read:

organ. This is shown in all three figures by the elevated baselines of the curves. The results of the experiments with cyproheptadine and mianserin are summarized in table 1. In this table are listed the pA_2 and pD_2' values of mianserin and cyproheptadine with respect to the response to ACh, 5-HT and tryptamine. From this table it can be seen, that the pA_2 of mianserin towards ACh is considerably lower than of cyproheptadine. From the same table it can be seen .....

EUROPEAN JOURNAL OF PHARMACOLOGY 26 (1974) 220–230. NORTH-HOLLAND PUBLISHING COMPANY

RECEPTORS INVOLVED IN THE ACTION OF 5-HT AND TRYPTAMINE ON THE ISOLATED RAT STOMACH FUNDUS PREPARATION

Abraham L. FRANKHUIJZEN* and Ivan L. BONTA

Department of Pharmacology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands

Received 27 September 1973 Accepted 15 January 1974

A.L. FRANKHUIJZEN and I.L. BONTA, *Receptors involved in the action of 5-HT and tryptamine on the isolated rat stomach fundus preparation*, European J. Pharmacol. 26 (1974) 220–230.

Participation of α-adrenoceptors in the effects of 5-HT and tryptamine on the isolated rat stomach fundus preparation was investigated. The effects of recognized anti-5-HT agents were also investigated to determine whether or not the same type of receptors was involved in the response of the rat fundus to 5-HT and tryptamine.

Methysergide was shown to be a very active, slowly reversible inhibitor of the 5-HT-induced contractions. The degree of inhibition was dependent on the time of pre-incubation and reached its maximum after approximately 2 hr.

The α-adrenolytic agent piperoxan appeared to be a competitive antagonist of 5-HT. Piperoxan was able to prevent or reduce the anti-5-HT activity of mianserin, cyproheptadine and methysergide, indicating a common site of action. All antagonists affected the 5-HT contractions more than those induced by tryptamine. It is concluded, that the effect of 5-HT on the isolated rat stomach fundus is the result of an interaction of 5-HT with one type of receptor. This receptor probably is the classical D-tryptamine receptor. The response to tryptamine is the result of an interaction by tryptamine with two different types of receptors. One type of receptor is identical to the receptor mediating the response to 5-HT, whilst the other type of receptor is more resistant to inhibition by methysergide, piperoxan, cyproheptadine or mianserin, and might be identical with the PRT-receptor, described earlier as being resistant to inhibition by phenoxybenzamine.

Phenoxybenzamine-ResistanT (PRT) receptor
Stimulatory α-adrenoceptor
Anti-5-HT
D-Tryptamine receptor
Anti-tryptamine
Methysergide
Piperoxan

1. Introduction

The potency of mianserin in counteracting the responses to 5-HT in a number of in vivo and in vitro pharmacological preparations was comparable to cyproheptadine (Van der Burg et al., 1970; Vargaftig et al., 1971). In contrast to cyproheptadine, mianserin failed to inhibit the 5-HT- and tryptamine-induced contractions of the isolated rat stomach fundus preparation (Frankhuijzen and Bonta, 1973). These experiments were performed in the presence of the α-adrenolytic piperoxan to prevent the inhibitory action of 5-HT on α-adrenoceptors (Offermeier and Ariëns, 1966). In receptor-protection experiments, both mianserin and piperoxan were able to prevent the inhibition of the response to 5-HT by phenoxybenzamine (Frankhuijzen and Bonta, 1973). According to Ariëns et al. (1964) non-competitive antagonists are not able to protect specific receptors against irreversible blockade by phenoxybenzamine. From the literature it is known that the D-tryptamine receptor in some respects bears resemblance to α-adrenoceptors. Vane (1960), Innes (1963) and Innes and Kohli (1969) described excitatory effects of sympathomimetic amines which they ascribed to an action on 5-HT receptors. On isolated strips of cat spleen

* This paper comprises part of a Doctors thesis in Pharmacology to be submitted by A.L.F. at the Erasmus University Rotterdam.

contractions due to 5-HT seemed to be mediated by adrenoceptors (Innes, 1962). With respect to the isolated rat fundus Abramets (1969) concluded from cross-tachyphylaxis and receptor-protection experiments, that the D-tryptamine receptor and the α-adrenoceptor were very similar.

One of the possibilities proposed to explain the apparent contradictory results with mianserin and piperoxan was that the α-adrenolytic properties of mianserin (Saxena et al., 1971) and of piperoxan in some way might be involved (Frankhuizen and Bonta, 1973). The aim of the present experiments was to investigate the possible role of α-adrenoceptors in the mediation of the effect of 5-HT on the rat fundus. In view of this and the above mentioned concept of Ariëns et al. (1964) with respect to the receptor protection, we repeated the experiments described by Offermeier and Ariëns in which they investigated the influence of piperoxan on the response of the rat fundus to 5-HT.

The anti-5-HT drug, methysergide was found to have no adrenolytic action (Von Fanchamps et al., 1960). This property of methysergide made it very suitable for the investigation of the possible participation of α-adrenoceptors in the response of the rat fundus to 5-HT.

In addition we looked to determine whether or not piperoxan affected the inhibition of the response to 5-HT and of tryptamine by several recognized anti-5-HT drugs. From our own experiments (Frankhuijzen and Bonta, 1973) and from literature (Barlow and Khan 1959a,b; Barlow, 1961; and Winter and Gessner, 1968) indications can be found for an action of tryptamine on other than D-tryptamine receptors. We investigated this possibility further by comparing the effects of the antagonists towards the responses of the rat fundus to 5-HT and to tryptamine.

2. Materials and methods

2.1. Preparation of the isolated rat stomach fundus strips

Experiments were carried out on rat stomach fundus strips from male albino Wistar rats weighing 200–300 g, killed by decapitation. The stomach was dissected and strips were cut as described by Vane (1957). 2 strips were cut from each fundus. The strips were set up in an organ bath at 37°C containing Tyrode solution, bubbled with a gas mixture containing 95% O_2–5% CO_2. The composition of the Tyrode solution was (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.6, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.6. Contractions were recorded with a Harvard electronic recording module. One end of a strip was attached to a lever of a Harvard Heart/Smooth muscle transducer loaded with 1 g weight. Before testing, the strips were allowed to equilibrate for 60 min, and during this period the bathing fluid was changed every 15 min. During the experiments, the bathing fluid was changed every 5 min. In all experiments the cumulative dose–response technique was used as described by Van Rossum (1963). Dose–response curves were recorded after a 25 min interval, the same agonist being administered every 50 min. In this way constant responses were obtained and no tachyphylaxis was observed to 5-HT. At the end of an experiment one dose–response curve was recorded for tryptamine. This procedure was chosen because, after the administration of high doses of tryptamine, the organ did not return to its original baseline even after 1 hr, or after stretching the tissue. The experimental procedure for a typical experiment is shown in fig. 1 so as to illustrate the exact scheme of drug administration.

2.2. Evaluation of antagonism

pA_2 and pD'_2 values were calculated according to the method of Ariëns et al. (1956) and Van Rossum (1963). A pA_2 value indicates the affinity of a competitive antagonist for the receptor and is denoted as the negative logarithm of the molar concentration of the antagonist which causes a 2-fold increase of the agonist concentration to obtain the same response. A pD'_2 value indicates the affinity of a non-competitive antagonist for the receptor, and is denoted as the negative logarithm of the molar concentration of the antagonist which causes a 50% reduction of the maximal effect of the agonist. Although the pA_2 and pD'_2 values are defined upon the assumption that an equilibrium state has been reached, pA_2 and pD'_2 values are also used in the experiments with methysergide in which the equilibrium state is not reached. In these experiments the parameters however only express the

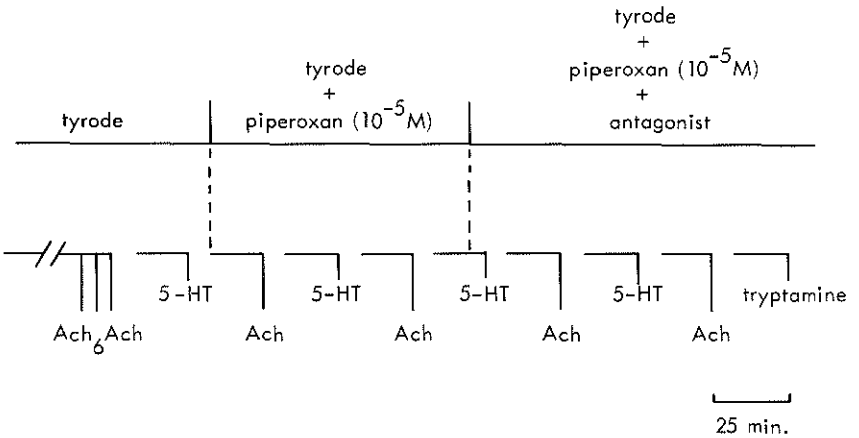


Fig. 1. Scheme of drug administration, illustrating the experimental procedure used. After 1 hr of equilibration of the tissue, 2 contractions to Ach were recorded, followed by cumulative dose–response curves every 25 min of resp. Ach and 5-HT. A dose–response curve for tryptamine was recorded at the end of the experiment. Ach_6 = acetylcholine, 10^{-6} M. The antagonists were dissolved in the Tyrode solution bathing the tissue.

degree of antagonism, although the values are calculated from equations valid only at equilibrium. Student's *t*-test was used for the statistical evaluation of the results.

2.3. Drugs

The following drugs were used: acetylcholine iodide (Fluka, A.G.), serotonin creatinine sulphate (Fluka A.G.), tryptamine hydrochloride (Fluka A.G.) methysergide hydrogen maleinate (Deseril, Sandoz), mianserin hydrochloride (Organon), cyproheptadine hydrochloride (Periactin, Merck Sharp and Dohme) and piperoxan (May and Baker). The solution of the drugs in Tyrode was prepared on the day of the experiment. Drugs were added to the 5 ml organ bath in a volume of 0.1 ml. The antagonists were dissolved in the stock Tyrode solution bathing the strips. All concentrations mentioned refer to the final concentration of the drug in the organ bath and are expressed in terms of molarity.

3. Results

3.1. Influence of methysergide on the responses to Ach, 5-HT and tryptamine

Methysergide, in a concentration of 10^{-9} M, had no effect on the Ach-induced contractions of the isolated rat stomach fundus preparation, even after 2 hr pre-incubation of the strips with methysergide. The same concentration of methysergide inhibited the 5-HT-induced contractions after only 5 min pre-incubation (fig. 2a). The 5-HT log dose–response curve was shifted to the right in the presence of methysergide. This shift was accompanied by a decrease of the maximal height of the curve. Both effects of methysergide on the 5-HT curve increased when the pre-incubation time was extended. The time dependence of the degree of inhibition of the maximal contraction height of 5-HT by methysergide is shown in fig. 2b. From this figure it can be seen that a maximal degree of antagonism was obtained after approximately 2 hr pre-incubation with methysergide. This long period of time necessary for the development of maximal inhibition of the response of the fundus to 5-HT is in accordance with results obtained by Barlow and Khan (1959a).

The tryptamine-induced contractions were also inhibited by methysergide (fig. 3). Pre-incubation with methysergide resulted in a shift of the tryptamine curve to the right. The maximal contraction height of tryptamine was however much less affected by methysergide compared with 5-HT. In table 1, are listed the pA_2 and pD'_2 values of methysergide towards both 5-HT and tryptamine at different pre-incubation times of methysergide. From this table it

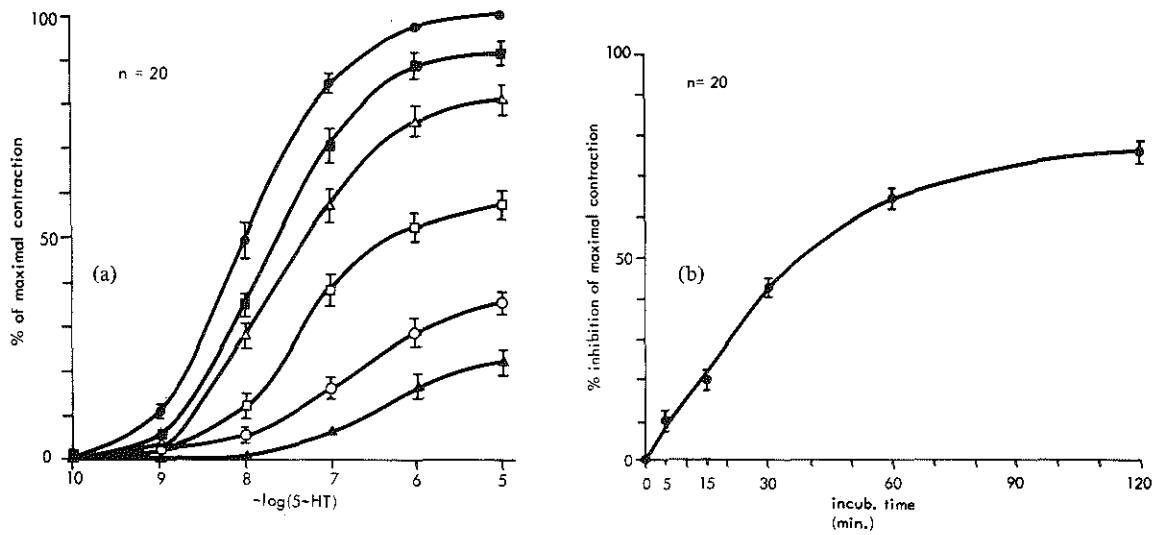


Fig. 2. (a) Inhibition of the response of the rat fundus to 5-HT by methysergide (10^{-9} M); influence of the pre-incubation time with methysergide. ●——● Log dose–response curve of 5-HT alone, ■——■ id. with methysergide (10^{-9} M) administered 5 min prior to 5-HT, or ○——○ id. 15 min or □——□ id. 30 min or ○——○ id. 60 min or ▲——▲ id. 120 min. Each curve represents the mean of 20 experiments. The vertical bars indicate the S.E.M. (b) Dependence of the percentage inhibition of the maximal response of the rat fundus to 5-HT on the pre-incubation time with methysergide (10^{-9} M). Each point of the curve represents the mean value ± S.E.M. (indicated by vertical bars) of 20 experiments.

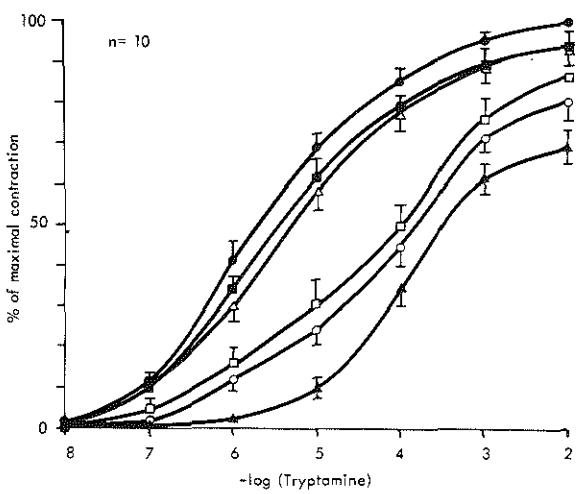


Fig. 3. Influence of methysergide (10^{-9} M) on the response of the rat fundus to tryptamine; influence of the pre-incubation time with methysergide. ●——● Log dose–response curve of tryptamine alone, ■——■ id. with methysergide (10^{-9} M) administered 5 min prior to tryptamine, or △—△ id. 15 min. or □——□ id. 30 min, or ○——○ id. 60 min or ▲——▲ id. 120 min. Each curve represents the mean of 10 experiments. The vertical bars in the figure indicate the S.E.M.

Table 1

The effect of the pre-incubation time of methysergide on the pA_2 and pD'_2 value of methysergide towards the response of the rat fundus to 5-HT and tryptamine. The values given in the table represent the mean ± S.E.M. of 20 (5-HT) or 10 (tryptamine) experiments.

Pre-incubation time of methysergide (10^{-9} M) (min)	Agonist			
	5-HT		Tryptamine	
	pA_2	pD'_2	pA_2	pD'_2
5	8.9 ± 0.4	8.0 ± 0.2	8.4 ± 0.7	7.7 ± 0.3
15	9.1 ± 0.3	8.39 ± 0.08	8.8 ± 0.6	7.9 ± 0.2
30	9.5 ± 0.5	8.88 ± 0.05	10.4 ± 0.2	8.3 ± 0.2
60	10.1 ± 0.3	9.27 ± 0.08	10.5 ± 0.3	8.4 ± 0.1
120	10.4 ± 0.4	9.55 ± 0.08	10.6 ± 0.3	8.6 ± 0.1

Table 2

Percentage recovery of the response of the rat fundus to 5-HT from the inhibition by methysergide (10^{-9} M); influence of piperoxan (10^{-5} M). The mean values of the % recovery of the response after the washout of methysergide are given ± S.E.M. The numbers of experiments are given in parentheses.

Pre-incubation time (min) of methysergide (10^{-9} M)	Recovery time (min) after washout of methysergide (10^{-9} M)				
	50	50 in presence of piperoxan (10^{-5} M)	100	100 in presence of piperoxan (10^{-5} M)	200
5 (5)	- 6.3 ± 0.4	94 ± 6	0 ± 0.4		
15 (7)	- 33 ± 1	61 ± 2	28 ± 1		
30 (10)	20.0 ± 0.8	53 ± 3	42 ± 2		
60 (7)			51 ± 2	65 ± 2	64 ± 3

can be seen that there is an increase of the pA_2 and pD_2' value of methysergide when the time of pre-incubation is extended. The pA_2 values towards 5-HT and tryptamine are comparable. The pD_2' values towards tryptamine are however, with the exception of the pD_2' value after 5 min pre-incubation time, significantly ($p < 0.01$–0.001) lower than towards 5-HT.

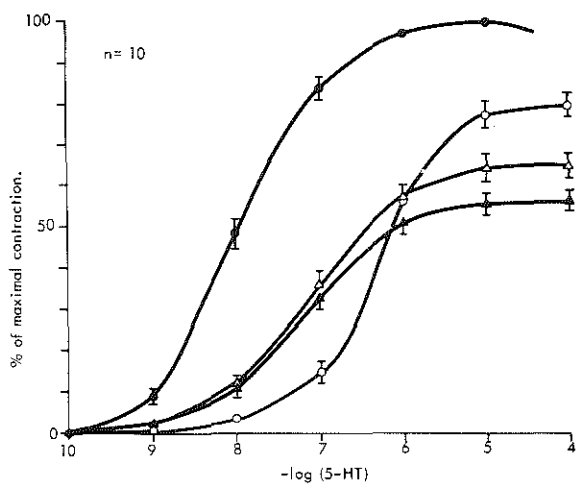


Fig. 4. Recovery of the response of the rat fundus to 5-HT from inhibition by methysergide (10^{-9} M, 30 min pre-incubation); influence of piperoxan (10^{-5} M). ●——● Log dose–response curve of 5-HT alone, ▲——▲ id. with methysergide (10^{-9} M) administered 30 min prior to 5-HT, or △——△ id. 50 min after the washout of methysergide, or ○——○ id. 50 min after the washout of methysergide, in the presence of piperoxan (10^{-5} M). Each curve represents the mean of 10 experiments. The S.E.M. are given by the vertical bars in the curves.

3.2. Recovery of the response to 5-HT from inhibition by methysergide: influence of piperoxan

The possibility was investigated as to whether methysergide exerted a reversible non-competitive type of antagonism towards 5-HT. Therefore the recovery of the response of the fundus to 5-HT was recorded at different time intervals after washing the strips from methysergide (10^{-9} M). As a result of these experiments, the antagonism of methysergide appeared to be of a very slowly reversible type. Even 200 min after washing the preparation from methysergide the percentage recovery of the maximal contraction of the fundus to 5-HT was only 64 ± 3% (table 2). The influence of the α-adrenolytic piperoxan on the recovery of the 5-HT response from methysergide inhibition was twofold. In the presence of piperoxan (10^{-5} M) the maximum of the 5-HT log dose–responses curve was increased (table 2). This increase was accompanied by a shift of the log dose–response curve to the right (fig. 4).

3.3. Influence of piperoxan (10^{-5} M) on the contractions induced by Ach, 5-HT and tryptamine

Because of the shift of the 5-HT curve to the right in the presence of piperoxan in the recovery experiments, the influence of piperoxan on the response of the fundus to 5-HT and tryptamine was investigated. Ach was included in these experiments to see whether an effect of piperoxan was limited to tryptamine receptors or not.

Administration of piperoxan resulted in a parallel

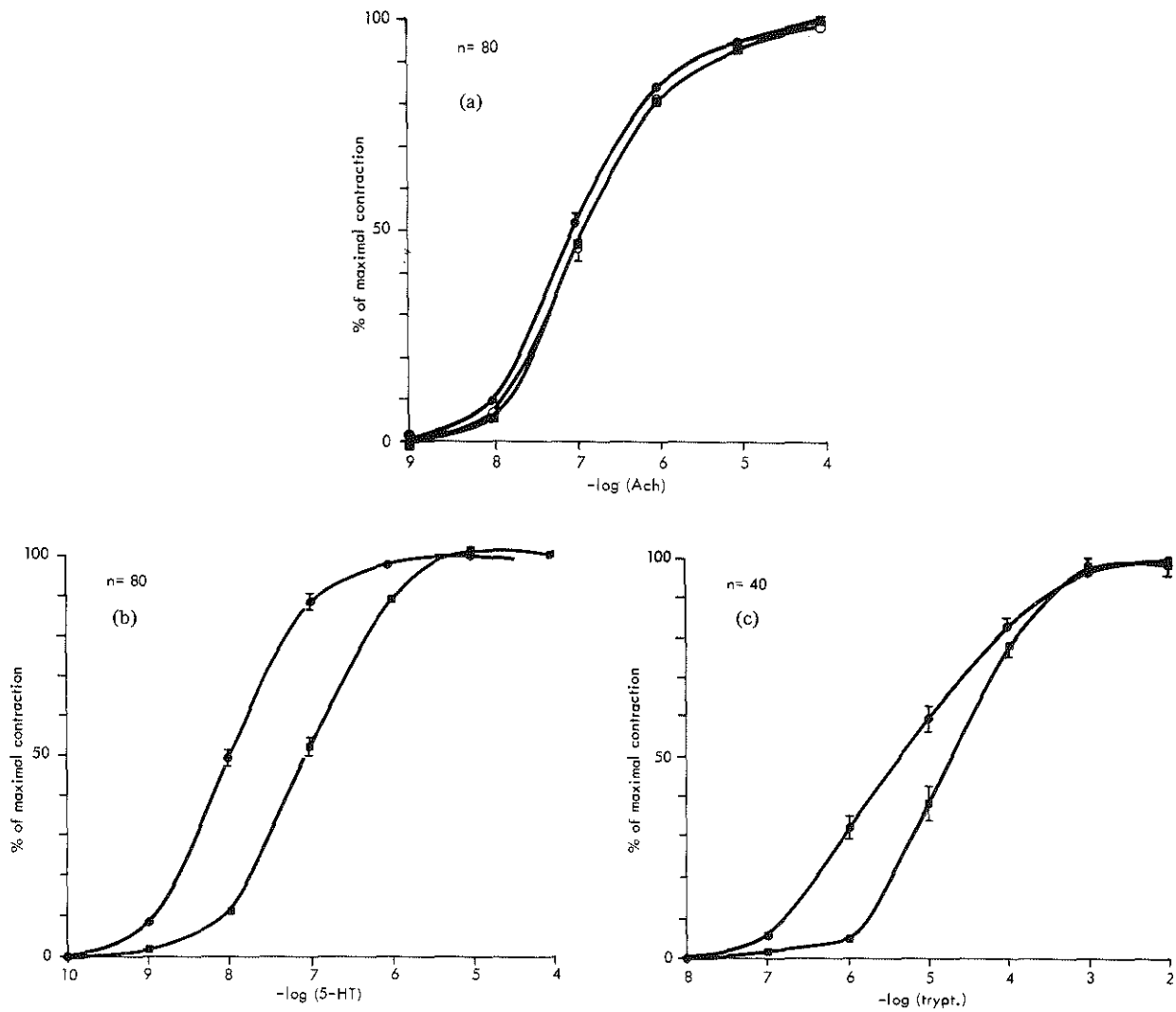


Fig. 5 (a) Influence of piperoxan (10^{-5} M) on the response of the rat fundus to Ach. ●——● Log dose–response curve of Ach alone, ■——■ id. with piperoxan (10^{-5} M) administered 20 min prior to Ach, or ○——○ id. 70 min prior to Ach. Each curve represents the mean of 80 experiments. The vertical bars in the figure indicate the S.E.M. (b) and (c) Influence of piperoxan (10^{-5} M) on the response of the rat fundus to 5-HT (b) and tryptamine (c). (b) ●——● Log dose–response curve of 5-HT alone, ■——■ id. with piperoxan (10^{-5} M) administered 45 min prior to 5-HT; (c) ●——● log dose–response curve of tryptamine alone, ■——■ id. with piperoxan (10^{-5} M) administered 145 min prior to tryptamine. Each curve represents the mean of 80 (5-HT) or 40 experiments (tryptamine). The vertical bars in the figures indicate the S.E.M.

shift to the right of the Ach log dose–response curve (fig. 5a). The difference between the pD_2' values (0.15 log units) of the Ach curve without, and in the presence of, piperoxan was not statistically significant, although it was observed in every experiment. Furthermore it could be reversed in the absence of piperoxan. The response of the fundus to 5-HT and tryptamine was much more affected. Pre-incubation of strips with piperoxan (10^{-5} M) for 45 min resulted in a parallel shift to the right of the 5-HT log dose–response curve of 0.90 ± 0.07 log units (fig. 5b). Extension of the pre-incubation time of piperoxan

Table 3

pA_2 values of piperoxan towards the responses of the rat fundus to Ach, 5-HT and tryptamine. The values represent the mean ± S.E.M. of 80 (Ach and 5-HT) or 40 (tryptamine) experiments.

Pre-incubation time (min) of piperoxan (10^{-5} M) (min)	Agonist	pA_2
20	Ach	4.51 ± 0.25
70	Ach	4.51 ± 0.25
45	5-HT	5.84 ± 0.08
95	5-HT	5.90 ± 0.11
145	Tryptamine	5.40 ± 0.15

did not result in a significant increase of the pA_2 value of piperoxan towards 5-HT (table 3). The action of piperoxan on the response to tryptamine (fig. 5c) appeared to be different from that of 5-HT. Although pre-incubation with piperoxan resulted in a shift of the tryptamine curve to the right, the pA_2 value of piperoxan towards tryptamine was significantly ($p < 0.001$) lower than towards 5-HT (table 3). Moreover, the slope of the tryptamine curve in the presence of piperoxan at 50% of the maximal contraction was increased by a factor of 1.5.

3.4. Influence of piperoxan (10^{-5} M) on inhibition by methysergide of the response to 5-HT and tryptamine

To obtain information as to whether or not piperoxan and methysergide have a common site of action, the influence of piperoxan on the methysergide inhibition of the contractions induced by 5-HT and tryptamine was investigated.

Pre-incubation of rat fundus strips with methysergide (10^{-9} M) for 1 hr in the presence of piperoxan did not affect the 5-HT log dose–response curve. Neither a shift to the right nor a decline of the curve was observed as it had been in the absence of piperoxan (fig. 6a). In the case of tryptamine, the inhibition by methysergide (10^{-9} M) after 2 hr pre-incubation was largely prevented by piperoxan (fig. 6b). The results of these experiments are summarized in table 4a and b. In these tables are listed the pD_2' values and the % maximal contraction of, respectively, the contractions induced by 5-HT and by tryptamine, without and in the presence of piperoxan and methysergide separately or in combination. From these tables it can be seen that pre-incubation of the fundus strips with methysergide in the presence of piperoxan did not result in a significant further decrease of the pD_2' values of 5-HT and tryptamine. The decrease of the maximal contraction of 5-HT and tryptamine by methysergide was largely prevented by piperoxan.

3.5. Inhibition of the response to 5-HT by cyproheptadine and mianserin: influence of piperoxan

To determine whether or not the influence of piperoxan was limited only to the anti-tryptamine actions of methysergide, two other recognized D-tryptamine receptor antagonists, mianserin and cyproheptadine, were used. It was investigated whether the actions of these two antagonists were affected by piperoxan in a way similar to methysergide. Pre-

Table 5

Influence of piperoxan (10^{-5} M) on the pA_2 and pD_2' values of cyproheptadine and the pA_2 values of mianserin towards the responses of the rat fundus to Ach, 5-HT and tryptamine. The values given represent the means of 10 experiments ± S.E.M.

Agonist	Antagonist					
	Cyproheptadine				Mianserin	
	pA_2		pD_2'		pA_2	
		In presence of piperoxan (10^{-5} M)		In presence of piperoxan (10^{-5} M)		In presence of piperoxan (10^{-5} M)
Ach	8.19 ± 0.10	8.03 ± 0.10			5.94 ± 0.40	6.05 ± 0.35
5-HT	7.83 ± 0.11	7.50 ± 0.10	5.70 ± 0.10	5.43 ± 0.09	7.05 ± 0.21	
Tryptamine	7.20 ± 0.17	6.87 ± 0.11			6.83 ± 0.28	

incubation of strips with mianserin (10^{-6} M) for 1 hr in the presence of piperoxan did not affect the 5-HT and tryptamine log dose–response curves (Frankhuijzen and Bonta, 1973). Pre-incubation with mianserin in the absence of piperoxan resulted in a parallel shift to the right of the 5-HT curve, indicating a competitive type of antagonism. The response of rat fundus strips to tryptamine in the absence of piperoxan was also affected by mianserin. The shift to the right of the tryptamine curve was however not parallel as it had been for 5-HT. Administration of mianserin resulted in a 2.4-fold increase in the slope of the curve at 50% of the maximal contraction height. In the case of cyproheptadine, the inhibition of the response of the fundus to 5-HT and tryptamine was reduced in the presence of piperoxan (table 5).

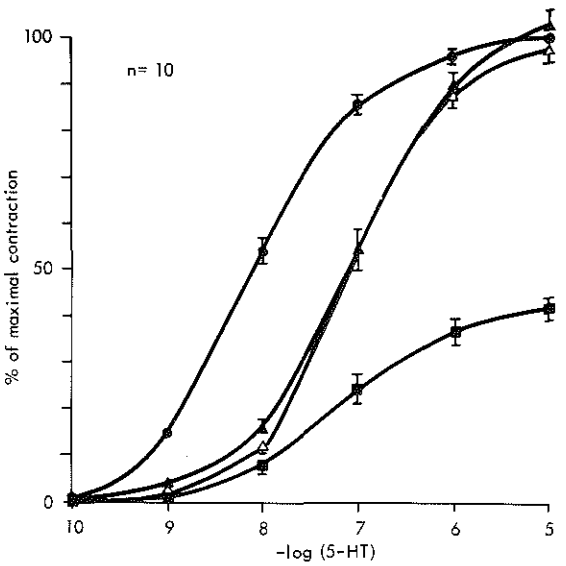


Fig. 6a and table 4a. Influence of methysergide on the response of the rat fundus to 5-HT alone, and in the presence of piperoxan (10^{-5} M). ●——● Log dose–response curve of 5-HT alone, ■——■ id. with methysergide (10^{-9} M) administered 60 min prior to 5-HT, or △——△ log dose–response curve of 5-HT in the presence of piperoxan (10^{-5} M), or ▲——▲ id. methysergide (10^{-9} M) administered 60 min prior to 5-HT in the presence of piperoxan (10^{-5} M). The values given in table 4a and each curve in fig. 6a, represents the mean of n experiments ± S.E.M. (in fig. 6a represented by vertical bars).

Table 4a

n	Antagonist	Conc. (M)	pD'_2 (5-HT)	% of max. contraction
88			8.05 ± 0.10	
44	Piperoxan	10^{-5}	7.05 ± 0.10	100 ± 1
12	Methysergide	10^{-10}	7.65 ± 0.15	71 ± 3
12	Piperoxan + methysergide	10^{-5} 10^{-10}	7.10 ± 0.15	94 ± 2
10	Methysergide	10^{-9}	7.20 ± 0.15	42 ± 3
10	Piperoxan + methysergide	10^{-5} 10^{-9}	7.00 ± 0.15	103 ± 3

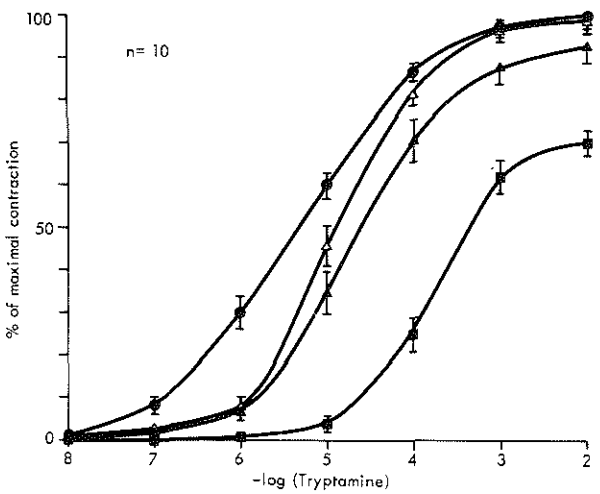


Fig. 6b and table 4b. Influence of methysergide on the response of the rat fundus to tryptamine alone, and in the presence of piperoxan (10^{-5} M) ●——● Log dose–response curve of tryptamine alone, ■——■ id. with methysergide (10^{-9} M) administered 2 hr prior to tryptamine, or △——△ log dose–response curve of tryptamine in the presence of piperoxan (10^{-5} M) or ▲——▲ id. with methysergide (10^{-9} M) administered 2 hr prior to tryptamine in the presence of piperoxan (10^{-5} M). The values given in the table and each curve in the figure represent the means of n experiments ± S.E.M. (in the figure represented by vertical bars).

Table 4b

n	Antagonist	Conc. (M)	pD'_2 (tryptamine)	% of max. contraction
40			5.25 ± 0.10	100
20	Piperoxan	10^{-5}	4.90 ± 0.10	99 ± 3
10	Methysergide	10^{-9}	3.60 ± 0.15	70 ± 3
10	Piperoxan + methysergide	10^{-5} 10^{-9}	4.70 ± 0.15	93 ± 4

4. Discussion

The spasmogenic action of both 5-HT and tryptamine is the result of an interaction with specific tryptamine receptors. These receptors are classified by Gaddum and Picarelli (1957) into two types: M- and D-tryptamine receptors. The M-tryptamine receptors are sited in the intramural parasympathetic ganglion cell (Brownlee and Johnson, 1963) and are blocked by morphine. The D-tryptamine receptors are localized in the membrane of smooth muscle cells and are blocked by phenoxybenzamine. In the isolated rat stomach fundus preparation only the D-tryptamine type of receptor is present (Vane, 1957; Offermeier and Ariëns, 1966). This is in contrast to the guinea-pig stomach, in which both types of tryptamine receptor are present (Yamaguchi, 1972). The contraction of the rat fundus by 5-HT is believed to be the result of an interaction of 5-HT with two kinds of receptors: D-tryptamine receptors and inhibitory α-adrenoceptors, as has been described by Offermeier and Ariëns (1966). However, according to the results of the experiments described in this paper, this model for the action of 5-HT on the rat fundus is no longer tenable. Methysergide was very active in antagonizing the 5-HT-induced contractions of strips of the rat fundus without affecting the response to Ach. Pre-incubation of the strips with 10^{-9} M methysergide resulted in a shift to the right of the 5-HT log dose–response curve. This shift was accompanied by a decrease of the maximal contraction height of the curve. The degree of antagonism appeared to be dependent on the time of pre-incubation and reached its maximum after about 2 hr. One possible explanation is, that methysergide exhibits a dualism in antagonism (Ariëns et al., 1964) with respect to 5-HT, behaving as a competitive as well as a non-competitive antagonist. However, according to the model for the action of 5-HT as proposed by Ariëns and Offermeier (1966) an alternative explanation would be that a pure competitive antagonism of methysergide exists towards the interaction of 5-HT with the D-tryptamine receptors. The decrease of the maximal contraction height of the log dose–response curve is then caused by the inhibitory action of 5-HT on α-adrenoceptors becoming more prominent. This possibility was supported by the finding that administration of the α-adrenolytic piperoxan, after washout of methysergide in the recovery experiments, resulted in an increase of the maximal contraction height to 5-HT.

Pre-incubation of fundus strips with piperoxan resulted however in a parallel shift to the right of the 5-HT log dose–response curve, indicating a competitive type of antagonism. This action of piperoxan is completely contradictory to the 5-fold potentiation of the maximal height of the 5-HT curve by piperoxan found by Offermeier and Ariëns (1966). In their experiments however strips were used from which the mucosa was removed, while the experiments described in this paper were performed on intact strips. Horn and Zweifach (1963) studied the influence of splitting the intact fundus strip into two layers at the level of the submucosa on the subsequent response to 5-HT. They found that the 5-HT-induced increase in tension of the serosal strip did not differ significantly from the tension increase produced in the intact strip. This means that the discrepancy between the results obtained by Offermeier and Ariëns and the results presented in this paper cannot be explained by a simple difference in the preparation. The competitive antagonism of piperoxan towards 5-HT is compatible with the previously observed receptor protection exerted by piperoxan towards the 5-HT-induced contraction against the irreversible inhibition by phenoxybenzamine (Frankhuijzen and Bonta, 1973).

Pre-incubation of strips with piperoxan prevented the inhibition of the response to 5-HT by methysergide. This makes it most likely that the site of action of both piperoxan and methysergide is the same. This means also that methysergide probably exerts an almost irreversible competitive type of antagonism towards 5-HT on the isolated rat fundus preparation. From experiments similar to those described for piperoxan and methysergide, it is concluded that the sites of action of mianserin and cyproheptadine are identical to those of piperoxan and that they also are competitive antagonists of 5-HT.

Comparing the influence of the anti-5-HT agents on the response of rat fundus to 5-HT and tryptamine, striking differences were found. These differences were very obvious with piperoxan and mianserin. Administration of these drugs resulted in a parallel shift to the right of the 5-HT log dose–response curve. In the case of tryptamine however, this shift was not parallel. The slope of the tryptamine log

dose–response curve at 50% of the maximal contraction height was increased with a factor of about 1.5. in the presence of piperoxan and 2.4. in the presence of mianserin. If the responses of the rat fundus to 5-HT and tryptamine are mediated by the same type of receptor, administration of piperoxan or mianserin should have resulted in a parallel shift to the right of the tryptamine log dose–response curve. Because of the observed increase in the slope of the tryptamine curve after competitive inhibition of the tryptamine receptors, there should be a subsidiary action of tryptamine on other than D-tryptamine receptors.

Responses to tryptamine mediated via other than D-tryptamine receptors are described in literature (Wooley and Shaw, 1957; Barlow, 1961; Winter and Gessner, 1968). All came to the conclusion that although the responses to tryptamine and 5-HT are affected by the same classes of antagonists, it is apparent, that differences exist with respect to the antagonism towards the action of tryptamine and 5-HT.

An important difference between tryptamine and 5-HT is each agent's lipid solubility. The lipid solubility of 5-HT is very low. Hence 5-HT, unlike tryptamine, is unable to passively enter the cell. Barlow (1961) ascribed the differential character of the inhibition of 5-HT and tryptamine either to an interference with the transport of tryptamine through the cell membrane coupled with an inhibition of the D-tryptamine receptor, or the existence of separate tryptamine and 5-HT receptors. Provided that the effect of tryptamine on the rat fundus is mediated by one type of receptor located in the cell membrane, inhibition of the transport of tryptamine through the cell, or inhibition of MAO, should result in a parallel shift to the left of the tryptamine curve. This was actually found by Offermeier and Ariëns (1966). This means that an interference with the transport or metabolism of tryptamine cannot be the explanation of the increase of the slope of the tryptamine curve after incubation with piperoxan or mianserin.

Except for mianserin and cyproheptadine, there is no prominent structural relationship between the antagonists used in these experiments. Nevertheless, all exerted a competitive type of antagonism towards 5-HT. This makes it most likely that the response of the rat fundus to 5-HT is mediated by only one type of receptor. According to the inhibition of the 5-HT of receptor. Acording to the inhibition of the 5-HT-induced contractions by phenoxybenzamine together with the receptor protection provided by mianserin and piperoxan against the inhibition by phenoxybenzamine (Frankhuijzen and Bonta, 1973), this receptor is of the classical D-type of tryptamine receptor.

From literature it is known that 5-HT shows an affinity towards α-adrenoceptors. This was shown amongst others for the rat vas deferens (Offermeier and Ariëns, 1966; Nishino et al., 1970), isolated strips of cat spleen. (Innes, 1962) and also for the rat stomach fundus (Abramets, 1969). A possible explanation for this action is that the spatial arrangement of the α-adrenoceptor and the D-tryptamine receptor is very similar. Another possibility is, that the action of 5-HT is mediated by an α-adrenoceptor with excitatory properties. This type of receptor is described in the literature (Gagnon, 1970; Gagnon and Belisle, 1970; Haffner, 1971; Baily, 1971; and Guimarães, 1969), and also for the rat fundus (Ogle and Wong, 1971). An interaction of 5-HT with such a type of receptor is more likely, since many of the α-adrenolytics posess anti-5-HT properties. Regarding the antagonists we used, piperoxan is a recognized α-adrenolytic, while α-adrenolytic properties of mianserin have been described (Saxena et al., 1971). No such property has been described for methysergide (Von Fanchamps et al., 1960). α-Adrenolytic properties have been described for structurally closely related compounds such as 2-bromo-LSD (Palmer and Burks, 1971; Krishnamurty, 1971). Although in the literature several arguments can also be found against the possibility of D-tryptamine receptors to be similar to α-adrenoceptors with stimulating properties (Gagnon, 1972), it is not possible to exclude this possibility from the experiments described in this paper.

In conclusion, the results presented indicate that the response of the isolated rat stomach fundus preparation to 5-HT is mediated by only one type of receptor. This receptor probably is the classical D-tryptamine receptor, although the possibility of a stimulant α-adrenoceptor is not excluded. The response to tryptamine is mediated by the same type of receptor. A subsidiary action of tryptamine on a different kind of receptor is described.

References

Abramets, I.I., 1969, Similarity between D-serotonin receptors and α-adrenergic receptors, Byul. Eksp. Biol. Med. 67, 158.

Ariëns, E.J., J.M. van Rossum and J.M. Simonis, 1956, A theoretical basis for molecular pharmacology, Arzneim. Forsch. 6, 282.

Ariëns, E.J., G.A.J. van Os, J.M. van Rossum and A.M. Simonis, 1964, Drug–Receptor interaction: Interaction of one or more drugs with different receptor systems, in: Molecular Pharmacology, Vol. 1, ed. E.J. Ariëns (Academic Press, New York) pp. 412, 310.

Bailey, D.M., 1971, Inhibitory and excitatory effects of sympathomimetic amines on muscle strips from the stomach of the guinea pig, Brit. J. Pharmacol. 41, 227.

Barlow, R.B., 1964, Effects on amine oxidase of substances which antagonize 5-hydroxytryptamine more than tryptamine, Brit. J. Pharmacol. 16, 153.

Barlow, R.B. and I. Khan, 1959a, Actions of some analogues of tryptamine on the isolated rat uterus and on the isolated rat fundus strip preparations, Brit. J. Pharmacol. 14, 99.

Barlow, R.B. and I. Khan, 1959b, Actions of some analogues of tryptamine on the isolated rat uterus and the rat fundus strip preparations, Brit. J. Pharmacol. 14, 265.

Brownlee, G. and E.S. Johnson, 1963, The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum, Brit. J. Pharmacol. 21, 306.

Frankhuijzen, A.L. and I.L. Bonta, 1973, Effect of mianserin, a potent anti-serotonin agent, on the isolated rat stomach fundus preparation, European J. Pharmacol. 25, 40.

Gaddum, J.H. and Z.P. Picarelli, 1957, Two types of tryptamine receptors, Brit. J. Pharmacol. 12, 323.

Gagnon, D.J., 1970, Intestinal smooth muscle: Demonstration of catecholamines induced contraction mediated through α-adrenergic receptors, European J. Pharmacol. 10, 297.

Gagnon, D.J., 1972, Contraction of the rat colon by sympathomimetic amines: effect of methysergide and 5-HT desensitization, European J. Pharmacol. 17, 319.

Gagnon, D.J. and S. Belisle, 1970, Stimulatory effects of catecholamines on the isolate rat colon after β-adrenergic blockade with oxprenolol and propranolol, European J. Pharmacol. 12, 303.

Guimarães, S., 1969, α-Excitatory, α-inhibitory and β-inhibitory adrenergic receptors in the guinea-pig stomach, Arch. Intern. Pharmacodyn. Therap. 179, 188.

Haffner, J.F.W., 1971, The adrenergic receptors in the isolated rabbit stomach muscle, Acta Pharmacol. Toxicol. 29, 327.

Horn, L. and B.W. Zweifach, 1963, Some factors affecting the response of smooth muscle to chemical mediators, Angiol. 14, 139.

Innes, I.R., 1962, An action of 5-hydroxytryptamine on adrenaline receptors, Brit. J. Pharmacol. 19, 427.

Innes, I.R., 1963, Action of dexamphetamine on 5-hydroxytryptamine receptors, Brit. J. Pharmacol. 21, 427.

Innes, I.R. and J.D. Kohli, 1969, Excitatory action of sympathomimetic amines on 5-hydroxytryptamine receptors of gut, Brit. J. Pharmacol. 35, 383.

Krishnamurty, V.S.R., 1971, Receptors for sympathomimetic amines and 5-hydroxytryptamine in the rat aorta, Arch. Intern. Pharmacodyn. 189, 90.

Nishino, K., T. Irikura and I. Takayanagi, 1970, Mode of action of 5-hydroxytryptamine on isolated rat vas deferens, Nature 228, 564.

Offermeier, J. and E.J. Ariëns, 1966, Serotonin I. Receptors involved in its action, Arch. Intern. Pharmacodyn. 164, 192.

Ogle, C.W. and C.Y. Wong, 1971, The inhibitory and excitatory properties of α-adrenoceptors in the rat stomach, Life Sci. I 10, 153.

Palmer, G.C. and Th. F. Burks, 1971, Central and peripheral adrenergic blocking actions of LSD and BOL, European J. Phamacol. 16, 113.

Saxena, P.R., P. van Houwelingen and I.L. Bonta, 1971, The effects of mianserin hydrochloride on the vascular response evoked by 5-hydroxytryptamine and related vasoactive substances, European J. Pharmacol. 13, 295.

Van der Burg, W.J., I.L. Bonta, I. Delovelle, C. Ramon and B.B. Vargaftig, 1970, A novel type of substituted piperazine with high antiserotonin activity, J. Med. Chem. 13, 35.

Van Rossum, J.M., 1963, Cumulative dose–response curves. II. Technique for the making of dose–response curves in isolated organs and the evaluation of drug parameters, Arch. Intern. Pharmacodyn. 143, 299.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Brit. J. Pharmacol. 12, 344.

Vane, J.R., 1960, The actions of sympathomimetic amines on tryptamine receptors, in: Ciba Foundation Symposium on Adrenergic Mechanisms, eds. J.R. Vane, G.E.W. Wolstenholme and M.O'Connor (Churchill, London) p. 356.

Vargaftig, B.B., I.L. Coignet, C.J. de Vos, H. Grijssen and I.L. Bonta, 1971, Mianserin hydrochloride: Peripheral and central effects in relation to antagonism against 5-hydroxytryptamine, European J. Pharmacol. 16, 366.

Von Fanchamps, A.W. Doepfner, H. Weidemann and A. Cerletti, 1960, Pharmakologische Charakterisierung von Deseril, einem Serotonin-Antagonisten, Schweiz. Med. Wochenschr. 37, 1040.

Winter, J.C. and P.K. Gessner, 1968, Phenoxybenzamine antagonism of tryptamines, their indene isosteres and 5-hydroxytryptamine in the rat stomach fundus preparation, J. Pharmacol. Exptl. Therap. 162, 286.

Wooley, D.W. and E. Shaw, 1957, Differentiation between receptors for serotonin and tryptamine by means of the exquisite specificity of antimetabolites, J. Pharmacol. Exptl. Therap. 121, 13.

Yamaguchi, T., 1972, Effects of 5-hydroxytryptamine on isolated strips of the guinea-pig stomach, Brit. J. Pharmacol. 44, 100.

ANALYSIS OF ERGOTAMINE - 5-HT INTERACTION ON THE ISOLATED RAT STOMACH PREPARATION

Abraham L. FRANKHUIJZEN

Department of Pharmacology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands

ABSTRACT

The effect of ergotamine on the isolated rat stomach and its influence on the response to ACh and 5-HT were investigated. The log dose-response curve of ergotamine was bell-shaped. Extension of the incubation time of ergotamine resulted in a parallel shift to the left of the curve. The response to ergotamine was inhibited by methysergide and piperoxan. Incubation with ergotamine resulted in a decrease of the pD_2-value of 5-HT together with a marked suppression of the maximum of the 5-HT curve. The response to ACh was affected in accordance with the prediction of an action of ACh and ergotamine on different receptors. The prolonged receptor stimulation by 5-HT or ACh resulted in a decrease of the apparent affinity towards their receptors. Incubation with ACh resulted in a parallel shift to the right of the 5-HT curve. However, no inconsistency with the theoretical prediction of an action on separate receptors was observed with the ACh curve in the presence of 5-HT. It is concluded that ergotamine is a partial agonist on the D-tryptamine receptors of the isolated rat stomach. The marked decrease of the maximum of the 5-HT curve by ergotamine is probably caused by the slowly reversible character of its antagonism. The parallel shift to the left of the ergotamine curve with the extension of the

incubation time and the persistence of its antagonism both are probably caused by a slow diffusion into and from the biophase.

1. INTRODUCTION

It has been argued that ergotamine may owe its therapeutic effectiveness in migraine to a selective constriction of the external carotid vessels (Saxena and de Vlaam-Schluter, 1974). The smooth muscle stimulatory action of ergot alkaloids seems to be mediated either by α-adrenoceptors (Innes, 1962) or by D-tryptamine receptors (Dyer, 1974). Another type of tryptamine receptor different from the classical D-tryptamine receptor, according to the classification by Gaddum and Picarelli (1957), seems to be involved in the mediation of the constrictor response of the external carotid vessels to ergotamine and 5-HT (Saxena and de Vlaam-Schluter, 1974) and probably also to the anti 5-HT drugs methysergide (Saxena, 1974) and mianserin (Saxena et al., 1971).
Tryptamine receptors different from the classical D-tryptamine receptors also seem to be present in the isolated rat stomach (Winter and Gessner, 1968; Frankhuijzen and Bonta, 1974a, b), although the response to 5-HT appears to be mediated only by the classical D-tryptamine receptors (Frankhuijzen and Bonta, 1974b).
Because of the importance ascribed to the agonistic response to ergotamine in migraine therapy and the occurrance of a non D-type of myogenic tryptamine receptor it seemed of interest to investigate in detail the effect of ergotamine on the isolated rat stomach preparation and its influence on the response to 5-HT.

2. MATERIALS AND METHODS

2.1. Experimental preparation

The rat stomach strips were prepared according to the method described

by Vane (1957) from Wistar adult male rats weighing 200-300 g killed by decapitation. Two strips were cut from the ruminal part of the stomach: one set of strips served as a blank in order to correct the responses of the other strips for changes in sensitivity and tone. The strips were set up in a 5 ml organ bath maintained at 37°C containing Tyrode solution and bubbled with a gas mixture containing 95% O_2 - 5% CO_2. The Tyrode buffer solution was of the following composition (mM): NaCl 136.8; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.6; NaH_2PO_4 0.4; $NaHCO_3$ 11.9 and glucose 5.6. The contractions were amplified 3 times using a Harvard heart/smooth muscle transducer loaded with 1.0 g weight and recorded with a Harvard electronic module 350.
Usually cumulative dose-response curves according to the method of Van Rossum (1963) were obtained. Only in the case of the ergotamine curves in which each concentration of ergotamine was left in contact with the tissue for a definite time the single dose-response technique was used. The total volume of the agonist added to the organ bath did not exceed 15% of the bath volume. The procedure used in the experiments was essentially the same as described before (Frankhuijzen and Bonta, 1974b).

2.2. Theoretical log dose-response curves

Two different types of drug-receptor interactions are involved in the experiments to be presented: "competitive interaction", in which two or more drugs act on one receptor system and "functional interaction" in which two or more drugs act on different independant receptors. The appropriate equations used for the calculations of the log dose-response curves were derived from those given by Ariëns (1964). In some instances it was necessary to use the logistic function (Parker and Waud, 1971):

$$\frac{E_A}{E_M} = \frac{\alpha}{1 + (K_A/A)^p}$$

where α = intrinsic activity, A = concentration of drug A, K_A = dissociation constant of the drug-receptor complex and p = exponent to admit correction for the slope of the dose-response curve experimentally found. The exponent was graphically determined by plotting $\log(E_M - E_A/E_A)$ against -log A.

2.3. Drugs

The following drugs were used: acetylcholine iodide (Fluka A.G.), serotonin creatinin sulphate (5-HT, Fluka A.G.), ergotamine tartrate (Gynergeen, Sandoz), methysergide hydrogen maleinate (Deseril, Sandoz), piperoxan chloride (May and Baker). The solutions of the salts of the drugs were prepared freshly on the day of the experiment. Drug solutions were added to the 5 ml organ bath in a volume of 0.1 ml. All concentrations mentioned refer to the final concentration of the drug in the organ bath and are expressed in terms of molarity.

3. RESULTS

3.1. Influence of ergotamine on the response to 5-HT.

Incubation during 70 min of rat stomach strips with ergotamine (Fig. 1) resulted in a dose-dependant contraction, the maximum of which was reached at a bath concentration of about 10^{-7}M. A further increase to 10^{-6}M of the concentration did not result in a higher contraction. The response even seemed to be diminished.

The influence of the incubation with ergotamine on the 5-HT log dose-response curve was twofold. The pD_2-value of 5-HT decreased with increasing ergotamine concentration together with a marked decrease of the maximum of the 5-HT curve (Table 1).

Both the elevation of the tone of the preparation and the inhibition of the response to 5-HT persisted for more than 2 hr after the removal of ergotamine from the bathing fluid.

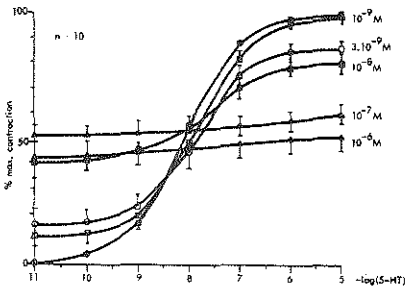


Fig. 1. Influence of incubation during 70 min with ergotamine on the response of rat stomach strips to 5-HT. ●——● Log dose-response curve of 5-HT; □——□ id. in the presence of ergotamine (10^{-9}M); o——o id. 3.10^{-9}M; ■——■ id., 10^{-8}M; Δ——Δ id., 10^{-7}M; ▲——▲ id., 10^{-6}M. Each curve represents the mean out of 10 experiments. The S.E.M. in each point of the curve is indicated by vertical bars.

Base-line	Ergotamine bath concentration (M)	% max.contr. of 5-HT	pD_2 (5-HT)
0 ± 0	0	100 ± 0	8.10 ± 0.05
11 ± 4	10^{-9}	98 ± 2	7.90 ± 0.10
16 ± 6	3.10^{-9}	86 ± 3	7.80 ± 0.15
40 ± 6	10^{-8}	80 ± 4	7.60 ± 0.15
51 ± 4	10^{-7}	60 ± 4	
42 ± 6	10^{-6}	51 ± 6	

Table 1. Influence of incubation during 70 min with ergotamine on the response of rat stomach strips to 5-HT. The values listed under base-line are expressed in % maximal contraction of 5-HT. All values given in the table represent the mean out of 10 exp.

3.2. Contraction of the rat stomach strip by ergotamine

The possibility of a partial agonistic action of ergotamine on D-tryptamine receptors was investigated using the cumulative dose-response technique. The ergotamine log dose-response curve was bell-shaped reaching its maximum at a bath-concentration of 3.10^{-6}M (Fig. 2).

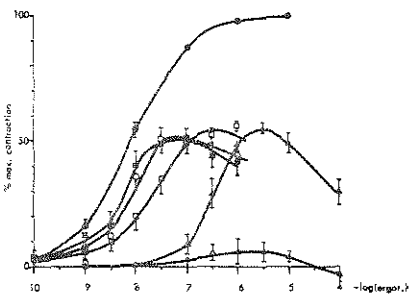


Fig. 2. Contraction of strips from rat stomach by ergotamine. ●——● Log dose-response curve of 5-HT; ▲——▲ id., of ergotamine; Δ——Δ id., after 20 min incubation with methysergide (10^{-8}M); ▣——▣ Log dose-response curve of ergotamine. Each concentration of ergotamine as indicated by the points of the curve was left in contact with the tissue for 20 min; o——o id., for 45 min; ■——■ id., for 70 min. Each curve represents the mean out of 10 experiments. The S.E.M. in each point in the curve is indicated by vertical bars.

Further increase of the ergotamine concentration resulted in a decrease of the contraction height. Incubation during 20 min with methysergide (10^{-8}M) resulted in an almost complete blockade of the ergotamine induced contraction.

A remarkable decrease of the pD_2-value of ergotamine of 1.75 ± 0.23 log units was observed in comparison with the curve obtained with single doses of ergotamine left in contact with the tissue for 70 min. In order

to confirm the influence of the duration of incubation on the pD_2-value of ergotamine, single dose-response curves were recorded after incubation with ergotamine for 20 and 45 min respectively. From Fig. 2 it can be seen that the ergotamine log dose-response curve was gradually shifted to the left with increasing incubation times. No significant change of the maximum height was observed, resulting in an increase of the pD_2-value of ergotamine.

3.3. Influence of ergotamine (10^{-8}M) on the response to ACh; comparison with its influence on the response to 5-HT.

During the incubation of the stomach strips with ergotamine the preparation is up till 70 min in a continuous state of contraction. The possibility was therefore investigated as to whether the continued elevated tone of the preparation did influence the contractility and/or sensitivity of the preparation. For this purpose cumulative dose-response curves of ACh and 5-HT were recorded after the incubation with one concentration of ergotamine (10^{-8}M).

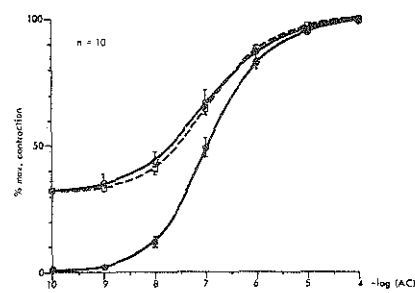


Fig. 3a. Influence of incubation during 45 min with ergotamine (10^{-8}M) on the response to ACh. ●——● Log dose-response curve of ACh; o——o id., after incubation with ergotamine; □- - -□ id., theoretical curve.

The log dose-response curves obtained experimentally were compared with the theoretical curves assuming a functional interaction between ergotamine and ACh (Fig. 3a) and a competitive type of interaction between ergotamine and 5-HT (Fig. 3b).

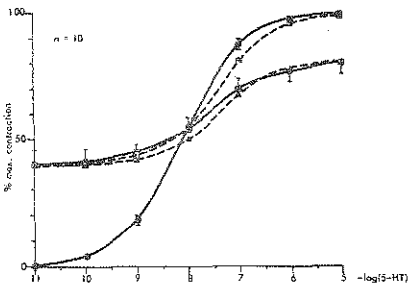


Fig. 3b. Influence of incubation during 70 min with ergotamine (10^{-8}M) on the response to 5-HT. ●——● Log dose-response curve of 5-HT; o——o id., in the presence of ergotamine (10^{-8}M); ▫--▫ id., theoretical curve; ▲--▲ id., theoretical curve corrected for the decline in maximal contraction height.

With respect to the ergotamine-5-HT interaction actually two theoretical log dose-response curves are given. In order to make a better comparison possible between the curve experimentally found and the theoretical curve, the original theoretical curve was transformed to a curve with the same maximum as the one experimentally obtained. The assumption was made that the decrease of the maximum of the 5-HT curve experimentally obtained was caused by the irreversibility of the action of ergotamine. The theoretical curve and the ACh-curve experimentally obtained were not significantly different. The mean value of the pD_2 of 5-HT in the presence of ergotamine was 7.60 $\pm$ 0.25. A pD_2-value of 7.35 was expected from the theory. Although no S.E.M. was calculated for the theoretical pD_2-value, the observed difference in pD_2-value was nevertheless not appreciable.

3.4. Influence of incubation with 5-HT (10^{-8}M) on the response to ACh and 5-HT.

In order to see as to whether the prolonged receptor stimulation by ergotamine had affected the response of the tissue to ergotamine and its effect on the 5-HT induced contraction, the same experiments were performed in which the effect of prolonged incubation with 5-HT on the response to ACh and 5-HT was investigated. A bath-concentration of 5-HT was chosen which was expected to result in a similar elevation of the tone of the preparation as with ergotamine (10^{-8}M). In order to see whether aspecific effects would occur, the influence of the incubation with 5-HT (10^{-8}M) was also investigated on the ACh induced response. Both the theoretical curve, assuming a synergistic action between 5-HT and ACh, and the curve experimentally obtained are depicted in Fig. 4a.

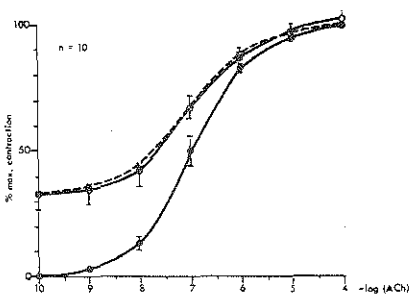


Fig. 4a. The influence of incubation with 5-HT (10^{-8}M) during 45 min on the response of rat stomach strips to ACh. ●——● Log dose-response curve of ACh; o——o id., in the presence of 5-HT (10^{-8}M); □---□ id., theoretical curve.

No significant difference was observed between the pD_2-value and the % maximal contraction of both curves. The incubation during 70 min with 5-HT (10^{-8}M) resulted in a marked shift to the right of the 5-HT log dose-response curve as compared with the theoretical curve (Fig. 4b).

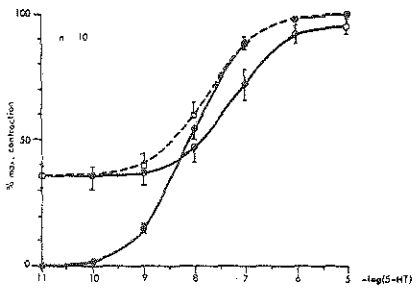


Fig. 4b. Influence of incubation of rat stomach strips during 70 min with 5-HT (10^{-8}M) on the response to 5-HT. ●——● Log dose-response curve of 5-HT; o——o id., after incubation with 5-HT (10^{-8}M); ▫--▫ id., theoretical curve.

Only a slight reduction of the maximum of the curve was observed, which, however, was not significant.

3.5. Influence of incubation with ACh (10^{-7}M) on the response to ACh and 5-HT.

It was investigated whether the tachyphylaxis towards the response to 5-HT of rat stomach strips after the prolonged exposure to 5-HT was specific for 5-HT or whether it was a more general phenomenon. For this reason the influence of the incubation with ACh of the preparation on the response to ACh and 5-HT was examined. As with ergotamine and 5-HT a concentration of ACh was chosen which was expected to result in a comparable increase in tone of the preparation. Again the curves experimentally obtained were compared with those calculated from the relevant equations. The incubation during 45 min with ACh (10^{-7}M) resulted in a parallel shift to the right of the log dose-response curve of ACh, if compared with the theoretical curve, without significantly affecting the maximum of the curve (Fig. 5a). This resulted in a highly

significant decrease of the pD_2-value of 0.55 ± 0.16 log units.

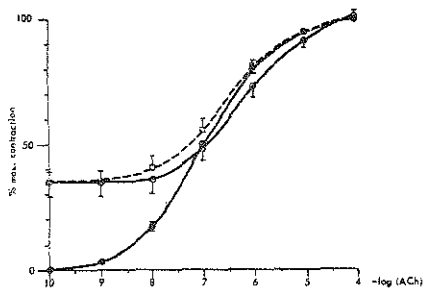


Fig. 5a. Influence of incubation during 45 min with ACh (10^{-7}M) on the response of rat stomach strips to ACh. ●——● Log dose-response curve of ACh; o——o id., after incubation with ACh (10^{-7}M); ▫--▫ id., theoretical curve.

Regarding the influence of incubation with ACh on the response to 5-HT (Fig. 5b) the decrease of the maximum of the curve of 4% ± 3 was not significant. The pD_2-value of 5-HT was, however, significantly ($p < 0.05$) reduced after the incubation with ACh with 0.45 log units.

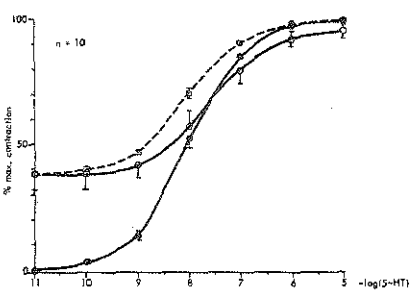


Fig. 5b. Influence of incubation during 70 min with ACh (10^{-7}M) on the response to 5-HT. ●——● Log dose-response curve of 5-HT; o--o id., after incubation with ACh. ▫--▫ id., theoretical curve.

Base-line	Antagonist	Agonist	% max. contraction	pD_2
0 ± 0		ACh	100 ± 0	7.00 ± 0.05
-3 ± 3	Piperoxan (10^{-5}M)	ACh	99 ± 2	6.80 ± 0.10
31 ± 6	Ergotamine (10^{-8}M)	ACh	101 ± 2	7.00 ± 0.20
12 ± 4	Piperoxan + Ergotamine	ACh	100 ± 3	6.70 ± 0.15
6	id., theor. values	ACh	100	6.80
0 ± 0		5-HT	100 ± 0	8.10 ± 0.05
0 ± 3	Piperoxan (10^{-5}M)	5-HT	101 ± 1	6.90 ± 0.10
40 ± 6	Ergotamine (10^{-8}M)	5-HT	80 ± 4	7.60 ± 0.25
26 ± 6	Piperoxan + Ergotamine	5-HT	95 ± 5	6.55 ± 0.15
22	id., theor, values	5-HT	>80	6.40

Table 2. Inhibition by piperoxan of the ergotamine induced contraction left in contact with the tissue for 45 respectively 70 min and its influence on the effect of ergotamine on the response to ACh and 5-HT. All values are given ± S.E.M. and represent the mean out of 10 experiments.

3.6. Influence of piperoxan on the effect of ergotamine on the response to ACh and 5-HT.

In order to make the differentiation possible between the receptors for ergot-alkaloids and D-tryptamine receptors the influence of piperoxan, which is a benzodioxane, on the effect of ergotamine was investigated (Table 2).

The response of the rat stomach strip to ergotamine (10^{-8}M) left in contact with the tissue for 45 respectively 70 min were strongly inhibited by piperoxan (10^{-5}M). The decrease of the pD_2-value of ACh after piperoxan was not significantly affected by ergotamine. With respect to the response to 5-HT the incubation with ergotamine resulted in a significant ($p < 0.05$) further decrease of the pD_2-value of 5-HT after piperoxan with 0.35 log units.

4. DISCUSSION

Incubation of rat stomach strips with ergotamine resulted in a contraction of the preparation. The magnitude of the submaximal contractions induced by ergotamine gradually increased with time reaching their maximum value after 45-70 min. Similar to 5-HT (Gaddum, 1957; Offermeier and Ariëns, 1966; Abramets, 1969; Dyer, 1974; Frankhuijzen and Bonta, 1974a, b) the log dose-response curve of ergotamine was bell-shaped. Methysergide, used in a concentration of 10^{-8}M and an incubation time of 20 min which was expected, according to its pD_2'-value (Frankhuijzen and Bonta, 1974b), to cause about 70% inhibition of the 5-HT induced contraction, nearly completely blocked the response to ergotamine. Therefore a subsidiary action of ergotamine on a tryptamine receptor different from the classical D-tryptamine receptor is excluded. For tryptamine it has been shown previously (Frankhuijzen and Bonta, 1974b) that the response mediated via this aberrant type of tryptamine receptor is very resistant to the inhibition by methysergide. The log dose-response curve

of 5-HT obtained after the incubation with ergotamine was not significantly different from the theoretical curve in which the assumption was made of an interaction of both ergotamine and 5-HT with the same receptor. Moreover piperoxan (10^{-5}M) which is shown to competitively antagonize the response to 5-HT (Frankhuijzen and Bonta, 1974b) inhibited the ergotamine induced contraction and affected the response to 5-HT in the presence of ergotamine in accordance with the supposition that all three drugs act on the same receptor. As pointed out above, the ergotamine-induced contraction of the rat stomach strip seems to be mediated solely by the classical D-tryptamine receptor. Several arguments can be found in the literature for the localization of the D-tryptamine receptors within the smooth muscle cell membrane (Born, 1962; Handschumacher and Vane, 1967; Born, 1970). The observed parallel shift to the left of the ergotamine log dose-response curve with increasing incubation times cannot for this reason be explained by an increased uptake of ergotamine into the smooth muscle cells. Several possible explanations can be given for the observed shift, all of which are based upon the assumption that the increase in the apparent affinity of rat stomach strips to ergotamine with time is caused by the low rate of increase of the contractile response. Possibly this is a reflection of a low rate of formation of the ergotamine/receptor-complex (Paton, 1961; Thron and Waud, 1968; Rang and Ritter, 1970; Lüllmann and Ziegler, 1973) or of a low rate of the translocation of Ca^{2+} across the cell membrane (Chang and Triggle, 1973).

Influences not related to 5-HT or its receptors were not involved in the effects observed with ergotamine on the response to 5-HT. The log dose-response curve of ACh obtained in the presence of ergotamine was nearly identical with the theoretical curve supposing an action of ergotamine and ACh on separate receptors.

From the literature (Gaddum, 1957; Waud, 1968; Katz and Thesleff, 1957; Rang and Ritter, 1970; Pluchino, 1972; Chang and Triggle, 1973b) it is known that prolonged receptor stimulation might result in desensitization.

Nonspecific desensitization (Cantoni and Eastman, 1946) of the isolated rat stomach preparation after the prolonged stimulation of the D-tryptamine receptors can be excluded because incubation with 5-HT affected the ACh log dose-response curve in a way similar to ergotamine, indicative for an action of 5-HT and ACh on different receptors. The 5-HT curve was, however, shifted to the right resulting in a 4 fold decrease in apparent affinity. Direct extrapolation of the desensitization induced by 5-HT to ergotamine is not allowed because of the uncertainty whether or not partial agonists are able to induce desensitization (Lüllmann and Ziegler, 1973; Chang and Triggle, 1973a, b). The contraction induced by ACh (10^{-7}M) gradually decreased with time and caused a parallel shift to the right of both the ACh and 5-HT log dose-response curve. The decrease of the contraction might have been caused by hydrolysis of the ACh, although the preparation was washed every 5 min after which ACh was administered again. Since the ACh log dose-response curve was shifted to the right after the incubation with ACh, tachyphylaxis seems to be a more appropriate explanation of the reduction of the response to ACh. Cholinergic mechanisms are supposed to be involved only in the action of 5-HT mediated by the neurotropic M-receptor (Gaddum and Picarelli, 1957; Gyermeck, 1966; Born, 1970). In literature however some support can be obtained for a cholinergic involvement also in the mediation of the action of 5-HT by D-tryptamine receptors (Leitch et al., 1957).

In conclusion the results of the experiments described clearly indicate a partial agonistic action of ergotamine on the D-tryptamine receptors of the isolated rat stomach preparation. In this respect no analogy exists, as with mianserin (Frankhuijzen and Bonta, 1974a; Saxena et al., 1971), with the results obtained by Saxena (1972) in which the selective vasoconstriction by ergotamine of the carotid vasculature seemed not to be mediated by D-tryptamine receptors.

Prolonged stimulation of the D-tryptamine receptors resulted in specific desensitization. Whether this phenomenon was also involved in the action

of ergotamine and to what extent remained uncertain.

REFERENCES

Abramets, I.L., 1969, Similarity between D-serotonin receptors and α-adrenergic receptors, Byul.Eksp.Biol.Med. 67, 158.

Ariëns, E.J., 1964, Molecular Pharmacology I (Academic Press, New York).

Born, G.V.R., 1962, The fate of 5-hydroxytryptamine in a smooth muscle and in connective tissue, J.Physiol. 161, 160.

Born, G.V.R., 1970, 5-Hydroxytryptamine receptors, in: Smooth Muscle, eds. Bülbring, E., A.F. Brading, A.W. Jones and T. Tomita, pp. 418-450, London: Edward Arnold.

Cantoni, G.L. and G. Eastman, 1946, On the response of the intestine to smooth muscle stimulants, J.Pharmacol.Exptl.Therap. 87, 392.

Chang, K.-J. and D.J. Triggle, 1973a, Quantitative aspects of drug-receptor interactions. I. Ca^{2+} and cholinergic receptor activation in smooth muscle: a basic model for drug-receptor interactions, J.Theor.Biol. 40, 125.

Chang, K.-J. and D.J. Triggle, 1973b, Quantitative aspects of drug-receptor interactions. II. The role of Ca^{2+}_{mem} in desensitization and spasmolytic activity, J.Theor.Biol. 40, 155.

Dyer, D.C., 1974, Evidence for the action of d-lysergic acid diethylamide, mescaline and bufotenine on 5-hydroxytryptamine receptors in umbilical vasculature, J.Pharmacol.Exptl.Therap. 188, 336.

Frankhuijzen, A.L. and I.L. Bonta, 1974a, Effect of mianserin, a potent anti-serotonin agent, on the isolated rat stomach fundus preparation, European J.Pharmacol. 25, 40.

Frankhuijzen, A.L. and I.L. Bonta, 1974b, Receptors involved in the action of 5-HT and tryptamine on the isolated rat stomach fundus preparation, European J.Pharmacol. 26, 220.

Gaddum, J.H., 1957, Drugs which antagonize the actions of 5-hydroxytryptamine on peripheral tissues, in: 5-Hydroxytryptamine, Ed. Lewis, G.P., pp. 195-205, Pergamon Press, New York.

Gaddum, J.H. and Z.P. Picarelli, 1957, Two types of tryptamine receptors Brit.J.Pharmacol. 12, 323.

Gyermeck, L., 1966, Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines, in: Handbook of experimental pharmacology, vol. XIX. 5-Hydroxytryptamine and related indolealkylamines, ed. Erspamer, V., Springer-Verlag, New York, pp. 471-528.

Handschumacher, R.E. and J.R. Vane, 1967, The relationship between the penetration of tryptamine and 5-hydroxytryptamine into smooth muscle and the associated contractions, Br.J.Pharmac.Chemother. 29, 105.

Innes, I.R., 1962, Identification of the smooth muscle excitatory receptors for ergot alkaloids, Brit.J.Pharmacol. 19, 120.

Katz, B. and S. Thesleff, 1957, A study of the desensitization produced by acetylcholine at the motor end-plate, J.Physiol.(London) 138, 63.

Leitch, J.L., D. Maryn, V.G. Debley and T.J. Haley, 1957, Influence of pH and atropine on the response of superfused tissues to 5-hydroxytryptamine and acetylcholine, J.Pharmacol.Exptl.Therap. 120, 408.

Lüllmann, H. and A. Ziegler, 1973, A transient state concept of drug receptor interaction, Naunyn-Schmiedebergs Arch.Pharmakol. 280, 1.

Offermeier, J. and E.J. Ariëns, 1966, Serotonin. I. Receptors involved in its action, Arch.int.Pharmacodyn. 164, 192.

Parker, R.B. and D.R. Waud, 1971, Pharmacological estimation of drug-receptor dissociation constants, Statistical evaluation, I. Agonists, J.Pharmacol.Exptl.Therap. 177, 1.

Paton, W.D.M., 1961, A theory of drug action based on the rate of drug-receptor combination, Proc.roy.Soc. B 154, 21.

Pluchino, S., 1972, Direct and indirect effects of 5-hydroxytryptamine and tyramine on cat smooth muscle, Naunyn-Schmiedebergs Arch. Pharmakol. 272, 189.

Rang, H.P. and J.M. Ritter, 1970, On the mechanism of desensitization at cholinergic receptors, Mol.Pharmacol. 6, 357.

Saxena, P.R., P. van Houwelingen and I.L. Bonta, 1971, The effects of mianserin hydrochloride on the vascular response evoked by 5-hydroxytryptamine and related vasoactive substances, European J.Pharmacol. 13, 295.

Saxena, P.R., 1972, The effects of antimigraine drugs on the vascular responses by 5-hydroxytryptamine and related biogenic substances on the external carotid bed of dogs: Possible pharmacological implications to their antimigraine action, Headache 12, 44.

Saxena, P.R. and G.M. de Vlaam-Schluter, 1974, Role of some biogenic substances in migraine and relevant mechanism in antimigraine action of ergotamine. Studies in an experimental model for migraine, Headache 13, 174.

Saxena, P.R., 1974, Selective vasoconstriction in carotid bed by methysergide: Possible relevance to its antimigraine effect, European J.Pharmacol. 27, 99.

Thron, C.D. and D.R. Waud, 1968, The rate of action of atropine, J.Pharmacol.Exptl.Therap. 160, 91.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Brit.J.Pharmacol. 12, 344.

Van Rossum, J.M. and F.G. Van den Brink, 1963, Cumulative dose-response curves, Arch.int.Pharmacodyn. 143, 299.

Waud, D.R., 1968, Pharmacological receptors, Pharmacol.Rev. 20, 49.

Winter, J.C. and P.K. Gessner, 1968, Phenoxybenzamine antagonism of tryptamines, their indene isosteres and 5-hydroxytryptamine in the rat stomach fundus preparation, J.Pharmacol.Exptl.Therap. 162, 286.

ROLE OF PROSTAGLANDINS IN TONE AND EFFECTOR REACTIVITY OF THE ISOLATED RAT STOMACH PREPARATION

Abraham L. FRANKHUIJZEN and Ivan L. BONTA

Department of Pharmacology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands

ABSTRACT

The influence of indomethacin (2 µg/ml) on the tone and the response to ACh, 5-HT and tryptamine of the stomach strip preparation from normal and Essential Fatty Acid Deficient (EFAD) rats was examined. The pD_2 value of ACh and 5-HT, but not of tryptamine, was significantly lower on the preparations obtained from EFAD rats than on those from normal rats. Contradictory results were obtained with respect to the influence of indomethacin on the pD_2 value of ACh, 5-HT and tryptamine. No significant difference in initial tone and contractility of the stomach strips of both groups of rats was observed. However, the tone of the strips from normal rats increased with time. Preparations from EFAD rats showed a gradual decrease of the tone with time. Pre-incubation with indomethacin reduced the tone of strips from both groups of rats to a comparable extent.

Thus further support is provided for the proposed role of prostaglandins in the maintenance of the tone of isolated smooth muscle. The possibility is raised that prostaglandins might not be predominantly involved in the generation of the initial tone and also not substantially contribute to the effector reactivity of the organ.

1. INTRODUCTION

There now exists compelling evidence that the prostaglandins may well have a physiological role in modulating autonomic transmission. Prostaglandins are probably liberated at a large number of autonomic effector sites and they subsequently influence both the release of transmitter material and the reactivity of the effector organ to the transmitter (cf. Hedqvist, 1973; Brody and Kadowitz, 1974). ACh (Ramwell et al., 1966a; Coceani et al., 1967; Coceani et al., 1968; Barles et al., 1968) as well as 5-HT (Ramwell et al., 1966b; Coceani et al., 1968; Alabaster and Bakhle, 1970; Holmes, 1970) and tryptamine (Alabaster and Bakhle, 1970) increase the release of prostaglandins. This possibility implicates that prostaglandins are involved in the contractile responses to ACh, 5-HT and tryptamine indirectly, if released in a subcontractile dose, by influencing the reactivity of the effector organ and/or directly if released in a contractile dose.

We intended to investigate this possibility using the isolated rat stomach strip preparation (Vane, 1957), because of its high sensitivity towards 5-HT and prostaglandins. Furthermore, enzymes which synthetize prostaglandins have been isolated from homogenates of the rat stomach (Pace-Asciak et al., 1968; Pace-Asciak, 1972).

Indomethacin, a compound which strongly inhibits prostaglandin biosynthesis (Vane, 1971), can be used to determine whether local generation of prostaglandins affected the contractile response to ACh, 5-HT or tryptamine.

It has been shown that Essential Fatty Acid (EFA)-deficiency causes a prostaglandin deficiency (Van Dorp, 1971; Haddeman and Hornstra, personal communication; Tan and Privett, 1973). By using EFA-deficient (EFAD) rats, effects similar to the administration of indomethacin on the tone and contractile responses of the preparations obtained from normal rats, were to be expected.

2. MATERIALS AND METHODS

Male rats of an inbred strain of the Medical Faculty were used. Pregnant mothers of these rats were placed on an Essential Fatty Acid Deficient (EFAD) diet five days before the expected delivery. The EFAD food contained 4% hydrogenated cocos fat while the control diet had 3.5% of its calories as linoleic acid. After weaning the newborn rats were kept on EFAD diet food.

2.1. *Preparation of the stomach strip*

The EFAD rats of 275-325 g and non-deficient controls of the same age of 375-425 g were killed by decapitation. The stomach was dissected out and two strips of about 2 mm width were cut from the ruminal part of the stomach (Vane, 1957), whereby much care was taken not to introduce differences in the length of the strips.
Threads were attached as close as possible to the ends of the strips. Strips were set up in an organ bath maintained at 37°C, containing Krebs solution and bubbled with a gas mixture containing 95% O_2 - 5% CO_2. The composition of the Krebs solution was (mM): NaCl 118.0; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.17; NaH_2PO_4 1.17; $NaHCO_3$ 25.0 and glucose 5.6. The length of the strips was determined by putting both ends of the strip together. The deflection recorded was divided by the magnification of the recording system, which consisted of a Harvard heart/smooth muscle transducer and a Harvard electronic recording module 350.

2.2. *Experimental procedure*

Before starting the experiment the strips were allowed to equilibrate for 60 min during which the bathing fluid was changed every 15 min. For the experiments in which the contractile response of the preparation to PGE_1 and $PGF_{2\alpha}$ were compared with those of ACh and 5-HT a 25 min time schedule of drug administration was used as described previously (Frankhuijzen and Bonta, 1974).

In the experiments in which the influence of indomethacin on the tone and contractile response of the preparation was investigated, 4 strips were used at the same time: 2 strips (strip A_1 and A_2) were obtained from one stomach (A) of a rat kept on the control diet, the two other strips (strip B_1 and B_2) were obtained from one stomach (B) of an EFAD rat. Twenty min after indomethacin was administered to strips A_2 and B_2 all 4 preparations were washed 3 times after which indomethacin was administered again. The strips were pre-incubated with indomethacin for 120 min. Thereafter, the response to ACh or 5-HT was recorded and 25 min later the response to tryptamine. The length of the preparations was measured at the end of each experiment.
For the statistical evaluation of the results these were tested two-sided at significance level 0.05. In the case of two independant samples (see 3.1. and 3.2.) the Wilcoxon-Mann-Whitney U test was used. In the case of two related samples (see 3.3.) the Student's t-test was used.

2.3. Drugs

The following drugs were used: acetylcholine iodide (ACh, Fluka A.G.), serotonin creatinine sulphate (5-HT, Fluka A.G.), tryptamine hydrochloride (Fluka A.G.), indomethacin hydrochloride (Indocid, Merck Sharpe and Dohme), prostaglandins E_1 and $F_{2\alpha}$ (PGE_1 and $PGF_{2\alpha}$, Upjohn). The solutions were prepared freshly on the day of the experiment. Drug solutions were added to the 15 ml organ bath in a volume of 0.15 ml. All concentrations mentioned refer to the final concentration of the drugs in the organ bath and are, except for indomethacin, expressed in terms of molarity.

3. RESULTS

3.1. Response to PGE_1 and $PGF_{2\alpha}$ of the stomach strip obtained from normal rats: comparison with the response to ACh and 5-HT.

Cumulative dose-response curves of successively ACh, PGE_1, 5-HT and $PGF_{2\alpha}$

were recorded using a 25 min time schedule. No significant difference in the slope of the log dose response curves was observed (Fig. 1).

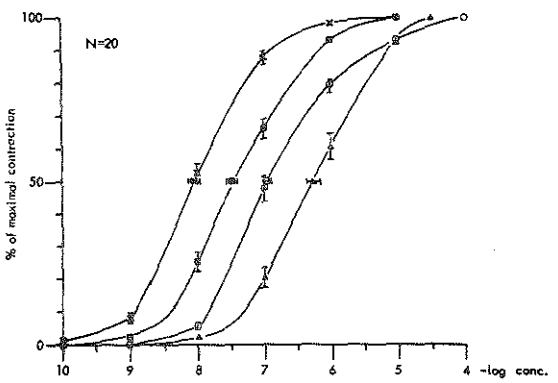


Fig. 1. Log dose-response curves of 5-HT (x——x), PGE_1 (■——■), ACh (o——o) and $PGF_{2\alpha}$ (Δ—— Δ) of stomach strips from normal rats. The curves represent the mean out of 20 experiments. The bars in the curves indicate the S.E.M.

From table 1 it can be seen that the tone of the preparation gradually increased in time. The maximum of the $PGF_{2\alpha}$ curve was 4% $\pm$ 2 higher ($p < 0.05$) and of PGE_1 4% $\pm$ 2 lower ($p < 0.05$) than the maximum of the 5-HT curve, which on its turn was 5% $\pm$ 2 lower ($p < 0.005$) than the maximum of the ACh curve. No significant difference was observed between the maximal heights of the ACh and $PGF_{2\alpha}$ curves. Rat stomach strips showed the highest affinity towards 5-HT followed by PGE_1 and PGE_2. The lowest affinity was observed for $PGF_{2\alpha}$, which was even lower than for ACh.

t (min)	Base-line	Agonist	% maximal contraction (relative to ACh)	pD_2
0	0 ± 0	ACh	100 ± 0	6.96 ± 0.08
25	2 ± 1	PGE_1	91 ± 3	7.46 ± 0.08
50	6 ± 1	5-HT	95 ± 3	8.04 ± 0.08
75	5 ± 1	$PGF_{2\alpha}$	99 ± 4	6.26 ± 0.10
Splawinsky et al. (1973)		PGE_2	95	7.36 (7.51 - 7.29)

Table 1. Characteristics of the log dose-response curves of ACh, PGE_1, 5-HT and $PGF_{2\alpha}$ successively recorded with 25 min interval in the order as given in the table. The mean values obtained from 20 preparations are given ± S.E.M. The values for PGE_2 are also given and were derived from Splawinski et al. (1973). The pD_2 value of PGE_2 was corrected for the difference in sensitivity of the preparation as appeared from the comparison of the pD_2 value of $PGF_{2\alpha}$ given by Splawinsky et al.

3.2. *Influence of an EFAD diet on the response to ACh, 5-HT and tryptamine.*

Feeding of rats with an EFAD diet reduced the pD_2 value of the stomach strips of ACh and 5-HT without significantly affecting the slope of the log dose-response curves (Fig. 1a, b). No significant change was observed with respect to the response to tryptamine (Fig. 2c).
From the values given in table 2 it follows that the pD_2 value of ACh was reduced with 0.32 ± 0.10 log units ($p < 0.05$) and of 5-HT with 0.29 ± 0.13

log units ($p < 0.05$). The decrease of the pD_2 value of tryptamine was not significant ($p > 0.20$). No significant difference was observed in the length of the preparations obtained from normal and EFAD rats, nor in the maximal response to ACh, 5-HT or tryptamine.

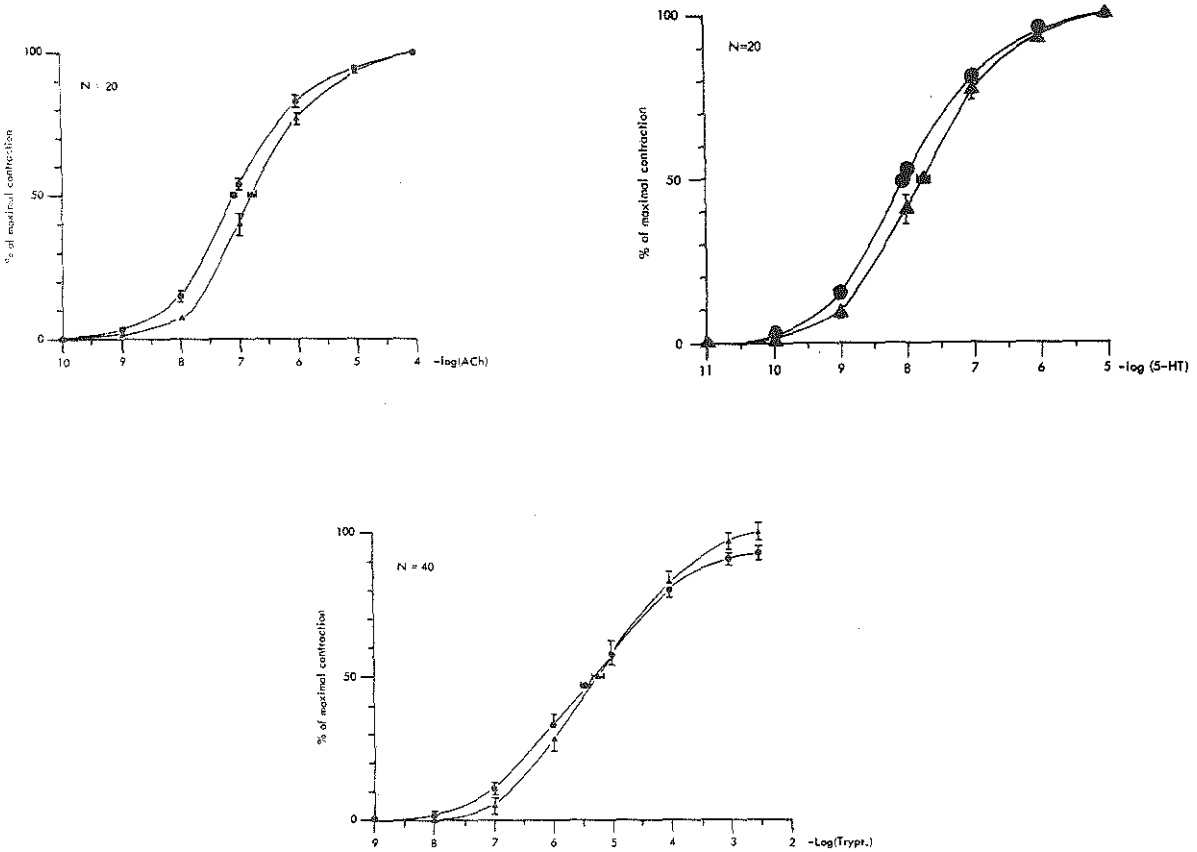


Fig. 2. Log dose-response curves of ACh (a), 5-HT (b) and tryptamine (c) of stomach strips obtained from normal (o——o) and EFAD rats (Δ——Δ). Each curve represents the mean out of 20 (ACh and 5-HT) or 40 (tryptamine) experiments. The bars in the curves indicate the S.E.M.

	Agonist	pD_2	Length of the preparation (mm) L	% maximal contraction %L
Normal rats	ACh	7.09 ± 0.06	30 ± 1	52 ± 2
EFAD rats	ACh	6.77 ± 0.08	31 ± 2	56 ± 1
Normal rats	5-HT	8.04 ± 0.08	25 ± 1	47 ± 2
EFAD rats	5-HT	7.75 ± 0.10	26 ± 1	46 ± 2
Normal rats	Trypt.	5.40 ± 0.09	28 ± 1	45 ± 2
EFAD rats	Trypt.	5.29 ± 0.09	29 ± 1	46 ± 3

Table 2. Influence of EFA-deficiency on the tone and contractile response of rat stomach strips. The contractility of the preparations (%L) is expressed as the % maximal contraction induced by the agonist relative to the length (L) of the preparations. The values represent the mean ± S.E.M. out of 20 (ACh and 5-HT) or 40 (tryptamine) experiments.

3.3. *Influence of indomethacin on the tone and the response to ACh, 5-HT and tryptamine of stomach strips from normal and EFAD rats.*

From table 3 it can be seen that the base-line of the preparations obtained from normal rats shifted upwards with time, indicating an increase in tone ($p < 0.05$ - $p < 0.02$). No significant difference was observed between the increase in tone after the administration of ACh, 5-HT or tryptamine. However, the tone of the preparations obtained from EFAD rats did not increase after ACh ($p > 0.5$) or even decreased with time after 5-HT ($p < 0.02$) and tryptamine ($p < 0.05$). Pre-incubation during 120-145 min with indomethacin (2 µg/ml) resulted in a decrease of the tone also of the preparations obtained from EFAD rats. Remarkable is that the base-

line of the preparations from both normal and EFAD rats in the presence of indomethacin descended to the same level (table 3).

Strip	t (min)	Base-line	Antagonist	Agonist	% maximal contraction	pD_2	$pD_2(t=150)-pD_2(t=0)$
$A_{1,2}$ (20)	0	0 ± 0		ACh	100 ± 0	7.09 ± 0.07	
A_1 (10)	150	8 ± 5			101 ± 3	7.13 ± 0.08	0.04 ± 0.06 (N.S.)
A_2 (10)	150	-17 ± 7	Indometha-cin		101 ± 2	6.92 ± 0.04	-0.17 ± 0.04 (P < 0.005)
$B_{1,2}$ (20)	0	0 ± 0		EFAD ACh	100 ± 0	6.77 ± 0.09	
B_1 (10)	150	-3 ± 3		EFAD	102 ± 3	6.83 ± 0.08	0.06 ± 0.06 (N.S.)
B_2 (10)	150	-16 ± 4	Indometha-cin	EFAD	101 ± 3	6.75 ± 0.13	-0.02 ± 0.07 (N.S.)
$A_{1,2}$ (20)	0	0 ± 0		5-HT	100 ± 0	8.04 ± 0.11	
A_1 (10)	150	12 ± 4			100 ± 2	8.12 ± 0.11	0.08 ± 0.05 (P < 0.20)
A_2 (10)	150	-15 ± 6	Indometha-cin		102 ± 2	7.96 ± 0.15	-0.08 ± 0.06 (P < 0.20)
$B_{1,2}$ (20)	0	0 ± 0		EFAD 5-HT	100 ± 0	7.75 ± 0.14	
B_1 (10)	150	-9 ± 4		EFAD	100 ± 2	8.06 ± 0.14	0.31 ± 0.08 (P < 0.005)
B_2 (10)	150	-11 ± 7	Indometha-cin	EFAD	102 ± 2	7.79 ± 0.07	0.04 ± 0.07 (N.S.)
$A_{1,2}$ (40)	0	0 ± 0		Trypt.	96 ± 4	5.40 ± 0.08	
A_1 (20)	175	9 ± 4			94 ± 3	5.46 ± 0.09	0.06 ± 0.12 (N.S.)
A_2 (20)	175	-23 ± 5	Indometha-cin		89 ± 2	5.39 ± 0.10	-0.01 ± 0.13 (N.S.)
$B_{1,2}$ (40)	0	0 ± 0		EFAD Trypt.	98 ± 4	5.29 ± 0.08	
B_1 (20)	175	-12 ± 3		EFAD	102 ± 5	5.34 ± 0.07	0.05 ± 0.11 (N.S.)
B_2 (20)	175	-24 ± 5	Indometha-cin	EFAD	98 ± 4	4.89 ± 0.10	-0.40 ± 0.13 (P < 0.005)

Table 3. Influence of pre-incubation with indomethacin (2 µg/ml) during 120 (ACh and 5-HT) or 145 min (tryptamine) on the tone and the characteristics of the log dose-response curves of ACh, 5-HT and tryptamine of stomach strips obtained from normal (A) and EFAD (B) rats. The values given in this table represent the mean ± S.E.M. The number of experiments is given between parathesis. The changes of the initial pD_2 value of tryptamine with time is given after 175 min pre-incubation.

Neither the maxima of the log dose-response curves recorded from the preparations obtained from normal rats nor those from EFAD rats changed significantly in the course of the experiments or were affected by the pre-incubation with indomethacin.
With respect to the pD_2 values no such uniform effects were observed as with the tone and maximal response (table 3). Together with the increase in tone of the preparations from normal rats the pD_2 value of all three agonists increased with time. However, this increase was statistically not significant. The increase with time of the pD_2 value of ACh and tryptamine of the preparations from EFAD rats was of the same magnitude as from normal rats but also not significant. The pD_2 value of 5-HT of strips from EFAD rats was however significantly ($p < 0.005$) increased after 150 min.
Pre-incubation of strips from both normal and EFAD rats with indomethacin generally decreased the pD_2 values of all three agonists if compared with the blanks (table 3). With respect to the response to ACh of strips from normal and to tryptamine of EFAD rats the pD_2 value was significantly ($p < 0.005$) decreased even below its initial value.

4. DISCUSSION

The tone of the stomach strip preparation obtained from normal rats gradually increased with time. A similar increase of the tone with time was described for the isolated rabbit jejunum (Ferreira et al., 1972) and guinea-pig ileum (Botting and Salzmann, 1974). The finding that pre-incubation with indomethacin (2 µg/ml) during 2 hr gradually decreased the tone of the preparation confirmed the results obtained by Eckenfels and Vane (1972) on the same preparation and is in agreement with the results obtained by Farmer et al. (1972) and Botting and Salzmann (1974) on respectively the tracheal smooth muscle and isolated segments of the ileum of the guinea-pig and of Ferreira et al. (1972) on the isolated rabbit jejunum. Because of the correlation observed between the level

of prostaglandin release and the degree of inherent smooth muscle activity (Posner, 1970; Ferreira et al., 1972; Botting and Salzmann,1974; Vane and Williams, 1973) and the effect of prostaglandin antagonist (Bennet and Posner, 1971; Davison et al., 1972; Ganesan and Karim, 1973) it is generally accepted that the intramural generation of prostaglandins contribute in maintaining spontaneous activity of isolated smooth muscle tissue.

The tone of the stomach strip preparation obtained from EFAD rats on the contrary gradually decreased with time. EFA-Deficiency has been shown to be associated with prostaglandin deficiency as reflected in the difference between the PGE_2 content of tissues obtained from EFAD and normal animals (Van Dorp, 1971; Tan and Privett, 1973; Haddeman and Hornstra, personal communication). If this also holds for the ruminal part of the rat stomach then the decrease of the tone with time of the stomach strips from EFAD rats would provide further support for the involvement of prostaglandins in the maintenance of the tone.

In line with this a lower initial tone of the stomach preparations obtained from EFAD rats as compared with those obtained from normal rats was to be expected. Under standardized conditions the strips should then show a difference in length. Indeed, the strips from EFAD rats were 1 mm longer than those from normal rats. This difference in length however, was statistically not significant ($p > 0.20$). However, such a small difference in length, which is about 5% of the length of the preparation, might be too small to be accurately measured by the procedure used. Pre-incubation during 2 hr with indomethacin (2 µg/ml) gradually decreased the tone of stomach strips obtained from EFAD rats and normal rats to the same extent. According to reports in literature (Northover, 1971; Sorrentino et al., 1972; Farmer et al., 1972), it does not seem very likely that part of the loss of the tone was caused by a non-specific smooth muscle relaxation by indomethacin. Thus, it appears that prostaglandins are not predominantly involved in the generation of the initial tone of the preparation. However, the stomach strips from EFAD

rats were evidently still able to generate prostaglandins, although at a lower rate available.

This might have been sufficient for the generation of the initial tone but not for the maintenance of the tone in time. Prostaglandin deficiency did not affect the contractility of the preparation. No significant difference was observed between the maximal responses to ACh, 5-HT and tryptamine of the preparations obtained from normal and EFAD rats. Moreover, pre-incubation with indomethacin of stomach strips obtained from either normal or EFAD rats did not affect the maximal response to ACh, 5-HT and tryptamine. These results provide additional evidence against a non-specific smooth muscle relaxation by indomethacin.

It is known that 5-HT (Ramwell et al., 1966b; Coceani et al., 1968; Alabaster and Bakhle, 1970; Holmes, 1970), tryptamine (Alabaster and Bakhle, 1970) as well as ACh (Ramwell et al., 1966a; Coceani et al.,1967; Coceani et al., 1968; Bartles et al., 1968) all increase the release of prostaglandins. According to Coceani et al. (1968), 5-HT in a concentration of 5.10^{-5}M, increases the rate of prostaglandin release from the isolated rat stomach from 0.3 ng PGE_1 equiv./min till 2.5 ng PGE_1 equiv./min. Extrapolating these data to the stomach strip and assuming a very rapid increase of the prostaglandin release, the administration of cumulative doses of 5-HT should have increased the prostaglandin level till a bath concentration equivalent to about 10^{-9}M PGE_1. According to the log dose-response curve of PGE_1, this still is a subcontractile concentration.

Prostaglandins may influence the reactivity of the effector organ (c.f. Brody and Kadowitz, 1974) probably as the result of a general effect on membrane enzyme activities (Johnson and Ramwell, 1973; Coceani et al., 1969) which will facilitate the translocation of membrane-bound Ca^{2+} (Ca^{2+}_{mem}) (Coceani and Wolfe, 1966; Coceani et al., 1969; Horton, 1969; Eagling et al., 1972; Kirtland and Baum, 1972; Carafoli and Crovetti, 1973; Greenberg et al., 1973; Greenberg and Long, 1973). In the concept of Chang and Triggle (1973), the intrinsic activity is a reflection of the

ability of a drug to translocate Ca^{2+}_{mem}. Apparently, ACh, 5-HT and tryptamine, being full agonists, initiated a Ca^{2+}-translocation in such an effective manner that an additional facilitation by prostaglandins was without effect.

The prostaglandins might however be involved effectively in the mediation of the response to submaximal concentrations of ACh and 5-HT. This is supported by the fact that the pD_2 value of ACh and 5-HT of stomach strips obtained from normal rats was significantly higher than those obtained from EFAD rats. Moreover, the pD_2 values were decreased after pre-incubation with indomethacin if compared with the blanks. However, the pD_2 values of ACh and 5-HT of stomach strips obtained from EFAD rats were also decreased after pre-incubation with indomethacin.

In contrast with 5-HT no significant difference was observed between the pD_2 values of tryptamine of stomach strips obtained from normal and EFAD rats. This supports the suggestion that part of the response to tryptamine on the isolated rat stomach preparation is mediated via other than D-tryptamine receptors (Winter and Gessner, 1968; Frankhuijzen and Bonta, 1974). Thus, obviously prostaglandins are not substantially involved in the subsidiary action of tryptamine. This conclusion is in agreement with the finding that pre-incubation with indomethacin did not affect the pD_2 value of tryptamine of strips obtained from normal rats. However, indomethacin significantly reduced the pD_2 value of tryptamine of strips from EFAD rats.

The present results provide additional evidence for the proposed role of prostaglandins in the maintenance of the tone of isolated smooth muscle. The possibility is put forward that prostaglandins are not predominantly involved in the generation of the initial tone. No definite answer can be given whether prostaglandins are involved in the mediation of the responses to ACh, 5-HT and tryptamine. The prostaglandins anyway did not substantially contribute to the mediation of the responses, although according to others (Sorrentino et al., 1972; Fried et al, 1969; Park et al., 1972; Sanner et al., 1973; Eakins et al., 1970) such a

contribution, although minimal, appears to be real.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. J. Bruinvels and Dr. J. Noordhoek for their useful comments on the manuscript and to Drs. H.J.A. Schouten for advice on the statistical evaluation of the data. They also wish to acknowledge technical assistance from Mrs. J.C. Sierevogel and secretarial help from Mrs. M.M. Thornton-Krom and Miss A.C. Heymeriks.

REFERENCES

Alabaster, V.A. and Y.S. Bakhle, 1970, The release of biologically active substances from isolated lungs by 5-hydroxytryptamine and tryptamine, Brit.J.Pharmacol. 40, 582.

Ariëns, E.J. (Ed.), 1956, Drug-Receptor Interaction, In: Molecular Pharmacology, Vol. I (Academic Press, New York), pp. 176.

Bartles, J.W., W. Vogt and G. Willie, 1968, Prostaglandin release from and formation in perfused frog intestine, Arch.Pharmak.exp. Pathol. 259, 153.

Bennet, A. and J. Posner, 1971, Studies on prostaglandin antagonists, Brit.J.Pharmacol. 42, 584.

Botting, J.H. and R. Salzmann, 1974, The effect of indomethacin on the release of prostaglandin E_2 and acetylcholine from guinea-pig isolated ileum at rest and during field stimulation, Brit.J. Pharmacol. 50, 119.

Brody, M.J. and P.J. Kadowitz, 1974, Prostaglandins as modulators of the autonomic nervous system, Fed.Proc. 33, 48.

Carafoli, E. and F. Crovetti, 1973, Interactions between prostaglandin E_1 and calcium at the level of the mitochondrial membrane,

Arch.Biochem.Biophys. 154, 40.

Chang, K.-J. and D.J. Triggle, 1973, Quantitative aspects of drug receptor interactions, I. Ca^{2+} and cholinergic receptor activation in smooth muscle: a basic model for drug-receptor interactions, II. The role of Ca^{2+}_{mem} in desensitization and spasmolytic activity, J.Theor.Biol. 40, 125, 155.

Coceani, F. and L.S. Wolfe, 1966, On the action of prostaglandin E_1 and prostaglandins from brain on the isolated rat stomach, Can.J. Physiol.Pharmacol. 44, 933.

Coceani, F., C. Pace-Asciak, F.S. Volta and L.S. Wolfe, 1967, Effect of nerve stimulation on prostaglandin formation and release from the rat stomach, Am.J.Physiol. 213, 1056.

Coceani, F., C. Pace-Asciak and L.S. Wolfe, 1968, Studies on the effect of nerve stimulation on prostaglandin formation and release in the rat stomach, In: Prostaglandin Symposium of the Worchester Foundation for Experimental Biology, Interscience, 39.

Coceani, F., J.J. Dreifuss, L. Puglisi and L.S. Wolfe, 1969, Prostaglandins and membrane function, in: Prostaglandins, Peptides and Amines, Eds. P. Mantegazza and E.W. Horton, Academic Press (New York), p. 73.

Davison, P., P.W. Ramwell and A.L. Willis, 1972, Inhibition of intestinal tone and prostaglandin synthesis by 5,8,11,14 tetranoic acid, Brit.J.Pharmacol. 46, 547.

Eagling, E.M., H.G. Lovell and V.R. Pickles, 1972, Interaction of prostaglandin E_1 and calcium in the guinea-pig myometrium, Brit. J.Pharmacol. 44, 510.

Eakins, K.E., S.M.M. Karim and J.D. Miller, 1970, Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate, Brit.J.Pharmacol. 39, 556.

Eckenfels, A. and J.R. Vane, 1972, Prostaglandins, oxygen tension and smooth muscle tone, Brit.J.Pharmacol. 45, 451.

Farmer, J.B., D.G. Farrar and J. Wilson, 1972, The effect of indomethacin on the tracheal smooth muscle of the guinea-pig, Brit.J. Pharmacol. 46, 536.

Ferreira, S.H., A.G. Herman and J.R. Vane, 1972, Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum, Brit.J.Pharmacol. 44, 328.

Frankhuijzen, A.L. and I.L. Bonta, 1974, Receptors involved in the action of 5-HT and tryptamine on the isolated rat stomach fundus preparation, European J.Pharmacol. 26, 220.

Fried, J., T.S. Santhanakrishnan, J. Shimizu, C.H. Lin, S.H. Ford, B. Rubin and E.O. Grigas, 1969, Prostaglandin antagonists: Synthesis and smooth muscle activity, Nature 223, 208.

Ganesan, P.A. and S.M.M. Karim, 1973, Polyphloretin phosphate temporarily potentiates prostaglandin E_2 on the rat fundus, probably by inhibiting PG 15-hydroxydehydrogenase, J.Pharm.Pharmac. 25, 229.

Greenberg, S., P.J. Kadowitz, F.P.J. Diecke and J.P. Long, 1973, Effect of prostaglandin $F_{2\alpha}$ on responses of vascular smooth muscle to serotonin, angiotensin and epinephrine, Arch.int.Pharmacodyn. 206, 5.

Greenberg, S. and J.P. Long, 1973, Enhancement of vascular smooth muscle responses to vasoactive stimuli by prostaglandin E_1 and E_2, Arch.int.Pharmacodyn. 206, 94.

Hedqvist, P., 1973, Prostaglandin mediated control of sympathetic neuroeffector transmission, Adv.Biosci. 9, 461.

Holmes, S.W., 1970, The spontaneous release of prostaglandins into the cerebral ventricles of the dog and the effect of external factors on this release, Brit.J.Pharmacol. 38, 653.

Horton, E.W., 1969, Hypothesis on physiological role of prostaglandins, Physiol.Rev. 49, 122.

Johnson, M. and P.W. Ramwell, 1973, Prostaglandin modification of membrane-bound enzyme activity: a possible mechanism of action,

Prostaglandins 3, 703.

Kirtland, S.J. and H. Baum, 1972, Prostaglandin E_1 may act as a "calcium ionophore", Nature New Biol. 236, 47.

Northover, B.J., 1971, Mechanism of the inhibitory action of indomethacin on smooth muscle, Brit.J.Pharmacol. 41, 540.

Pace-Asciak, C., K. Morawska, F. Coceani and L.S. Wolfe, 1968, The biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ in homogenates of the rat stomach, in: Prostaglandins Symposium of the Worcester Foundation for Experimental Biology, Intersci., 371.

Pace-Asciak, C., 1972, Prostaglandin synthetase activity in the rat stomach fundus: activation by l-norepinephrine and related compounds, Biochim.Biophys.Acta 280, 161.

Park, M.K., Ch. Rishor and D.C. Dyer, 1972, Vasoactive actions of prostaglandins and serotonin on isolated human umbilical arteries and veins, Can.J.Physiol.Pharmacol. 50, 393.

Posner, J., 1970, The release of prostaglandin E_2 from the bovine iris, Brit.J.Pharmacol. 40, 163.

Ramwell, P.W., J.E. Shaw, W.W. Douglas and A.M. Poisner, 1966a, Efflux of prostaglandin from adrenal glands stimulated with acetylcholine, Nature 210, 273.

Ramwell, P.W., J.E. Shaw and R. Jessup, 1966b, Spontaneous and evoked release of prostaglandins from frog spinal cord, Am.J.Physiol. 211, 998.

Sanner, J.H., R.A. Mueller and R.M. Schultze, 1973, Structure-activity relationship of some dibenzoxazepine derivatives as prostaglandin antagonists, Adv.Biosci. 9, 139.

Sorrentino, L., F. Capasso and M. Di Rosa, 1972, Indomethacin and prostaglandins, European J.Pharmacol. 17, 306.

Splawinsky, J.A., A.S. Nies, B. Sweetman and J.A. Oates, 1973, The effects of arachidonic acid, prostaglandin E_2 and prostaglandin $F_{2\alpha}$, on the longitudinal stomach strip of the rat. J.Pharmacol. Exptl.Therap. 187, 501.

Tan, W.C. and O.S. Privett, 1973, Studies on the synthesis in the vesicular glands of Essential Fatty Acid-Deficient and hypophysectomized rats, Biochim.Biophys.Acta 296, 586.

Van Dorp, D., 1971, Recent developments in the biosynthesis and the analysis of prostaglandins, Ann.N.Y.Acad.Sci. 180, 181.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Brit.J.Pharmacol. 12, 344.

Vane, J.R., 1971, Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs, Nature New Biol. 231, 232.

Vane, J.R. and K.I. Williams, 1973, The contribution of prostaglandin production to contractions of the isolated uterus of the rat, Brit.J.Pharmacol. 48, 629.

Winter, J.C. and P.K. Gessner, 1968, Phenoxybenzamine antagonism of tryptamines, their indene isosteres and 5-hydroxytryptamine in the rat stomach fundus preparation, J.Pharmacol.Exptl.Therap. 162, 286.

SUMMARY AND CONCLUSIONS

It has been shown that although the responses to 5-HT and tryptamine are affected by the same classes of antagonists, differences exist with respect to the antagonism towards the action of tryptamine and 5-HT. The primary aim of this investigation was to ascertain whether the same types of receptors were involved in the action of these amines on the isolated rat stomach preparation. Since it has been demonstrated that both 5-HT and tryptamine are able to enhance the release of prostaglandins, the possibility of the involvement of prostaglandins in the contractile responses to 5-HT and tryptamine was also investigated.

Mianserin and cyproheptadine are two structurally related antagonists of 5-HT. However, in contrast to cyproheptadine, mianserin in concentrations up to 10^{-6}M, failed to inhibit the 5-HT and tryptamine-induced contractions of the isolated rat stomach preparation. By increasing the concentration of mianserin the organ responded by contraction which was not affected by antagonists of ACh, histamine or 5-HT or by α- and β-adrenoceptor antagonists. These experiments were performed in the presence of the α-adrenolytic piperoxan to prevent the inhibitory action of 5-HT on α-adrenoceptors. Nevertheless, in receptor-protection experiments, both mianserin and piperoxan were able to prevent the inhibition of the contractile response to 5-HT by phenoxybenzamine. However, in separate experiments it could be demonstrated that both mianserin and piperoxan competitively antagonised the response to 5-HT. In view of the affinity of piperoxan to the 5-HT receptor, its interaction with other 5-HT antagonists was studied as well. From these experiments it was concluded that cyproheptadine as well as methysergide and ergotamine act as competitive antagonists to 5-HT and tryptamine on the isolated rat

stomach preparation. The 5-HT induced contractions were more affected than those induced by tryptamine. From thexe experiments it was concluded that the response to 5-HT was the result of an interaction with one type of receptor, probably the classical D-tryptamine receptor. The response to tryptamine seemed to be the result of an interaction with two different types of receptors. One type is identical with the receptor mediating the response to 5-HT, while the subsidiary action of tryptamine is mediated by another type of receptor which is more resistant to inhibition. This type of receptor might be identical with the PRT (Phenoxybenzamine ResisTant)-receptor, known to be more resistant to inhibition by phenoxybenzamine. Moreover, in contrast with the 5-HT induced contraction, inhibition of the prostaglandin biosynthesis did not affect the tryptamine-induced contraction. In this way additional support was obtained for the suggestion of a divergence in the mediation of the responses towards 5-HT and tryptamine. At the same time these experiments excluded the possibility of a substantial contribution of prostaglandins in the subsidiary action of tryptamine. Despite seeming analogy between the constrictor response of the external carotid vessels and the contractile response of the isolated rat stomach to ergotamine and mianserin, only for the contractile response to mianserin, receptors other than the classical D-tryptamine receptors appear to be involved. Prolonged receptor stimulation resulted in a specific desensitization. Since in the analysis of the effect of ergotamine on the response to 5-HT prolonged pre-incubation times were used, the possible involvement of receptor desensitization cannot be excluded.

SAMENVATTING EN CONCLUSIES

Alhoewel er in kwalitatief opzicht overeenkomst bestaat tussen het antagonisme van de effecten van 5-HT en tryptamine, zijn er in kwantitatief opzicht verschillen. Daarom werd met behulp van enkele 5-HT antagonisten nagegaan of dezelfde typen receptoren betrokken waren bij de door 5-HT en tryptamine geïnduceerde contracties van strips van de rattemaag. Daar men heeft aangetoond dat zowel 5-HT als tryptamine in staat zijn de afgifte van prostaglandinen te verhogen, werd tevens onderzocht in hoeverre deze betrokken zijn bij de effekten van deze aminen.

Mianserine en cyproheptadine zijn twee antagonisten van 5-HT, die wat betreft hun structuur sterke overeenkomsten vertonen. Toediening van mianserine (10^{-6}M) gaf, in tegenstelling tot cyproheptadine, echter geen remming van de door 5-HT en tryptamine geïnduceerde contractie van strips van de rattemaag. Verhoging van de concentratie van mianserine resulteerde in een contractie van het orgaan, die niet beïnvloed werd door anticholinergica, anti-histaminica, 5-HT-antagonisten of α- en β-adrenolytica. Teneinde de auto-inhibitie van 5-HT via α-adrenerge receptoren te voorkomen werden deze experimenten uitgevoerd in aanwezigheid van het α-adrenolyticum piperoxan. In receptorbeschermingsexperimenten echter bleken zowel mianserine als piperoxan in staat de remming van 5-HT door phenoxybenzamine te kunnen tegengaan. De conclusie dat mianserine en piperoxan blijkbaar affiniteit hebben voor de "5-HT receptor" werd nog eens bevestigd doordat beide afzonderlijk in staat waren de respons van 5-HT competitief te antagoneren. Derhalve werd eveneens de invloed van piperoxan onderzocht op de

remming van de 5-HT en tryptamine contractie door cyproheptadine, methysergide en ergotamine. Uit deze experimenten bleek dat zowel cyproheptadine als methysergide en ergotamine op competitieve wijze de 5-HT en tryptamine effecten antagoneerden. De door 5-HT geïnduceerde contractie werd echter sterker geantagoneerd dan die veroorzaakt door tryptamine. Deze resultaten toonden aan dat de 5-HT respons tot stand komt via een interactie met één type receptor. Deze receptor is waarschijnlijk de klassieke D-tryptamine receptor, terwijl het door tryptamine veroorzaakte effect vermoedelijk tot stand komt via een interactie met twee verschillende typen receptoren. Van deze twee typen receptoren is er één gelijk aan die waarmee 5-HT een interactie geeft. De tweede component van de tryptamine contractie verloopt via een ander type receptor, die mogelijk identiek is aan de PRT (Phenoxybenzamine ResisTant)-receptor, waarvan beschreven is, dat deze geen affiniteit voor phenoxybenzamine bezit. Bovendien bleek, dat de door tryptamine geïnduceerde contractie, in tegenstelling tot die van 5-HT, niet beinvloed werd door remming van de biosynthese van prostaglandinen. Ook dit gegeven wijst op een verschillend mechanisme voor de door 5-HT en de door tryptamine geïnduceerde contracties. Deze experimenten sluiten tevens de mogelijkheid uit dat prostaglandinen in belangrijke mate betrokken zijn bij de tweede component van de tryptamine respons. Geen overeenkomst bleek er te bestaan tussen de effecten van ergotamine en mianserine op het vaatbed van de carotis externa en op de strips van de rattemaag, met uitzondering van de door mianserine veroorzaakte contractie die niet via de D-tryptamine receptoren tot stand leek te komen. Ergotamine bleek wèl een partieel agonist te zijn op de D-tryptamine receptor. Langdurige receptor stimulering had specifieke desensitizering tot gevolg. Daar bij de experimenten met ergotamine langdurige pre-incubatie tijden werden gebruikt, bestaat de mogelijkheid dat ook hierbij receptor desensitizering is opgetreden.

CURRICULUM VITAE

Na het behalen in 1960 van het einddiploma HBS-B aan het Thorbecke Lyceum te Utrecht werd in 1962, na het volbrengen van de dienstplicht, de studie in de Scheikunde aan de Rijksuniversiteit te Utrecht aangevangen. Het kandidaats-examen, letter G, werd afgelegd in juni 1967, het doktoraal-examen Scheikunde met als hoofdvak Biochemie (Prof.Dr. L.L.M. van Deenen) en als bijvak Farmacologie (Prof.Dr. D. de Wied) in juni 1969.
Sinds september 1969 was de promovendus als wetenschappelijk medewerker verbonden aan de Afdeling Farmacologie van de Faculteit der Geneeskunde van de Erasmus Universiteit te Rotterdam, alwaar dit proefschrift onder leiding van Prof.Dr. I.L. Bonta werd bewerkt.
Sinds mei 1974 is de promovendus werkzaam op de Afdeling Farmacologie van de Faculteit der Geneeskunde van de Vrije Universiteit te Amsterdam.