Symposium on substance P

urednik Stern, Pavao

1961

Naučno društvo NR Bosne i Hercegovine

https://bastina.anubih.ba/items/4bb17a51-0c8c-429a-8f96-d5b3e81cf9c8

NAUČNO DRUŠTVO NR BOSNE I HERCEGOVINE

POSEBNA IZDANJA
Vol. I

ODJELJENJE MEDICINSKIH NAUKA
Knjiga 1

Urednik

P. STERN
redovni član Naučnog društva NR BiH

SIMPOZIJUM
O
SUPSTANCIJI P

održan 9. i 10. VI 1961. god.

SARAJEVO
1961

U. S. v. EULER AND F. LISHAJKO

PRESENCE OF SUBSTANCE P IN SUBCELLULAR PARTICLES OF PERIPHERAL NERVES

SP has been shown to be present in peripheral nerves in amounts which are characteristic for different kinds of nerves. Thus Pernow (1953) found amounts varying from 5 units SP/g in the phrenic nerve of dogs to 45 U./g in the dorsal roots. The amounts found in the sciatic nerve of the dog were 10—15 U./g. His results were essentially confirmed by Amin, Crawford and Gaddum (1954). Using butanol-extraction of acetone-dried powder Leach (1959) found lower figures (8.7 U./g) in dorsal roots of cattle.

Little is known so far about the location of SP in the axon. In view of the findings of Euler and Hillarp (1956) that noradrenaline occurs in subcellular particles in adrenergic nerves it appeared of interest to study whether SP might also be present in special structures which may be possible to separate from the rest of the nervous axonal tissue.

Methods

Sciatic and brachial nerves were removed from large dogs immediately after death. The animals had been maintained in chloralose anaesthesia for 1—6 hours. The nerves were freed from the sheath and squeezed between nylon cylinders in the cold according to a technique previously described (Euler 1958). The press juice and washing fluid (0.13 M K-phosphate, pH 7.0) were centrifuged for 5 min. at $600 \times g$ in order to remove larger particles, if occurring. The supernatant was centrifuged for 30 min. at $50{,}000 \times g$ in the cold and the sediment extracted with 1 ml 0.1 N H_2SO_4 and diluted with water to 6 ml. The clear extract was neutralized with sodium hydrogen carbonate to pH 6.2.

Assay. — The extracts were tested on the rabbit duodenum, the guinea pig ileum and the rabbit blood pressure. The majority of assays were made on the guinea pig ileum.

The general course of assay was the following. On the untreated preparation the extract was assayed against ACh, H and, in some cases against 5-HT and SP. After atropinization and treatment with an antihistaminic (Lergigan (R)) the assay was repeated against 5-HT and SP.

Atropine sulphate and Lergigan were added to a concentration of 10^{-5} g/ml in the bath. Higher concentrations often depressed the response to drugs in an unspecific way. The effect of 5-HT was annulled with LSD in a concentration of 10^{-7} g/ml. The biological activity remaining after LSD was tested against SP. The extract was then subjected to incubation with trypsin 0.2 mg per ml for periods up to 45 min. at 37° and pH 7.

During this treatment the activity gradually disappeared like that of SP and along a similar time course. The result strongly supports the assumption that the active substance found in the sediment is in fact SP. The contraction on the guinea pig ileum was often somewhat slower than that of SP, but on several occasions no difference could be noted.

Fig. 1 shows the effect of an extract of the nerve sediment obtained by high *g* centrifugation in comparison with ACh, H, 5-HT and SP standard before and after specific inhibitors and treatment with trypsin. An increase in the response to SP as well as to the extracts was observed after LSD (Fig. 2). An effect of this kind was first described by Krivoy (1957).

When assayed against a standard of SP (75 U./mg, made by Hoffmann la Roche and kindly placed at our disposal by Prof. J. H. Gaddum) the sediment was found to contain approximately 4—8 U./g of nerve. A certain loss in biological activity of the extract was noted after treatment of the test organ with atropine and with Lergigan (R), but not after LSD. This presumably indicates that some of the biological activity on the untreated or partially treated test organ was due to ACh and H. When the effect of the extract was increased after treatment with LSD it was not possible to state whether or not a loss of action due to the presence of 5-HT had occurred.

The loss in activity of the extract of the sediment after Lergigan (R) (4 U./g) corresponds to 1 µg histamine and the loss after atropine (3 U./g) to 0.06 µg ACh. These figures are, however, nominal and are based on the unproven assumption that the relative activities are unaltered and that no other compounds are present which may influence the response. (Table I).

TABLE I

BIOLOGICAL ACTION EQUIVALENTS OF SEDIMENT H IV FROM THE SCIATIC AND BRACHIAL NERVES OF THE DOG, PER G OF NERVE

	Tested directly	After Lergigan (R)	After Atropine	After LSD
H equ. µg/g	3	—	—	—
ACh equ. µg/g	0.24	0.25	—	—
5-HT equ. µg/g	—	—	6	—
SP equ. U./g	12	8	5	6

Equivalents: 1 U. SP = 0.25 µg H = 0.02 µg ACh.

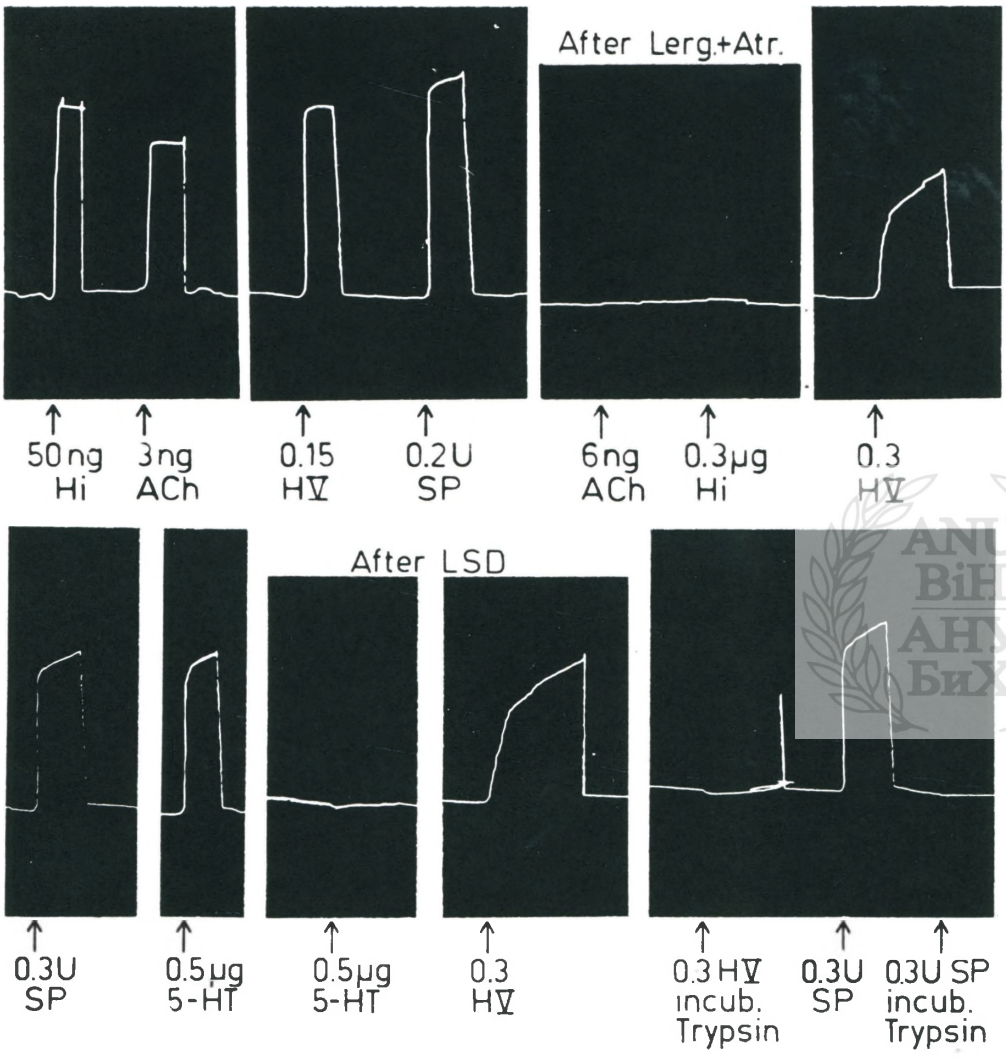


FIG. 1

Isolated guinea pig ileum. 3 ml bath, 38°C, Tyrode's solution. H V, extract of sediment from press juice of sciatic and brachial nerves of the dog, figures representing volumes in ml (1 g nerve = 3 ml).

Drugs administered as indicated (H = histamine, ACh = acetylcholine,, 5-HT = 5-hydroxytryptamine).

Lergigan (R), atropine, and LSD added to the bath fluid reservoir in a concentration of 10^{-7} g/ml.

ANU
BiH
АНУ
БиХ

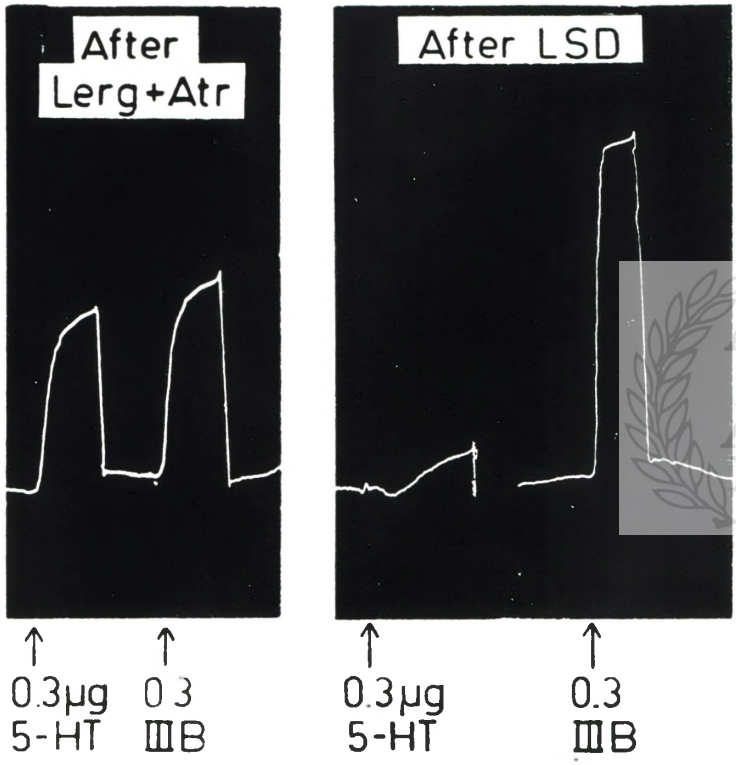


FIG. 2

Isolated guinea pig ileum. Atropine, Lergigan (R) as in Fig. 1. Effect of extract III B, prepared as extract H V, enhanced after LSD.

ANU
BiH
АНУ
БиХ

Tests on the rabbit intestine and the rabbit's blood pressure were in accord with the assumption that the active substance is SP.

Discussion

The functional role of SP in peripheral nerves is still not understood. Its presence in intraaxonal granules is, however, of interest since it suggests that the polypeptide is stored in a specific way and may be functionally related to the mechanisms of nerve action. The SP in the sedimented granules occurs in a bound or protected form since addition of the untreated sediment to the organ bath has a much weaker action than after extraction with acid. This is in agreement with the catecholamine stored in granules of adrenergic nerves (Euler 1958). The proportion of SP found in the granules is fairly high, and may be estimated at about 50 per cent of the total amount. The general occurrence of SP in nervous tissue suggests an action which may be related to some function of a more general nature or to the metabolism of the nerve. Its presence in the nervous system of fish seems to support this concept.

Summary

The subcellular sediment obtained on high *g* centrifugation of press juice from the sciatic and brachial nerves of the dog contains an active factor having biological actions and chemical properties suggesting its identity with SP, as shown by the action of specific inhibitors and treatment with trypsin.

The active substance is present in the sediment in a bound or protected form or can be released by extraction with acids.

The amounts of the SP-like substance found in the sediment is 4—8 U./g of nerve, indicating that up to 50 per cent, and perhaps more, is present in a granular fraction.

The loss of biological activity of the extract of the sediment after treatment with specific inhibitors suggests the presence of H and ACh in small amounts.

The presence of SP in a bound form in the neurons points to some action of SP in the general function of the nerve.

PRISUTNOST SP U SUPCELULARNIM ČESTICAMA PERIFERNIH ŽIVACA

Supcelularni sediment dobiven prilikom ultracentrifugiranja istiještenog soka n. ischiadicus i n. brachialis psa sadrži faktor čija biološka i kemijska svojstva sugeriraju identitet sa SP, kako pokazuju djelovanja specifičnih inhibitora i obrada tripsinom. Aktivna supstancija u sedimentu nalazi se u vezanoj ili zaštićenoj formi, iz koje se može osloboditi ekstrakcijom pomoću kiselina. Količine supstancije slične SP nađene u sedimentima iznose 4—8 jed./g tkiva, što pokazuje da je do 50% te količine, a, možda, i više, u granularnoj frakciji. Gubitak biološke aktivnosti ekstrakata poslije obrade specifičnim inhibitorima sugeriše prisutnost malih količina H i ACh. Postojanje SP u neuronima u vezanoj formi ukazuje na izvjesnu ulogu ove supstancije u općoj nervnoj funkciji.

REFERENCES

AMIN A. H., T. B. B. CRAWFORD AND J. H. GADDUM (1954) — J. Physiol. 126, 596—618.
EULER U. S. v. (1958) — Acta physiol. scand., 43, 155—166.
EULER U. S. v. AND N. A. HILLARP (1956) — Nature 177, 44—45.
KRIVOY W. A. (1957) — Brit. J. Pharmacol. 12, 361--364.
LEACH E. (1959) — J. Physiol. 149, 34P—35P.
PERNOW B. (1953) — Acta physiol. scand. 29, Suppl. 105.

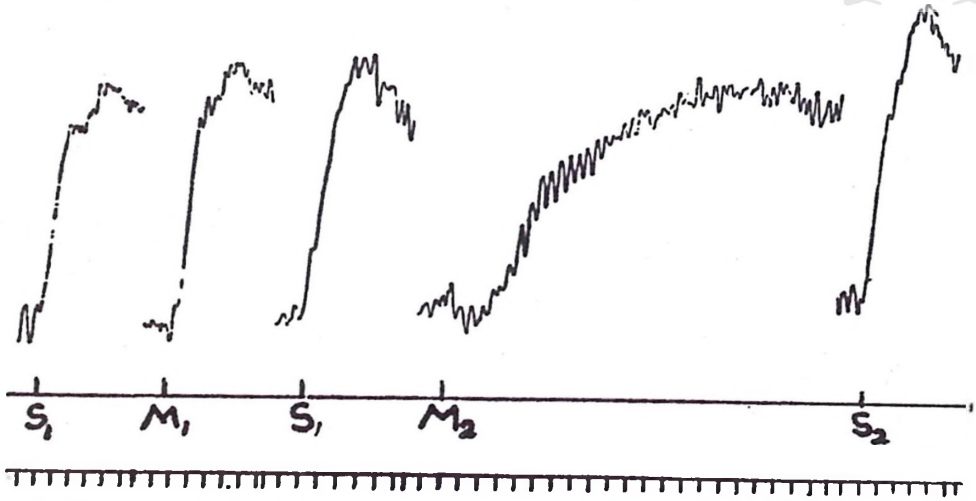
DISCUSSION

GADDUM: Am I right in supposing that these residues contained both microsomes and mitochondria? C. O. Hebb found that cholinacetylase in the CNS was in the mitochondrial fraction, whereas cholinacetylase in the sciatic nerve was in the microsome fraction. It would be interesting to know whether there is a similar difference for SP.

v. EULER: We have not studied in particular the presence of mitochondria and microsomes in the sediment of nerves containing SP but there is little doubt from experiments with splenic nerves that they are present.

ZETLER: The finding that the microsomal fraction of peripheral nerves is as such without biological activity unless it is treated with sulphuric acid contrasts to what I have seen with mitochondria prepared from mouse brain. The experiment shown in the following figure demonstrates that substance P can easily be extracted from mitochondria by boiling at pH 3 (HCl), furthermore, that the same amount of biological activity is very slowly released if the corpuscles are, without pretreatment, suspended in the bath fluid.

It can be assumed that substance P is present in brain mitochondria in an active form and is released during the breakdown of the mitochondrial membrane in Tyrode's solution at 32°C. Thus, substance P seems to be stored in the central nervous system and peripheral nerves in different ways. The small fraction of substance P present in mouse brain which cannot be extracted by mild methods [Zetler G. and G. Ohnesorge (1957) — Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. 231, 199] may also be stored in a way as suggested by Euler and Lishajko.



Isolated guinea pig ileum, bath volume 3 cm³, temperature 32°C (atropine 10^{-7}, mepyramine 10^{-9}, tryptamine $2/10^{-5}$, S_1 : 0.2 U. substance P, S_2 : 0.24 U. substance P. M_1 : 0.1 cm³ mitochondrial fraction of mouse brain, 3 minutes boiled at pH 3 (HCl) and neutralized with NaOH; M_2: the same dose of mitochondrial fraction, but neither acidified nor boiled. The doses of M_1 and M_2 correspond to about 3.5 mg mouse brain each (the cerebellum was removed before mincing). The time marker indicates 10 seconds.