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

D. B. BELESLIN AND B. Ž. RADMANOVIĆ

THE EFFECT OF SUBSTANCE P ON THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE CAT

It has been shown that SP, injected intra-arterially into the central end of the lingual artery while occluding the external carotid artery. potentiated the response of the nictitating membrane to submaximal stimulation of the preganglionic sympathetic nerve, while higher doses usually depressed the response. The stimulating action of ACh on the superior cervical ganglion »in situ« was also potentiated by SP (Beleslin, Radmanović and Varagić, 1960).

It was therefore of interest to reinvestigate the action of SP on the isolated superior cervical ganglion of the cat and to test whether changes in the output of ACh from the ganglion contribute to the failure of transmission.

Methods

Cats of both sexes, weighing 1.5 to 3 kg, were used. Anaesthesia in cats was induced with ether and maintained by intravenous injection of chloralose (100 mg/kg b. w.).

The superior cervical ganglion was prepared for perfusion following the conventional method, with the modifications suggested by Perry (1953). The contractions of the nictitating membrane were recorded with an isotonic lever fitted with a frontal writing point magnifying the movements of the membrane ten times. The cervical sympathetic chain was divided and, when stimulated electrically, its peripheral end was placed on shielded electrodes and covered with liquid paraffin. For stimulation an electronic stimulator delivering square wave pulses was used. The pulses had a duration of 0.8 msec. and a frequency between 2 and 5 per sec.

When ACh was to be collected, the post-ganglionic trunk was tied and eserine-salicylate, 10^{-6} g/ml, added to Locke's solution. The preganglionic nerve was stimulated intermittently, for 5 min. periods of stimulation, alternating with an equal period of rest. This procedure usually gave a series of samples containing an approximately equal amount of ACh. Five or six samples were usually collected.

ACh was assayed on the blood pressure of eviscerated, chloralosed cats.

SP was kindly supplied by Professor Gaddum, Dr. Lembeck and Dr. Pernow. SP supplied by Professor Gaddum and Dr. Pernow was a concentrate and contained 75 U./mg. SP supplied by Dr. Lembeck contained 5.6 U./mg.

Results

SP and sympathetic nervous stimulation. — Upon addition of SP in doses from 0.25 to 40 units to the fluid, perfusing preparations of the superior cervical ganglion, the changes in response of the nictitating membrane to submaximal stimulation of the preganglionic sympathetic nerve were recorded. In present experiments, when SP was added to the perfusing fluid, the contractions of the nictitating membrane in response to preganglionic nerve stimulation were usually depressed or blocked. Fig. 1 shows the responses of the nictitating membrane to stimulation of the cervical sympathetic trunk with stimulation periods of 5 sec. every 90 sec. After addition of 1 and 3 units of SP at P_1 and P_2 the response to preganglionic stimulation was unchanged. The responses of the nictitating membrane to preganglionic nerve stimulation at P_3 were slightly reduced after addition of 10 units of SP to the perfusing fluid. When 30 units of SP were added to the perfusing fluid (at P_4) the responses to preganglionic stimulation were completely abolished. The complete block lasted 12 min., after which the responses gradually returned to normal. The amount of SP which was added to the perfusing fluid did not, in itself, stimulate the ganglion, and caused no contraction of the nictitating membrane in the absence of preganglionic stimulation. The transient depression of the response of the nictitating membrane to preganglionic nerve stimulation was obtained even after small doses of SP. A typical experiment is shown in Fig. 2. At **P**, 0.5 units of SP was added to the perfusing fluid. The response to preganglionic stimulation was first reduced and after 15 min. completely abolished. The complete block lasted 5 min., after which the response gradually returned to normal. This type of response was obtained in 6 out of 9 experiments. The higher doses of SP (from 10 to 40 units) produced the block of the response to preganglionic nerve stimulation in 7 out of 10 experiments.

In present experiments the potentiation of the response of the nictitating membrane to preganglionic nerve stimulation was rarely observed after adding SP. After small doses of SP (from 0.25—2 units) its potentiating effect was observed only in 1 out of 9 experiments. Similar results were obtained with higher doses (from 10—40 units). This effect was observed only in 1 out of 10 experiments.

SP and ACh. — In present experiments, when SP was added to the perfusing fluid, the contractions of the nictitating membrane to ACh were depressed or blocked. A typical experiment is shown in Fig. 3. At **P** in **A**, 2 units of SP were added to the perfusing fluid

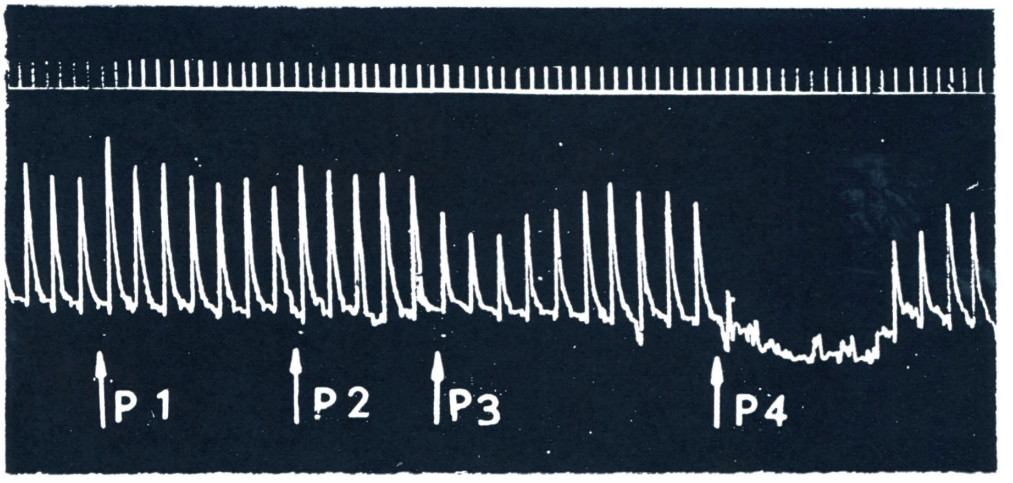

FIG. 1

Cat, chloralose. Contractions of the nictitating membrane to submaximal preganglionic stimulation of the cervical sympathetic nerve (5 pulses/sec., 0.8 msec., 7.5 mA). At P_1, P_2, P_3, and P_4 SP was added to perfusing fluid in doses of 1, 3, 10 and 30 units respectively. Time 1 min.

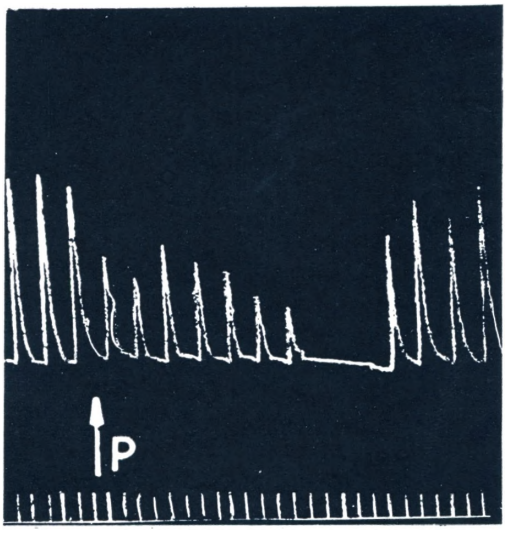

FIG. 2

Cat, chloralose. Contractions of the nictitating membrane to submaximal preganglionic stimulation of the cervical sympathetic nerve (5 pulses/sec., 0.8 msec., 7.5 mA). At 0.5 unit of SP was added to perfusing fluid. Time 1 min.

ANU
BiH
АНУ
БиХ

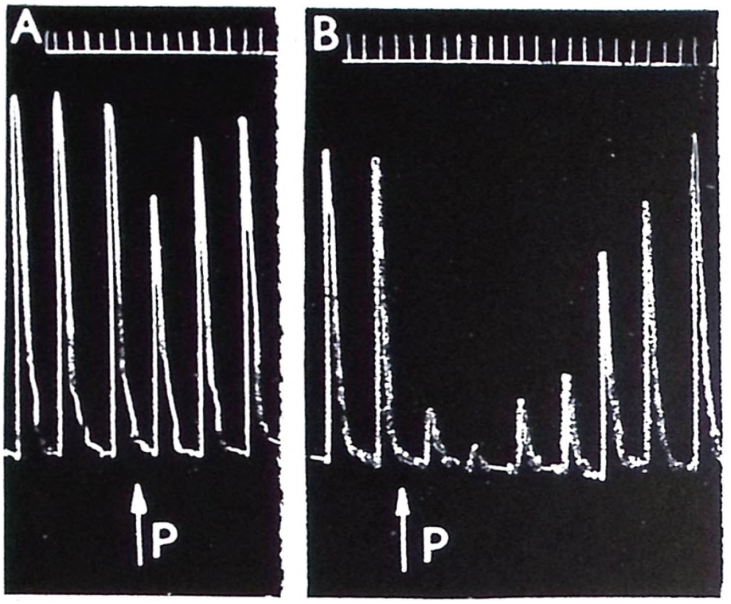

FIG. 3

Cat. chloralose. Contractions of the nictitating membrane to ACh (4 μg). At P in A, 2 units of SP was added to perfusing fluid. At P in B, 30 units of SP was added to perfusing fluid. Time, 1 min.

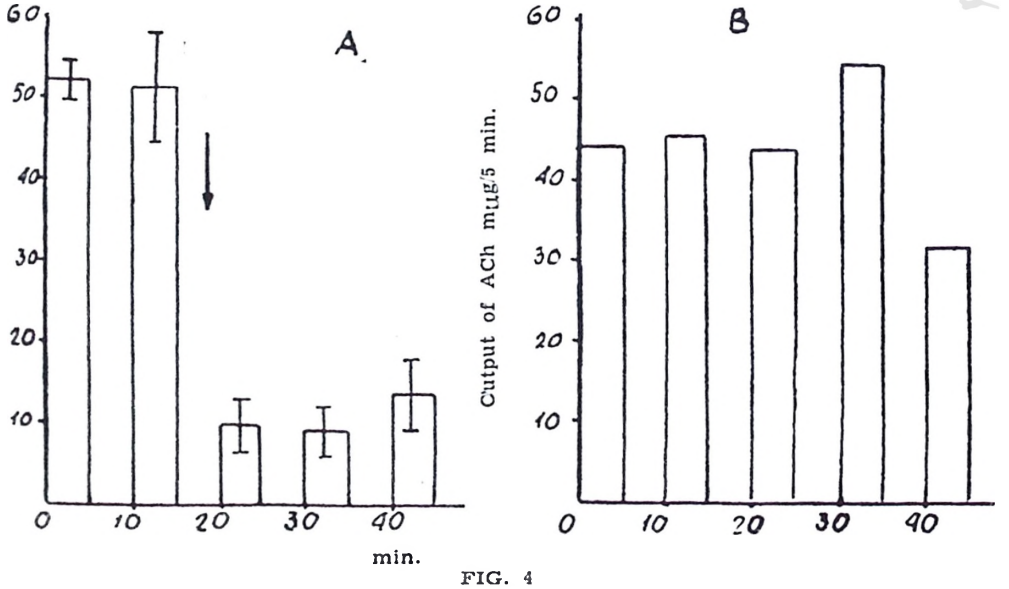

FIG. 4

Each block represents amount of ACh released during 5 min. stimulation of the cervical sympathetic nerve at 2/sec., 0.8 msec., 7.5 mA. At the arrow in A 20 units of SP was added to perfusing eserinized Locke's solution. In B the ganglion was perfused only with eserinized Locke's solution (no SP was added).

ANU
BiH
АНУ
БиХ

and produced a transient depression of the response to ACh. 'The higher dose of SP (30 units) in the same experiments at **P** in **B** produced complete block of the response of the nictitating membrane to ACh. The depression of the response of the nictitating membrane after small doses of SP (from 0.1 to 5 units) was observed in 8 out of 11 experiments. The higher doses of SP (from 10—30 units) usually produced the block of the response to ACh. This was observed in 3 out of 4 experiments.

The potentiation of the response of the nictitating membrane to ACh after small doses of SP (from 0.1 to 5 units) was observed in 2 out of 11 experiments, and only in 1 out of 4 experiments after a higher dose of SP (30 units).

The effect of SP on ACh output. — Hutter and Krista Kostial (1954) found that, when the preganglionic nerve was stimulated intermittently for 5 min. periods of stimulation alternating with equal periods of rest, gave a series of samples containing an approximately equal amount of ACh. These experiments prompted us to test the effect of SP on ACh output. Using the same procedure we found that SP in a dose of 20 units decreased the ACh output. At the arrow in Fig. 4, 20 units of SP was added to the perfusion fluid. The ACh output was reduced by about 80%. This reduction lasted 30 min.

Discussion

These experiments show that SP, when added to the perfusing fluid, mostly depressed, and rarely potentiated, the transmission in the superior cervical ganglion. The depression was usually observed after lower as well as after higher doses of SP. We have found in these experiments that the failure of ganglionic transmission produced by SP is accompanied by a decrease in the amount of ACh liberated as a result of preganglionic stimulation.

The effect of ACh on the superior cervical ganglion was usually depressed. Higher doses of SP usually depressed the response of the nictitating membrane to ACh.

In our present experiments there was no difference in action on the superior cervical ganglion of the cat between the concentrated SP, supplied by Prof. Gaddum and Dr. Pernow, and SP supplied by Dr. Lembeck.

We have found that SP reduced the output of ACh from nerve endings. The extent to which this action contributes to the failure of transmission cannot be determined from our experiments. Brown and Feldberg (1936) found that the amount of ACh, liberated by a series of maximal preganglionic volleys, is in excess of the minimum required to produce full excitation of the ganglion cells. The reduction of responses to ACh also suggests that some other mechanism might be involved in the action of SP. The decreased output of ACh may also be due to ACh synthesis or to a cholinesterase activity of SP.

In our previous work (Beleslin, Radmanović and Varagić, 1960) a potentiating effect of SP was observed in 5 out of 11 experiments, when it was injected intra-arterially into the central end of the lingual artery, and a depression was obtained in 4 out of 11 experiments.

It is very difficult to explain the mechanism by which SP potentiates the responses of the nictitating membrane to the preganglionic nerve stimulation or to ACh. SP probably acts by sensitisation of ACh receptors in the postganglionic neuron, or this effect is due to nonspecific action of impurities in SP itself. In our previous work we used unpurified SP (obtained by extraction of ox brain). This preparation contained usually about 2—3 U./mg. The potentiating effect of SP on the ganglion »in situ« might be connected with the presence of blood. The greater percentage of potentiation on the ganglion »in situ« observed in our previous experiments might also be due to the presence of some other active substances in unpurified SP.

Summary

(1) SP added to the perfusing fluid in lower and higher (0.25—40 units) doses usually depressed the response of the nictitating membrane to preganglionic sympathetic nerve stimulation.

(2) The stimulating action of ACh on the superior cervical ganglion, as judged by contraction of the nictitating membrane, was also depressed after adding SP to the perfusing fluid.

(3) SP in a dose of 20 units, which usually produced block of ganglionic transmission, reduced at the same time the output of ACh.

EFEKAT SP NA IZOLOVANI GORNJI VRATNI GANGLION MAČKE

Dodatak SP perfuzionoj tekućini u dozama 0.25—40 jed. obično smanjuju reakciju membranae nictitantis na preganglionarnu simpatičku stimulaciju.

Stimulativno djelovanje ACh na gornji vratni ganglion također je smanjeno poslije dodatka SP, sudeći po kontrakciji membranae nictitantis.

SP u dozi od 20 jed., koja je dovoljna da izazove blokadu ganglionarne transmisije, ujedno snižava izlučivanje ACh.

REFERENCES

BELESLIN D., B. RADMANOVIĆ AND V. VARAGIĆ (1960) — Brit. J. Pharmacol. 15, 10.
BROWN G. L. AND W. FELDBERG (1936) — J. Physiol. 88, 265.
HUTTER O. F. AND KRISTA KOSTIAL (1954) — J. Physiol. 124, 234.
PERRY W. L. M. (1953) — J. Physiol. 119, 439.

DISCUSSION

LEMBECK: The inhibition of the ganglion stimulation by SP seems to be an effect which does not appear immediately after the injection (like hexamethonium), but shows a slow onset and exerts its full effect not earlier than after some minutes.

KRIVOY: The original observation of Beleslin *et al.* that SP potentiates ganglion transmission have been repeated in my laboratories using electrophysiological techniques *in vivo* and *in vitro*, although the dose of SP was much smaller (20 i. U./ml).