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OCCURRENCE OF A SUBSTANCE P-LIKE POLYPEPTIDE IN FISH INTESTINE AND BRAIN

BY

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Substance P has been found in relatively high amounts in the intestine and brain of various animals, such as the monkey, horse, ox, pig, sheep, dog, cat, rabbit, and man (Euler and Gaddum, 1931; Douglas, Feldberg, Paton, and Schachter, 1951; Ehrenpreis and Pernow, 1952; Pernow, 1953, 1955; and others). It thus appears to be a regular constituent of these organs in mammals. No definite data are available, however, from other phyla, and it therefore seemed of interest to determine whether or not this highly active polypeptide also occurs in poikilothermic animals.

Substance P may be salted out from crude aqueous extracts by ammonium sulphate (Euler, 1936a, b; Pernow, 1953). A convenient and efficient method of purification, based on adsorption chromatography on aluminium oxide, has been described by Pernow (1953). The present paper reports some experiments made on purified extracts from the intestine and brain of fish.

METHODS

Extracts were prepared from the intestine of freshly caught cod (*Gadus calarias* and *G. morrhua*) and dogfish (*Squalus acanthias*). After removal of their contents the organs were immediately cut into small pieces, heated to boiling in 2 volumes of water, and sulphuric acid added to a final *p*H of 4. After 10 min. boiling the extract was filtered. To the cool filtrate ammonium sulphate was added to a final concentration of 2/3 saturation and the solution left overnight at 5° C. The precipitate was filtered off and pressed nearly dry between filter papers.

The crude extracts were purified according to Pernow (1953). The dry ammonium sulphate precipitate was dissolved in 4 parts of water, and methanol slowly added during continuous stirring to a final concentration of 70%. The voluminous precipitate contained only little activity and was discarded. The 70% methanol extract was run through a column of 5–12 g. aluminium oxide (British Drug Houses) at a rate of 0.5–1 ml./min. The column was washed with 70% methanol, followed by 10–20 ml. of decreasing concentrations of methanol (60%, 50%, etc.) and finally distilled water.

After evaporation of the methanol *in vacuo* the eluates were assayed on a strip of isolated guinea-pig intestine (Pernow, 1951) in a 3 ml. bath of oxygenated Tyrode solution at 38° C., containing atropine sulphate and an antihistamine ("Lergigan," Recip) each in a concentration of 1 : 2.5 million. A purified preparation of Substance P obtained from beef intestine, kindly placed at our disposal by Dr. B. Pernow, was used as standard, and the activity expressed in the P unit previously used in our laboratory. The extracts were also tested on rabbit isolated intestine, and on the blood pressure of the cat under pentobarbitone anaesthesia and of the rabbit under urethane anaesthesia.

The polypeptide nature of the active substance was checked by incubation with pure trypsin ("Tripur," Novo).

RESULTS

Preliminary tests showed that ammonium sulphate precipitates of aqueous extracts of cod and dogfish intestine and cod brain had the typical actions of Substance P, corresponding approximately to 4–6 standard units/g. of fresh organ, when tested on the guinea-pig isolated intestine, treated with atropine and lergigan.

Cod Intestine

Purification.—A batch of 200 g. cod intestine yielding 2.5 g. dry ammonium sulphate precipitate with a total activity of 1,100 U. (5.5 U./g. wet weight) was subjected to the purification procedure described by Pernow (1953). After extraction with 70% methanol the solution was passed through a column of 12 g. aluminium oxide. Some eluates were pooled, concentrated *in vacuo*, and made up to a concentration of 70% methanol and again run through an aluminium oxide column and eluted with decreasing concentrations of methanol. The results obtained are shown in Table I.

Biological Activity.—The effects of a partially purified extract from cod intestine, consisting of

TABLE I

BIOLOGICAL ACTIVITY IN ELUATES FROM A PARTIALLY PURIFIED EXTRACT OF COD INTESTINE CONTAINING 480 U. "P"

The assays were performed on guinea-pig gut in the presence of atropine and lergigan.

Methanol Concentration of Elution Fluid	Volume (ml.)	Activity (U.) after Passing Through Column
Concentrated eluates in 70% methanol	25	<10
70%	15	<10
60%	19	10
50%	27	378
40%	14	28
20%	15	<10
20%	16	<10
aq. dest.	15	<10
		Total 416 U.

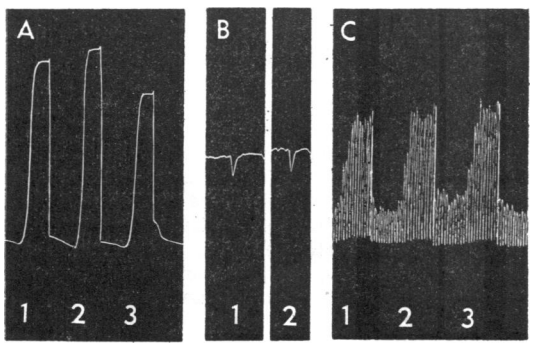

FIG. 1.—A. Isolated guinea-pig ileum. 3 ml. bath. 38° C. Atropine and lergigan 1 : 2.5 mill. in Tyrode solution. 1, 0.2 U. "P" standard. 2, 0.008 ml. purified cod intestine extract "C." 3, 0.007 ml. same extract. B. Blood pressure, rabbit, atropine. 1, 0.06 ml. extract "C." 2, 2 U. "P." C. Isolated rabbit jejunum. 15 ml. bath. Atropine 1 : 2.5 mill. in Tyrode solution. 38°. 1, 0.03 ml. extract "C." 2, 1 U. "P." 3, 0.04 ml. extract "C."

eluates obtained with 50% methanol and lower concentrations, concentrated *in vacuo*, were tested on various biological preparations and compared with a Substance P standard. The extract contained 9.3 U. "P"/mg. dry weight. The results are presented in Table II and Fig. 1. In all instances good agreement between the general type of effect of the cod intestinal extract and Substance P standard was obtained. No difference was noted

TABLE II

BIOLOGICAL ACTIVITY OF PURIFIED COD INTESTINE EXTRACT

Test Preparation	Effect	Activity (Units "P"/ml.)
Guinea-pig ileum (atropine-lergigan)	Contraction	24–27
Rabbit duodenum (atropine)	,,	25
Rabbit blood pressure (atropine)	Slight fall	20
Cat blood pressure (atropine, lergigan)	,, ,,	20–25

between the biological effects of the extract before and after atropine and lergigan, showing that for practical purposes it was free from choline and choline esters or histamine. Since the effect was completely abolished by treatment with trypsin, no other active substance of non-polypeptide nature (such as 5-hydroxytryptamine) seemed to be present.

Inactivation by Trypsin.—Incubation of purified cod intestinal extracts with pure trypsin 1 : 3,000 caused half-inactivation in about 10 min. at *p*H 5.5 at 38° C. in the same way as for Substance P from mammalian intestine. After treatment with trypsin the inactivation was proportional on all test preparations, proving the protein (polypeptide) nature of the active substance. Fig. 2 shows the activity of the extract on rabbit duodenum and guinea-pig ileum after treatment with trypsin.

A quantitative comparison of the cod intestinal extract and Substance P standard on the various test preparations showed that good agreement was obtained on guinea-pig ileum, rabbit duodenum, and on cat and rabbit blood pressure. An equally good agreement was also noted between the effects of Substance P standard and two other cod intestinal extracts on the test preparations used. All of the extracts were inactivated by trypsin.

Dogfish Intestine

From 13 dogfish weighing about 25 kg., 270 g. intestine were obtained, yielding 3.55 g. dry ammonium sulphate precipitate. After this was dissolved in water and methanol added to 70%, the clear supernatant after centrifugation contained 1,300 U. (4.8 U./g.).

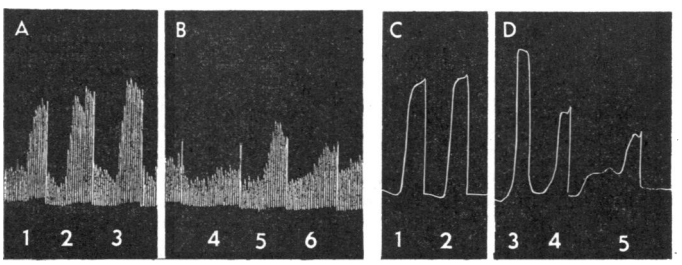

FIG. 2.—A and B. Isolated rabbit jejunum. 15 ml. bath. Atropine 1 : 2.5 mill. in Tyrode solution. 38° C. 1, 0.05 ml. purified cod intestine extract "B." 2, 1 U. "P." 3, 0.08 ml. extract "B." 4 (½ hr. later), 0.067 ml. extract "B" incubated with trypsin. 5, 0.2 U. "P." 6, 0.1 U. "P." C and D. Isolated guinea-pig ileum. 3 ml. bath. Atropine and lergigan 1 : 2.5 mill. in Tyrode solution. 1, 0.3 U. "P." 2, 0.02 ml. extract "B." 3 (45 min. later), 0.1 U. "P." 4, 0.05 U. "P." 5, 0.02 ml. extract "B" incubated with trypsin.

The methanol extract was run through an aluminium column as described above and eluted with decreasing concentrations of methanol. Most of the activity was found in the 20% methanol and aqueous fractions.

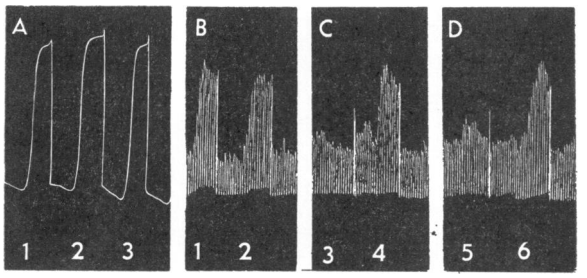

FIG. 3.—A. Isolated guinea-pig ileum. 3 ml. bath. Atropine and lergigan. 1, 0.1 ml. purified cod brain extract "D." 2, 0.2 U. "P." 3, 0.05 ml. purified dogfish intestine extract "E." B.–D. Isolated rabbit jejunum. 15 ml. bath. Atropine. 1, 0.4 ml. extract "D." 2, 1 U. "P." 3, 0.25 ml. extract "E" incubated with trypsin. 4, Same as 3 without incubation. 5, 0.18 ml. extract "D" incubated with trypsin. 6, 0.18 ml. "D."

After removal of the methanol the action of these fractions was tested on various biological preparations and good agreement was found on comparison with Substance P standard (Fig. 3). Treatment with trypsin abolished the effect completely.

Cod Brain

A small amount of cod brain, 15.3 g. from 13 cods weighing 15 kg., was extracted and precipitated with ammonium sulphate as above. An aqueous solution of the precipitate was used for direct assay on the same preparations as used for the extracts of intestine and showed the same type of actions. The activity was 6 U./g. fresh brain on all test preparations. After incubation with trypsin no effect was obtained on the test preparations.

Fig. 3 illustrates the effect of the cod brain extract, purified by adsorption on aluminium oxide and eluted with methanol in decreasing concentrations, on guinea-pig ileum and rabbit duodenum.

DISCUSSION

A factor has been found in ammonium sulphate precipitates of fish intestine and brain which stimulated the isolated intestine of the guinea-pig and rabbit and lowered the rabbit's blood pressure, thereby suggesting that this activity was due to Substance P. This assumption was strongly supported by the facts that, after purification, the factor had the same actions as Substance P qualitatively and quantitatively, even in the presence of atropine and antihistamine drugs, and was inactivated by trypsin in the same way as was Substance P. Evidence has recently been produced by Eliasson, Lie, and Pernow (1956) to show that Substance P in mammalian intestine is identical with that in brain. The present data have not revealed any differences between the Substance P activity from intestine and brain in fish.

It seems therefore that Substance P is not restricted to mammals but also occurs in considerable quantities in the corresponding organs of fish.

SUMMARY

1. A factor having, qualitatively and quantitatively, the same properties as Substance P has been shown to occur in the intestine of the cod and the dogfish and in cod brain.

2. The activity of the ammonium sulphate precipitate corresponded to 4.8–5.5 Units "P"/g. intestine and to 6 U. "P"/g. brain.

3. The active principle was purified by adsorption on aluminium oxide and elution with methanol in decreasing concentrations.

4. The active principle was inactivated by incubation with trypsin in a similar way to Substance P from mammalian intestine.

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