Baština Akademije nauka i umjetnosti Bosne i Hercegovine

Symposium on substance P

urednik Stern, Pavao

1961

Naučno društvo NR Bosne i Hercegovine

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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.

ANU BiH АНУ БиХ

SARAJEVO
1961

J. FRANZ, R. A. BOISSONNAS AND E. STÜRMER

PHARMACOLOGICAL PROPERTIES OF SUBSTANCE P ISOLATED IN AN APPARENTLY PURE STATE

SP was discovered 30 years ago by von Euler and Gaddum (1931). It is a potent biogenic polypeptide and appears to play an important rôle under physiological conditions (Schachter, 1960). The krown pharmacological properties of SP reported in the literature and at this symposium are based mainly on observations with crude SP preparations. Pernow (1953) by chromatography on aluminium oxide columns succeeded in preparing a strongly enriched SP preparation with an activity of 2,500—3,500 U./mg. Recently Franz, Boissonnas and Stürmer (1961) were able to isolate SP from horse intestine in an apparently pure state (activity 30—35,000 U.*)/mg).

This apparently pure substance P (SPp) was compared with a crude substance P extract (SPex: our starting material) on isolated smooth muscle preparations (guinea pig ileum and hen caecum) and on the blood pressure of the atropinized rabbit. The results are presented in Figs. 1—3.

It can be seen from the Figures that SPp was active in all these tests and that, within the limits of biological variation, the increase in activity in all three tests was proportional to the degree of purification, viz. about 1 : 2000 (SPex : SPp). These SP activities thus appear to be inherent properties of our highly purified material which is probably mainly responsible for the pharmacological activity of crude SP-extracts.

SP behaves differently from pure synthetic bradykinin on hen caecum: as is shown in Fig. 4, SP contracts hen caecum, while bradykinin causes relaxation. On treatment with chymotrypsin (0.05 mg SPp + 0.002 mg chymotrypsin in 0.012 ml, pH 9.5, 3 hours, 25°C) SPp and bradykinin are broken down, but only the former yields arginine. Under the same conditions, trypsin does not completely inactivate SPp; carboxypeptidases (A + B) which inactivate bradykinin, splitting off arginine, do not destroy the biological activity of SPp, nor is arginine split off. Total hydrolysis of SPp and bradykinin yields arginine and

*) We are indebted to Dr. Pernow who kindly supplied us with von Euler-Gaddum standard.

proline as common constituents, but SPp also yields leucine/isoleucine and alanine, amino-acids which are not found on hydrolysis of bradykinin.

Summary

Substance P was isolated in an apparently pure state from horse intestine by chromatography and electrophoresis (activity 30,000—35,000 units per mg). The activity of the purified substance (SPp) and the starting material (SPex) were compared on guinea pig ileum, hen caecum, and rabbit blood pressure. The ratio of activities, SPp:SPex, was 2000:1.

SPp and Bradykinin were readily distinguished in their pharmacological and chemical properties.

FARMAKOLOŠKA SVOJSTVA SP IZOLOVANE U OČITO ČISTOM STANJU

SP izolovana je u očito čistom stanju iz crijeva konja primjenom kromatografije i elektroforeze (aktivnost 30,000—35,000 jed./mg). Aktivnost prečišćene supstancije (SPp) i ishodnog materijala (SPex) uspoređene su na ileumu zamorca, caecumu kokoši i krvnom pritisku kunića. Aktivnosti stoje u odnosu SPp : SPex = 2000 : 1.

SPp i bradikinin se jasno razlikuju po svojim farmakološkim i kemijskim svojstvima.

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SCHACHTER M. Substance P. in (1960) — Polypeptides which affect smooth muscles and blood vessels (Pergamon Press, Oxford, London, New York, Paris.

DISCUSSION

LEMBECK: (1) Were the steps of purification carried out under low temperature?

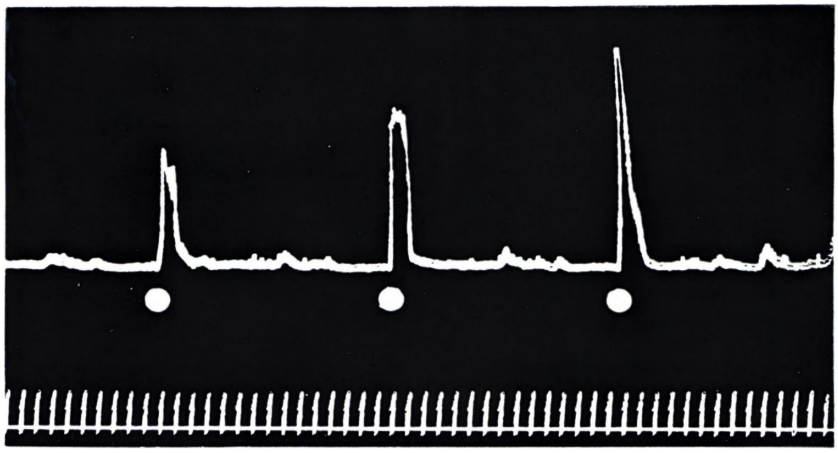
(2) In which steps of purification did the greatest loss occur?

STÜRMER: (1) The chromatography on aluminium oxide was carried out at low temperature.

(2) The greatest loss occurred in the ion exchange chromatography.

PERNOW: I want to ask you more about your experience of the stability of the pure preparation. When I worked on the purification of the substance and got a preparation which seems to have had one tenth of the activity of your most active fraction, I found it very unstable in a more purified stage. I tried to find out if this lability was due to oxidation or enzymatic activity, but I never found any consequence in the »spontaneous« loss of activity. Very often I found that if a higly purified preparation in aqueous solution was frozen down and then again thawed up a 50 per cent loss of activity or more was found.

STÜRMER: Some solutions of our highly purified SP retained their potency for about 4 days. However, sometimes a loss of biological activity was detectable within 24 hours. We do not know the reason for this.



atropine and thenalidine 10^{-6} g/ml added 5 min. before SP.

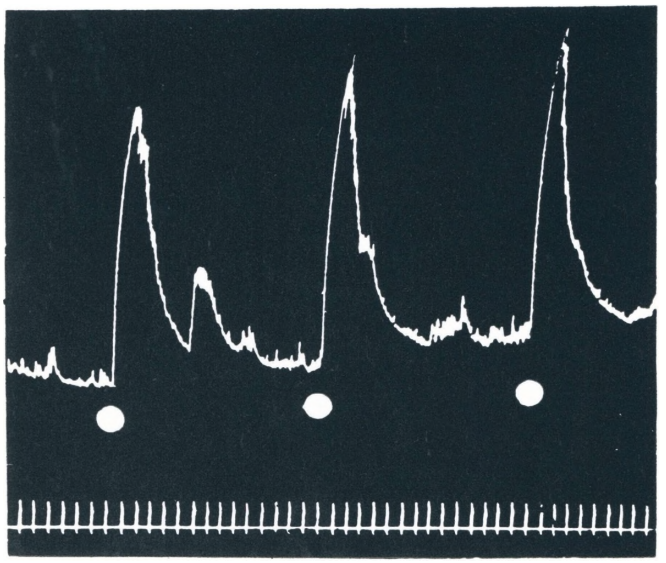
min.

0.3 U SPex	0.01 µg SPp	0.3 U/ml SPex
0.1 µg SPp	—	0.3 U
1 mg SPp	—	30,000 U

FIG. 1

Effects of a crude extract, SPex, (15 U./mg) in comparison with the highly purified SP-preparation (SPp) added (o) to isolated guinea pig ileum. Note that there is no difference between the action of crude and highly purified SP. Other details of method: Tyrode solution; 37° C; carbogen; all doses per ml; concentrations of atropine and thenalidine refer to the salts (sulphate and tartrate respectively).

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atropine and thenalidine 10^{-6} g/ml added 5 min. before SP.

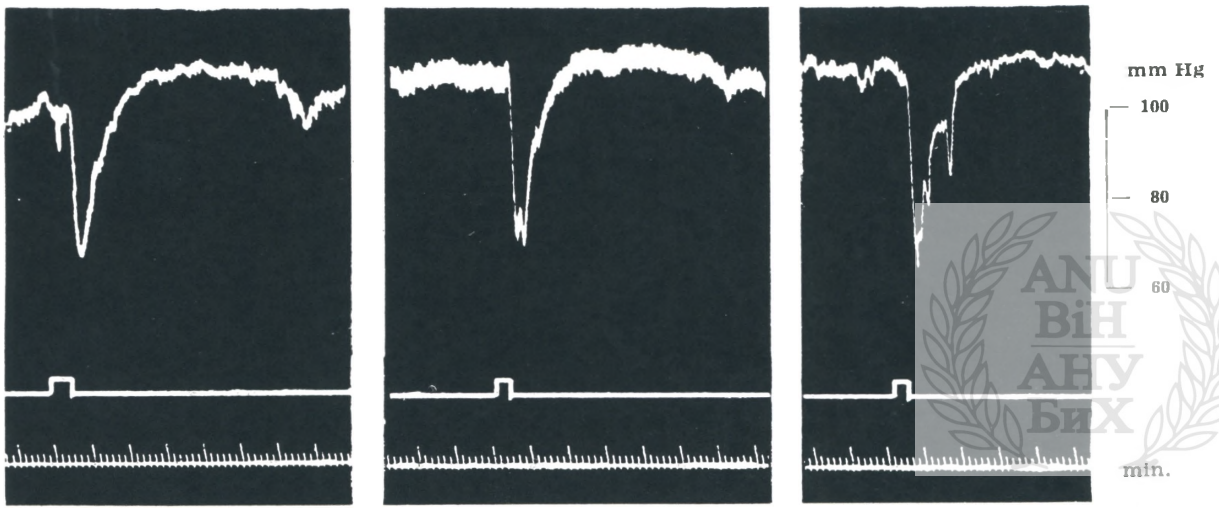
min.

0.3 U. SPex	0.1 µg SPp	0.3 U./ml SPex
0.01 µg	—	0.3 U.
1 mg SPp	—	30,000 U.

FIG. 2

Effects of a crude extract, SPex, 15 U./mg) in comparison with the highly purified SP-preparation (SPp) added (o) to isolated hen caecum. Note that there is no difference between the action of crude and highly purified SP. Method as described in Fig. 1.

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2.8 U. SPex	0.084 µg SPp	2.8 U./kg SPex
0.084 µg SPp	—	2.8 U.
1 mg SPp	—	33,000 U.

FIG. 3

Effects on intravenous injection (⨍) of a crude extract, (15 U./mg) in comparison with that of the highly purified SP-preparation (SPp) on the blood pressure of the rabbit recorded by a mercury manometer from the carotid artery.

Note that there is no difference between the action of crude and highly purified SP.

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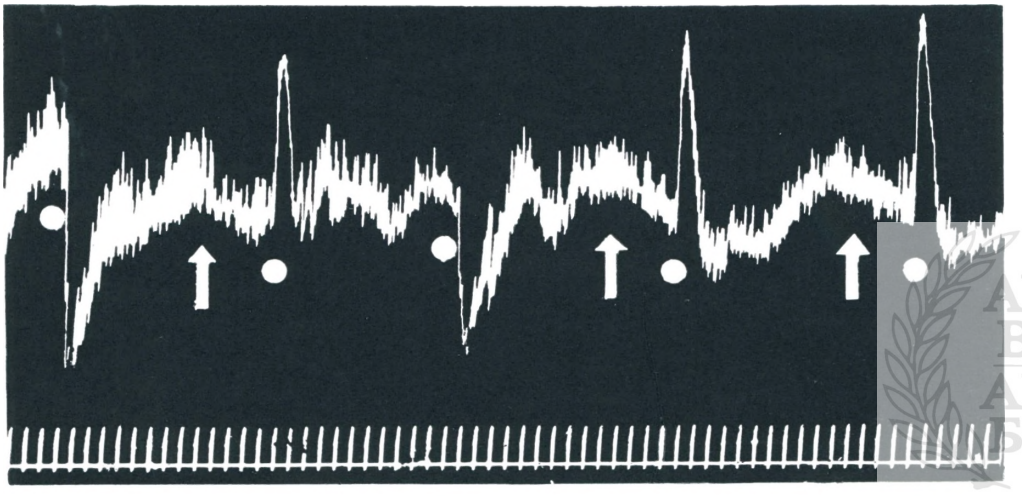


FIG. 4

Effects of SP and pure synthetic bradykinin (Br) added (o) to isolated hen caecum. Method as described in Fig. 1.

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HAEFELY: We found our purified SP preparation rather unstable when diluted in accordance with Pernow. It is surely not due to bacterial contamination and probably not to oxidation. Because of the lability of more purified SP we still prefer an impure preparation as standard. The purified SP was prepared by Hoffmann la Roche by counter current distribution. The molecule does not contain histidin.

PERNOW: Professor Gaddum mentioned the silicone treatment of the glasses in order to prevent losses of activity. I tried this once after having read in the Biochemical Journal a few years ago, that this technique prevented loss of activity of angiotensin, which was found to be easily adsorbed on the glass wall. In my experience this treatment, however, did not prevent the loss of activity of SP.

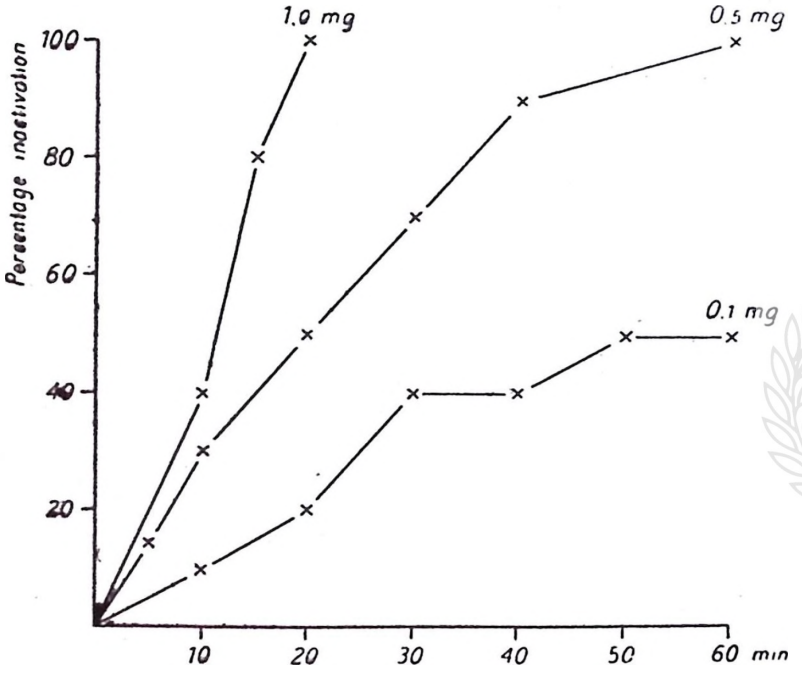


FIG. 1

Inactivation of SP by crystalline chymotrypsin. 0.5 mg of SP (100 U.) in Tyrode's solution was incubated with 0,1—200 µg trypsin in 1—35 min. at 38° C.

STÜRMER: We did not use silicone-treated glassware.