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U. S. v. EULER

DISTRIBUTION AND ACTION OF SUBSTANCE P IN FISH AND INVERTEBRATES

A knowledge of the distribution of a biologically occurring, active substance may often be of aid in understanding its function. This applies to the distribution in the animal series, the macrodistribution in various organs, as well as its microdistribution in the cell. It may be particularly important for substances with largely unknown functions like SP, occurring characteristically in intestinal smooth muscle and in nervous tissue.

The distribution of SP in the animal series is also of interest from the points of view of comparative physiology and may give valuable hints as to evolutionary mechanisms. If its first occurrence in an evolutionary chain can be correlated to a new function or requirement necessitated by altered environmental conditions or prompted by the need for greater achievements, particularly as regards the nervous system, some light may also be shed on its function in the more developed species.

For this reason some studies have been performed on fish and invertebrates (Euler and Östlund 1956, 1957 a, b; Dahlstedt et al. 1959) in order to obtain more information about the occurrence and distribution of SP in cold-blooded animals, since most studies have been made previously on material from warm-blooded animals.

Of previous studies in this field may be mentioned the finding by Correale (1956) that SP occurs in the brain of the frog in high amounts up to 250 U./g.

Material and methods

Preparation of extracts. — As material for the studies to be reviewed here the brain and intestine of teleosts and elasmobranchs have chiefly been used. In some instances extracts have been made of various portions of the brain or the spinal cord. Certain marine invertebrates have been extracted in total or in parts.

Extracts have been made by boiling the organs, after cutting into smaller pieces, in 5 volumes of water to which sulphuric acid was added to pH 4. This reaction was maintained by adding more sul-

phuric acid if necessary. After 10 min. boiling the extract was cooled and filtered on paper. The residue was washed with 1 volume of acidulated water. To the combined extracts ammonium sulphate was added to saturation according to the method of Euler (1936) suggesting its polypeptide nature. The precipitate was allowed to develop at + 5°C overnight, and was filtered off on paper. After repeated washing with 2/3 saturated ammonium sulphate, excess liquid was pressed off and the precipitate dried and powdered.

The further purification was performed according to Pernow (1953) by dissolving the powder in 4 volumes of water under heating to about 50°C and stirring. To the solution was added slowly 2.2 volumes of methanol under continuous stirring. The massive precipitate was allowed to settle in the cold overnight and was washed with 70 per cent methanol. It contained very little SP activity and was removed by centrifugation and discarded. The clear brownish solution, containing practically all of the SP activity, was passed over a column of aluminium oxide (10 g, diameter of column 2 cm, flow 1 ml per minute). The column was eluted with 20 ml portions of methanol in water in falling concentrations from 60 per cent to 20 per cent, followed by repeated elutions with water.

Bioassay. — The methanol present in the eluates was removed by evaporation in vacuo. The aqueous eluates were tested directly. All portions were assayed on the isolated guinea pig ileum according to Pernow (1953) and in most cases also on the isolated rabbit duodenum, the rabbit's blood pressure and the chicken rectal caecum, using purified preparations from cow intestine (100—1,000 U./mg) as standard, prepared according to Pernow (1953).

The specificity of the action was tested

(1) by parallel assays with the standard on different test preparations (Fig. 1) after treatment with atropine and an antihistaminic as outlined above,

(2) by incubating the samples and the SP standard with trypsin (Euler 1936) and comparing the rate of disappearance of action.

Test for biological action in fish. — The biological action of SP preparations from fish intestine and other sources were tested on isolated pieces of small intestine from the teleosts *Pleuronectes platessa* and *Labrus berggylta* and from the elasmobranch *Raja batis*.

The segments of intestine used were suspended in a 75-ml bath with deep sea water diluted 1:2 or 1:2.5 with distilled water for teleosts and 3:5 for elasmobranchs. To the latter solution was added 30 g urea per liter. All experiments were made at room temperature. The drugs were added directly to the bath.

In some instances the intestinal segment was suspended in air and washed with the bath solution at frequent intervals. Drugs were then applied by allowing a small volume of the bath solution, containing the

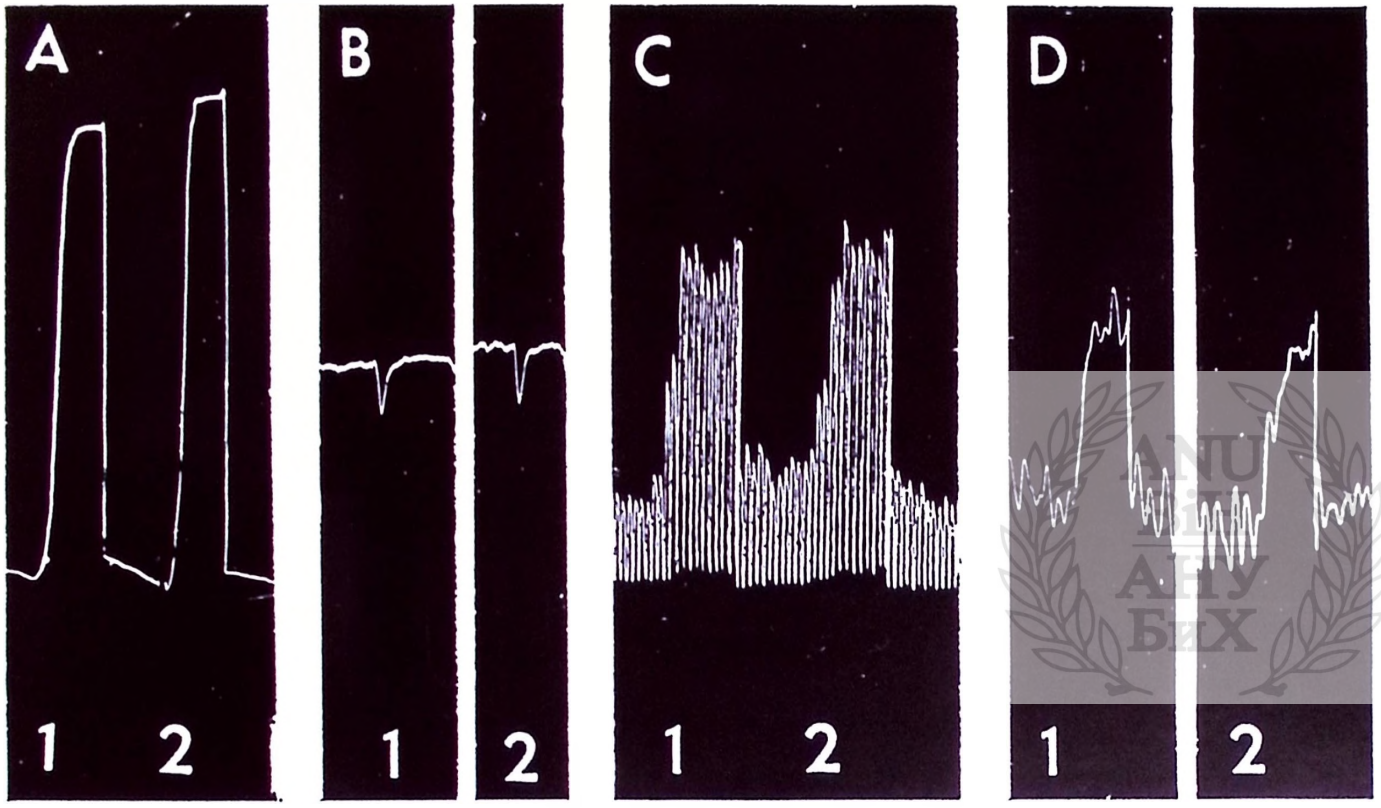


FIG 1

Effects of a purified extract of cod intestine compared with a standard prepared from beef intestine. **A.** Isolated guinea pig ileum, 3-ml bath, 38°C. Atropine and Lergigan (R) 1:2.5 mill. in Tyrode's solution. 1, 0.2 U. SP-standard. 2, 8 ml purified cod intestine extract »C«. **B.** Blood pressure, rabbit, atropine 1, 0.06 ml »C«, 2, 2 U. SP standard. **C.** Isolated rabbit jejunum, 15-ml bath, atropine 1:2.5 mill. 1, 1 U. SP standard. 2, 0.04 »C«. **D.** Isolated chicken rectal caecum, 15-ml bath. 1, 3 U. SP standard. 2, 0.09 ml »C«. [modified after Euler and Östlund (1956) Brit. J. Pharm. 11, 323.]

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drug, to flow directly on the isolated intestine in air, in a way similar to the superfusion technique of Gaddum (1953).

Results

Distribution. — As reported previously SP was found regularly and in relatively high amounts in intestine, brain, and spinal cord of teleosts and elasmobranchs and in smaller amounts in the cyclostome *Myxine glutinosa*. Table I gives some figures obtained.

TABLE I
AMOUNTS OF SP IN FISH ORGANS, UNITS PER GRAM.

	Teleosts		Elasmobranchs		Cyclostome
	Gadus callarias	*Esox lucius*	*Squalus acanthias*	*Raja batis*	*Myxine glutinosa*
Small intestine	5.5	0.7	4.8; 2	3	0.04
Ventricle				1.3	
Whole brain	6			4—9.6	3.3
Telencephalon			50	5.1	
Mid-brain and cerebellum			21	8.5	
Medulla oblongata			45	39	
Spinal cord			18	19—28	
Spinal cord, dorsal part				25	
Spinal cord, ventral part				15	

The following invertebrates were extracted in total and analysed for the presence of SP: *Mytilus edulis*, *Actiniaria* and *Ciona intestinalis*. In the two first-named species no activity which could be ascribed to SP was found in the purified extracts while *Ciona intestinalis* contained a small amount, 0.02 U./g, which corresponded to the effects of SP.

In the intestine of *Nephrops norwegicus* no SP action was found.

Effect of SP on fish intestine. — In view of the presence of SP in extracts of intestine of teleosts and elasmobranchs it appeared

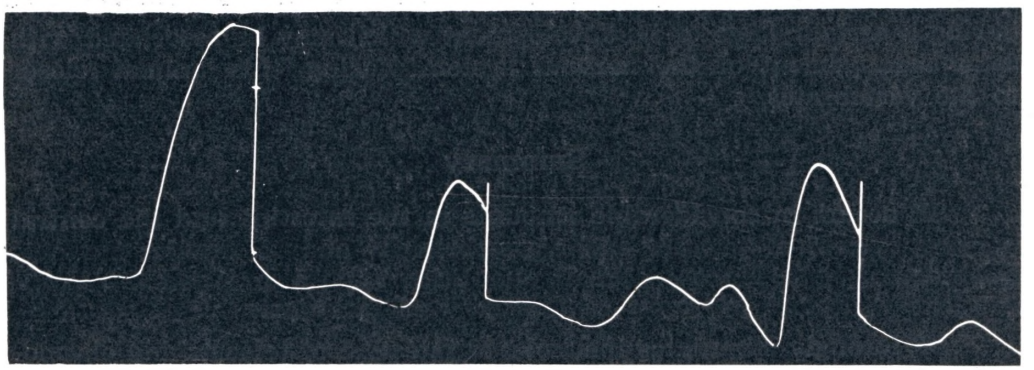


FIG. 2
Isolated small intestine of Pleuronectes platessa, mounted in air, intermittently washed with deep sea water 1:2. Temp. 22°C. Topical application of SP. From left: 0.25 U. SP standard in 0.005 ml, 0.1 U. SP standard in 0.01 ml, 10 µg ACh in 0.1 ml [after Dahlstedt et al. (1959) Acta physiol. scand. 47. 124].

of interest to study whether it exerted actions of similar kind as found in mammals.

It was found that SP, even in small amounts, had a stimulating effect on the isolated intestine. This was particularly obvious when the superfusion technique was applied. Thus 0.1 unit SP in 0.01 ml caused a marked contraction of the isolated small intestine of *Pleuronectes platessa* (Fig. 2). Considering the activity of pure SP (Franz, Boissonnas and Stürmer, 1961), which was found to be 30,000—35,000 U./mg, the total amount given in the case mentioned would correspond to 3.3 ng.

Discussion

The studies reported so far on the presence of SP in fish indicate that it is present in considerable amounts in the intestine and in the central nervous system in teleosts and elasmobranchs. The high amounts in the brain and spinal cord of elasmobranchs are particularly noteworthy.

In the cyclostome investigated, *Myxine glutinosa*, the amounts were smaller, especially in the intestine. It is tempting to relate the low content of SP in the hagfish intestine with the low degree of motility of this organ in comparison with the small intestine of teleosts in particular. If SP functions as a motility hormone of mammalian intestine — for which some support can be advanced — it seems possible that it exerts a similar function in fish in view of its strongly stimulating effect on this organ in teleosts and elasmobranchs.

Before the functional significance of SP in the brain and spinal cord of fish can be fruitfully discussed more knowledge is required as to the normal physiology of the CNS of different species and the action of SP on specified mechanisms.

Of certain interest appears the presence of SP in measurable quantities in *Ciona intestinalis*, since this invertebrate has a dorsal chord during a certain stage of its development which may be associated with some arrangement of the nervous system placing this animal closer to the vertebrates.

Summary

SP occurs in considerable amounts in the intestine and the central nervous system of teleosts and elasmobranchs and in smaller amounts in the cyclostome *Myxine*. Small quantities were also found in *Ciona intestinalis*, but so far none in *Mytilus* and *Actiniaria*.

SP strongly stimulates the isolated intestine of teleosts and elasmobranchs.

It is concluded that SP has a relatively wide distribution in the animal kingdom and exerts similar actions on intestinal smooth muscle as in mammals.

RAZDIOBA I DJELOVANJE SP KOD RIBA I BESKIČMENJAKA

SP se u znatnim količinama nalazi u crijevu i centralnom nervnom sistemu teleosta i elazmobranha, a u manjim količinama kod ciklostome Myxine. Male količine SP nadene su i kod Cionae intestinalis, a potpuni nedostatak SP kod Mytilusa i Actinariae.

SP snažno stimulira izolovano crijevo teleosta i elazmobranha. Zaključuje se da je SP široko rasprostranjen u životinjskom carstvu i da djeluje na glatku muskulaturu crijeva nižih životinja, slično kao kod sisavaca.

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DISCUSSION

KRIVOY: We have been able to cause electric catfish to discharge by bathing them in 0.05 U./ml of SP.

LEMBECK: We also did not find SP in avertebrates (molluscs, holoturia). In the different species of vertebrates SP occurs mainly in the central gray matter, the amount in the telencephalon depends on the same extent of the amount of the central grey matter which reaches the fore brain.