A Biological Active Peptide in the Skin of Lampreys (Eudontomyzon danfordi vladykovi), by G. Fischer and W. Albert. (Z.Naturforsch. 26b, 1021-1023, 1971) Translated by P.L. Nock.

In recent years interest in the production and description of kinin-type substances has been greatly intensified. So, for example, bradykinin, phyllokinin, physalaemin, ranatensin and caerulein could be extracted from the skin of amphibians as well as eledoisin out of the salivary glands of Eledon moschata. An examination of lampreys seemed to us particularly profitable in the search for the incidence of further kinins; on the one hand because of their exceptional phylogenetic position, and on the other hand because of the occurrence in humans of symptoms of poisoning after the animals have been consumed. We concluded from these toxic effects that the active principle could be discovered in the skin secretion.

Material and Methods

1. Animal subjects

Ammocoetes of different sizes and also adults of both sexes of the species Eudontomyzon danfordi vladykovi were readily available for our research. This species is found in many tributaries of the Danube in Austria, Czechoslovakia and Yugoslavia. It does not live parasitically, and does not perform any spawning migrations. The animals die a short time after spawning. They reach a maximum length of 20 cm.

2. Method of extraction

a) Preliminary attempts at extracting the skin secretion.

In order to induce an increased mucus secretion in the animals, they were subjected to ether fumes in a closed vessel for 30 minutes and finally washed off with an amount four times their weight of 99% methanol. This extract was evaporated to dryness at 40°C. The residue was absorbed in a physiological salt solution for biological testing.

b) Skin extracts.

750 g. of lampreys were skinned; we added 100 g. of undried skins to a fourfold volume of 99% methanol and extracted after 24 hours. There then followed two extractions with a fourfold volume of 80% methanol. These extracts were combined, evaporated and had the fat removed with an amount of 20 times their weight of chloroform/methanol 2 : 1 (v/v). The methanol phase, which had been brought to 200 ml., was next lyophilised in small portions of 5 ml.

3. Biological investigation of the extracts.

The biological tests followed on isolated rat uterus (in Dale saline solution), rat duodenum, guinea pig ileum and rabbit jejunum (in Tyrode saline solution) with the addition of the antagonist agents atropine, mepyraminmalaete (neocantergan) and bromlysergicacid diethylamide (BOL 148) in a volume of 0.1 µg/ml. The volumes of the organ baths amounted to 20 ml. for the rabbit jejunum, 8 ml. for the remaining organs. The temperature of the oxygenated bathing solution was 30°C for the rat uterus and 37°C for the other organs. A further biological examination followed on the blood pressure of narcotised rats (urethane 1.25 g/kg) in connection with the injection of 250 µg/kg of the above-named antagonisers.

4. Further characterisation of the biologically active principle

In order to be able to determine a peptide character of the biologically active substance, we undertook experiments involving digestion with trypsin, chymotrypsin and carboxypeptidase B. To trypsin 50 µg/0.1 ml was added, to chymotrypsin 5 µg/0.1 ml and to carboxypeptidase B 5 units/0.1 ml. extract of 0.25 g. tissue. To enable the enzymes to reach their optimum effect, the pH-value of the solutions

was brought to the corresponding level with 0.1N NaOH. As controls we provided alternately the same amounts of the extract, treated in the same manner, up to the addition of the enzyme. Testing on the isolated rat uterus followed.

5. Thin layer chromatography of the skin extracts

Plates were used to which silica gel H (Fa. Merck, Darmstadt) had been applied in a layer 2 mm. thick. For the separation, 50 µl extract from 0.1 g. of skin was applied in lines, and as reference substance 50 ng. of bradykinin. The medium employed was butanol - Glacial acetic acid - water (4:1:1). The chromatogramm was removed at intervals of 1 cm at right angles to the direction of flow, and each 1cm strip was tested for activity. These groups were extracted in 80% methanol. We used the individual evaporated extracts for further biological testing on the rat uterus.

Results

Additions of the extract obtained by the method described under 2a has the effect, as shown in Fig. 1, of causing dose-dependent contractions on the isolated rat uterus.

The active principle in this extract could, under the given conditions, be broken down in each case within 5 minutes by trypsin, chymotrypsin and carboxypeptidase.

As the experiments led one to suppose that the active principle is located in the skin, further investigations should be made on the study of extracts from the isolated skin. As chromatographic techniques were also provided for the closer characterisation of the active principle, the extract must have its fat removed, although as a consequence this has a greater loss of activity. By quantitative comparison of the extract (with the fat removed) with bradykinin, the f following equivalents could be determined on different compounds:

rat uterus	1.36 mg $\pm$ 0.3	contracting
rat duodenum	4.38 ng $\pm$ 1.91	relaxing
rabbit jejunum	1.69 mg $\pm$ 0.33	contracting

Table 1: quantitative comparison of effects of the extract from 1 g of undried lamprey skin with bradykinin (n=5).

The effect on the guinea pig ileum and rat blood pressure could unfortunately only be established qualitatively, since on account of the relatively insignificant effectiveness of these preparations and on account of the quite insignificant volume of original material, a comparison with bradykinin must be dispensed with. And so extracts of 0.5 g. lamprey skin first sparked off a contraction on the guinea pig ileum, while a depressant effect on blood pressure was first observed on amounts of 2.5 g. extracted from the skin.

For evidence of an exclusive localisation of the peptide in the skin, we also extracted and tested skinned animals. Here, however, we were not able to establish any kind of biological activity which could be linked to an effect of the peptide.

Fig.2 makes it clear that the eluates obtained by means of the method described under (5), the 5, 6, and 10 showed some biological activity for the isolated rat uterus. It was proved moreover that bradykinin shows the same R_f-value as the most active group (5-6) from the skin extract of the lampreys.

Discussion

Already the application of the mucuous extract obtained simply through rinsing the animals with methanol caused contractions on the isolated rat uterus and the porpoise ileum. This stimulating effect was also fully maintained after the application of the antagonisers compared with the usual occasional biologically active substances which occur in company with organ extracts. As the effect of these active components is disturbed by rypsin, chymotrypsin and carboxypeptidase B, a peptide must be concerned. The effect of the carboxypeptidase B afforded some indication of the group affiliation of this peptide, as

this enzyme affects bradykinin and peptides similar to bradykinin at once, but which in general does not affect tachykinin.

Analysis of the results of the fat-free skin extract resembles that of bradykinin and kallidin (relaxation of the rat duodenum). A blood pressure depressant effect was likewise apparent on the rat.

By means of thin-layer chromatography two active components were established, where the main activity comes up to the "Kinin" and the R_f-value of bradykinin corresponds to that of the peptide. The second, less effective group could not be identified at present. As however the equivalents of three preparations are not the same as bradykinin, either a substance resembling bradykinin or the interference through the second active peptide next to "bradykinin" must be accepted.

In order to be able to confirm the identity of both substances, further chemical investigations would be necessary. This however has had to be postponed on account of the inadequate amount of available material.

Summary.

In this paper two biological active peptides isolated from lampreys are described and characterized. We found that these peptides are located in the skin and are active on isolated organs and on blood pressure. These two peptides could be separated by chromatographic methods: it could be demonstrated that most of the activity is due to a bradykininlike peptide.

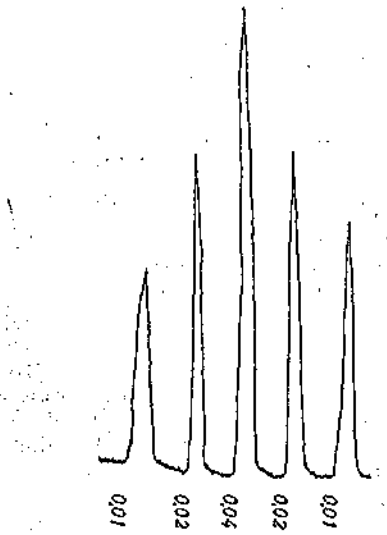

Abb. 1. Dosisabhängige Kontraktionen des Rattenuterus nach Verabreichung des Methanol-extrahierten Schleimes von Neunaugen. Dosierungen (Zahlen) ausgedrückt in ml Testextrakt (2,5 ml Testlösung entsprechen dem Extrakt aus 1 g Neunaugenfrischgewicht).

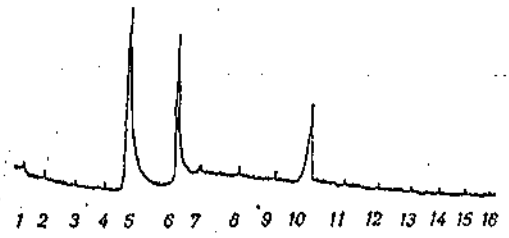

Abb. 2. Biologische Wirkungsprüfung der Eluate aus der Dünnschichtchromatographie. Zahlenreihe 1—16: Zusätze der einzelnen Eluate an den isolierten Rattenuterus.

Figures.

1) Dosage-dependent contractions of the rat uterus according to provision of the methanol-extracted mucus of lampreys. Dosages (numbers) expressed in ml. per test extract (2.5 ml. test solution correspond to the extract from 1 g. lampreys, fresh weight).

2) Examination of the biological activity of the eluate from the thin-layer chromotography. Numbers 1-16: applications of the individual eluate to the isolated rat uterus.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.