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.

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SARAJEVO
1961

V. VARAGIĆ, D. B. BELESLIN AND B. Ž. RADMANOVIĆ

SOME PERIPHERAL NEUROTROPIC EFFECTS OF SUBSTANCE P

The potent smooth muscle stimulating effect of SP was first described by Euler and Gaddum (1931) and later studied in detail by Pernow (1953). Meanwhile, SP is also a normal constituent of brain tissue and has been found to produce various effects on the central nervous system [Euler and Pernow (1954), Zetler (1960)]. Lembeck (1957) presented evidence that SP stimulated afferent nervous fibres. It was therefore decided to study peripheral neurotropic effects of SP.

SP and peristalsis. — During the investigation of the effect of cooling and of 5-HT on the peristaltic reflex of the isolated guinea pig ileum (Beleslin and Varagić, 1958a) it was decided to test the action of SP on the peristalsis. In order to mimic physiological conditions as closely as possible, SP was injected intraluminally. For this reason the original Trendelenburg's method (1917) for recording the peristaltic activity was slightly modified, in order to allow the intraluminal injections. It was found that the introduction of SP into the lumen of the guinea pig ileum caused an increase in the number and amplitude of the peristaltic waves. SP was also found to be able to restore peristalsis in preparations, in which it was abolished by fatigue (Fig. 1), by external or internal application of 5-HT (Fig. 2), or by lowering the temperature of the bath (Beleslin and Varagić, 1958b). Since the effect of SP was absent in preparations in which the mucous membrane was removed, and since hexamethonium abolished the effect of SP on peristalsis (Fig. 3), it was concluded that SP acted on the afferent nervous elements of the peristaltic reflex arc possibly through the sensory receptors in the mucosa.

In experiments, in which the antagonism of morphine-like analgesics and SP on the peristaltic reflex was studied, Medaković and Radmanović (1959) have found that the stimulating action of SP on the peristaltic reflex was inhibited by morphine and morphine-like analgesics (Fig. 4). The relative inhibitory potency of analgesics on the stimulating action of SP corresponded well to their relative analgesic potencies. On the other hand, the intraluminally introduced SP could not restore the peristaltic reflex previously abolished by morphine and morphine-like analgesics (Fig. 5). Medaković (1959) has also found that

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.

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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).

morphine, acting from the intestinal lumen, can paralyse the peristaltic reflex, possibly by acting on the sensory mucosal receptors. It was therefore concluded that morphine and SP acted at the same point. The surmountability in the interaction of SP and analgesics was not demonstrated in these experiments.

SP acting on the outside of the guinea pig ileum. — Large doses of SP (30—100 units), when acting on the outside of the isolated guinea pig ileum, abolished the peristaltic reflex, whereas small doses (4 units) occasionally potentiated peristalsis (Beleslin and Varagić, 1959a). This block could not be overcome by intraluminal injections of 5-HT or itself (Fig. 6). It was also found on the Magnus preparation that a high dose of SP can block the response of the gut to a small dose of the same substance.

Recently Radmanović (1961, in press) has found that small doses of SP when acting on the outside of the isolated guinea pig ileum could restore the peristaltic reflex previously abolished by *d*-tubocurarine (*d*-TC) (Fig. 7). On the other hand, previous addition of SP into the bath did not abolish the inhibitory effect of *d*-TC on the peristalsis. In similar circumstances 5-HT injected intraluminally failed to restore peristalsis which was abolished by *d*-TC. This effect of SP was attributed to its action on the nervous elements in the gut, possibly on the intestinal ganglia, because *d*-TC was found to have a ganglion blocking activity [Feldberg and Lin (1949), Paton and Zaimis (1949)].

SP and the amount of 5-HT in the gut. — 5-HT was known to affect peristalsis in a similar way as SP. It stimulates peristalsis

TABLE I

THE EFFECT OF SP ON THE AMOUNT OF 5-HT IN THE ILEUM, STOMACH AND SPLEEN OF THE RAT (BELESLIN AND VARAGIĆ, 1959b)

Number of experiments	Ileum (μg 5-HT/g)		P	Stomach (μg 5-HT/g)		P	Spleen (μg 5-HT/g)		P
	Controls	SP, 100 U./kg		Controls	SP, 100 U./kg		Controls	SP, 100 U./kg	
1	1130	1574		1126	2086		1871	2307	
2	1303	2742		1598	1860		1000	1498	
3	1215	1670		1056	1452		714	1918	
4	754	1336		1553	1558		857	1428	
5	720	2218		988	1684		1124	615	
6	826	1578		891	2072		1076	1198	
7	340	1800		1919	2374		1810	1995	
8	902	1460		711	1743		1082	1018	
9	1480	1572		951	2198		972	1047	
10				1694	2308		705	1869	
Mean	964	1773		1249	1933		1121	1489	
			0.01			0.01			0.1
st. error	118.1	146.1		119.9	96.2		128.4	191.8	

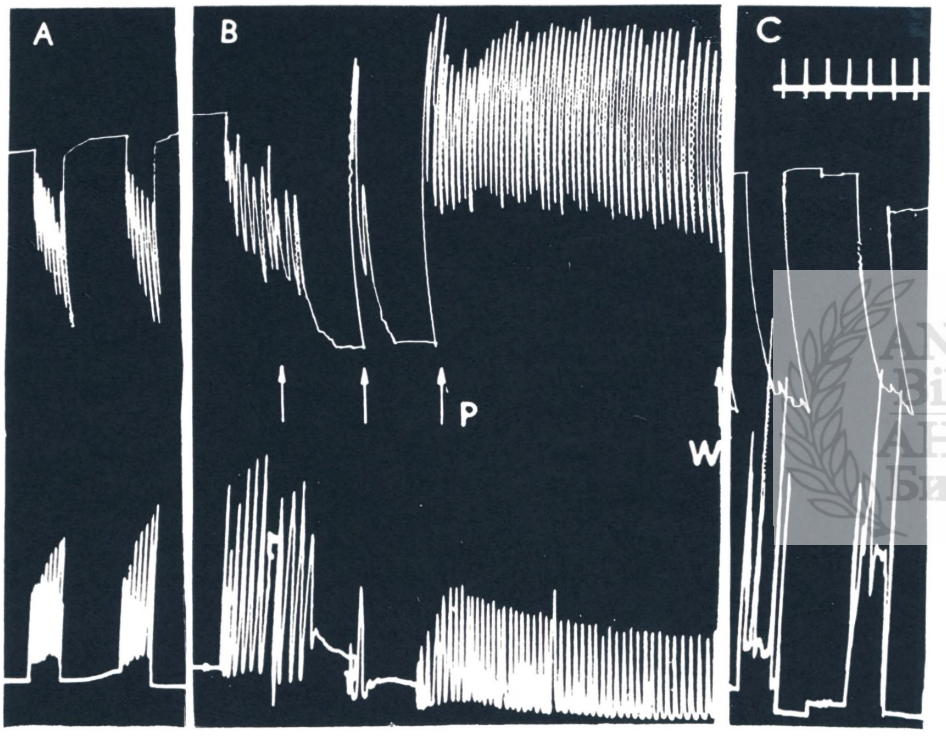


FIG. 1

The effect of SP on peristalsis in fatigued guinea pig ileum. **A**, normal peristaltic response to a raised intraluminal pressure. **B**, at first arrow, 0.2 ml Tyrode intraluminally (i. l.); at. **P**, 10 U. SP i. l.; at **W**, bath and intestine washed out. **C**, 110 min. after washing. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. Time, 1 min. (Beleslin and Varagić, 1958).

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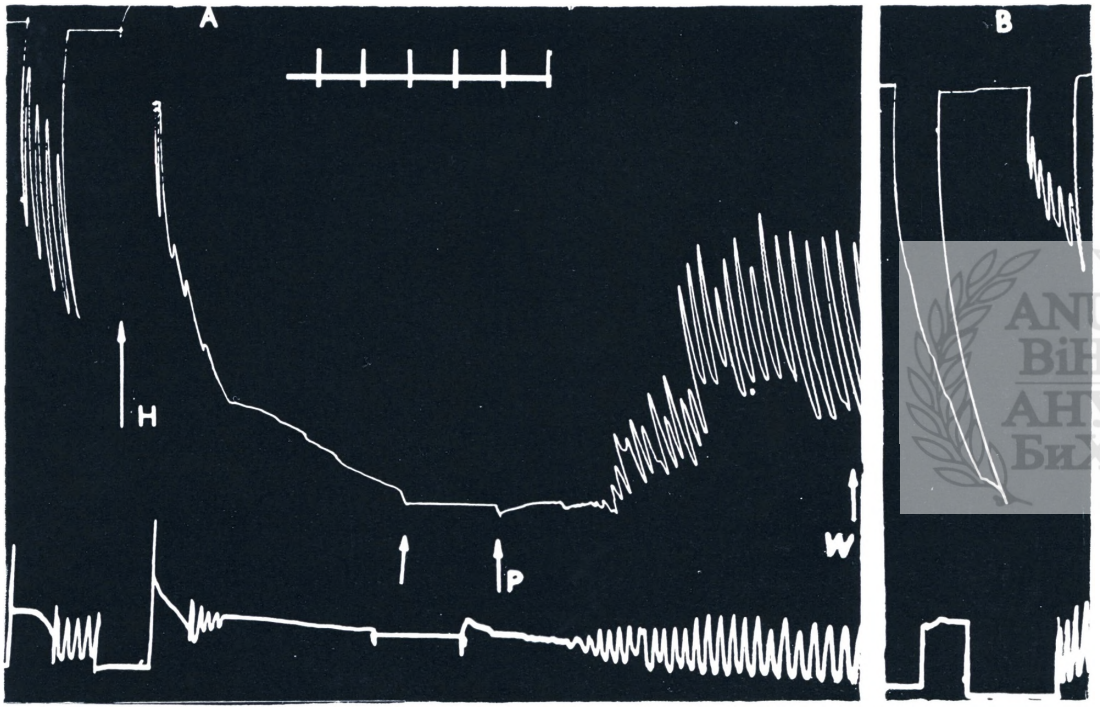


FIG. 2

Restoration of peristalsis by SP after inhibition by 5-HT. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. **A**, at **H**, 1 mg 5-HT added to bath; at arrow, 0.2 ml Tyrode intraluminally (i. l.); at **P**, 10 U. SP i. l.; **W**, bath and intestine washed out. **B**, peristalsis 25 and 140 min. after washing out. Time, 1 min. (Beleslin and Varagić, 1958).

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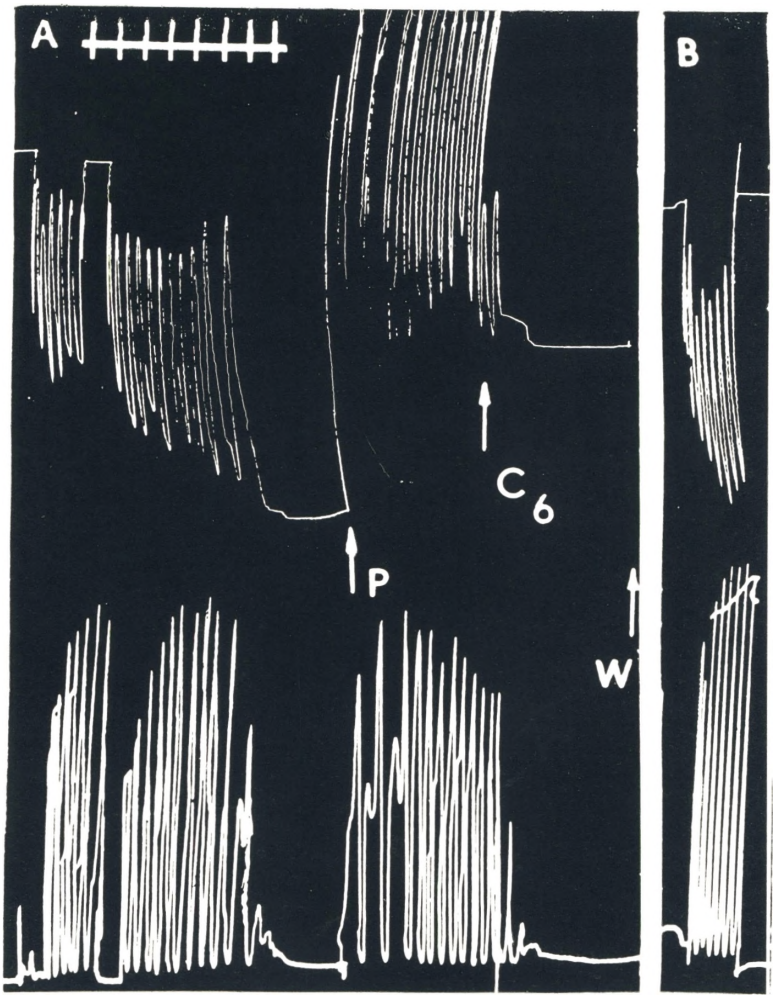


FIG. 3

The effect of hexamethonium (C_6) on peristalsis produced by SP. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. **A**, at P 10 U. SP i. l.; at C_6, 0.4 mg hexamethonium added to bath; at **W**, bath and intestine washed out. **B**, 45 min. after washing. Time, 1 min. (Beleslin and Varagić, 1958b).

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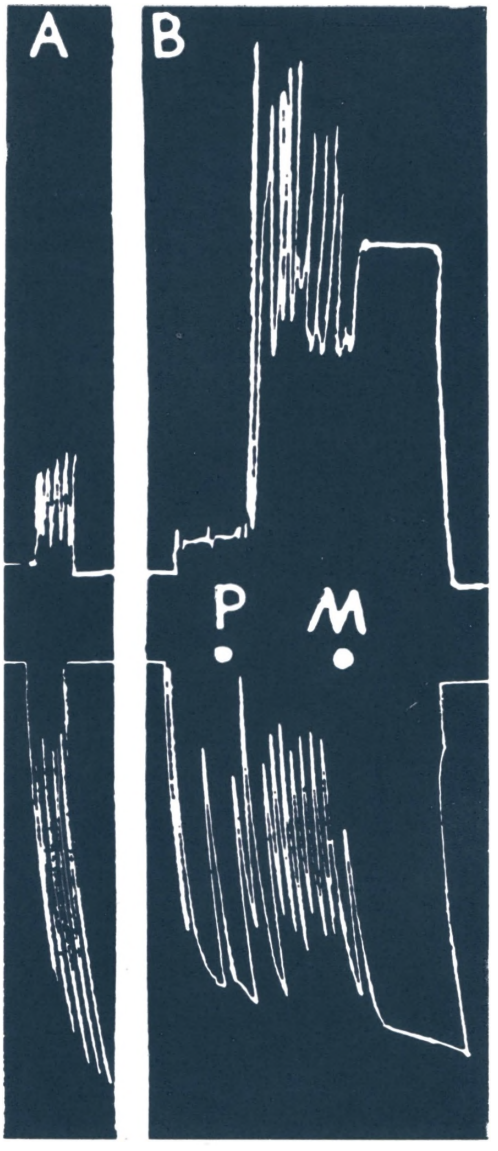


FIG. 4

The effect of morphine on peristalsis produced by SP. Upper record: contractions of longitudinal muscle. Lower record: peristalsis. **A**, control record. **B**, at **P** 20 U. SP i. l.; at **M**, 1 µg morphine i. l. (Medaković and Radmanović, 1959).

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FIG. 5

The effect of SP on peristaltic block produced by morphine. Upper record: contractions of longitudinal muscle. Lower record: peristalsis. **A**, control record. **B**, at **P** 20 U. SP i. l. Between **A** and **B** 1 μg morphine i. l. (Medaković and Radmanović, 1959).

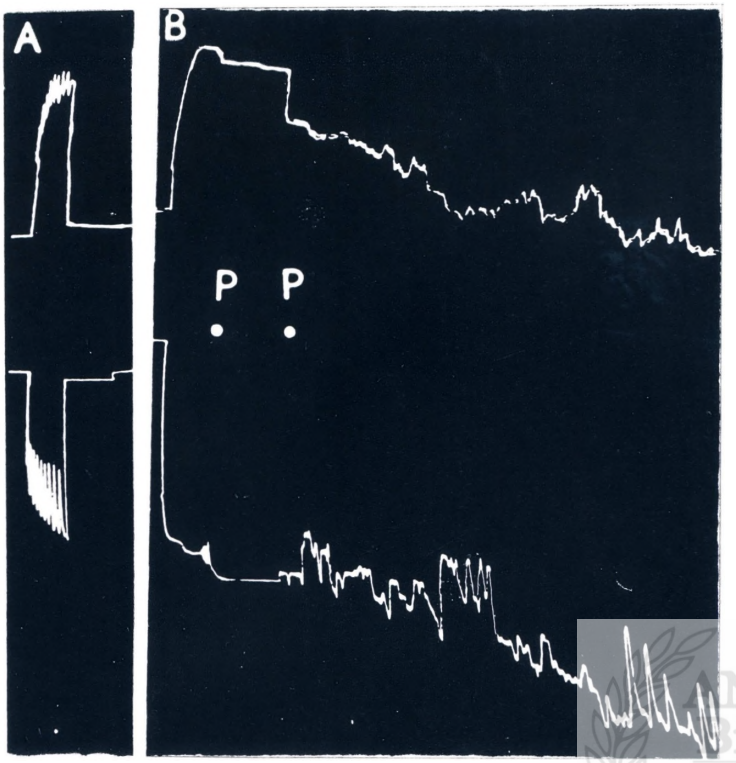
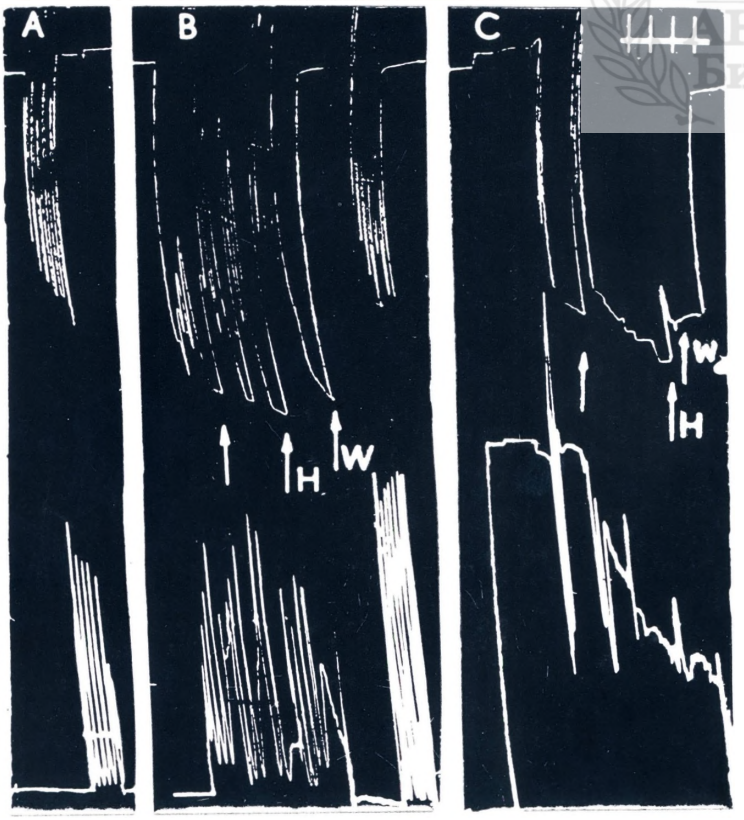


FIG. 6

The effect of 5-HT on the peristaltic block produced by SP. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. **A**, control record. Between **A** and **B**, 30 units and between **B** and **C**, 50 units of SP added to the bath. At the arrow, 0.2 ml Tyrode i. l.; at **H**, 2 μg 5-HT i. l.; at **W**, bath and intestine washed out. Time, 1 min. (Beleslin and Varagić, 1959a).



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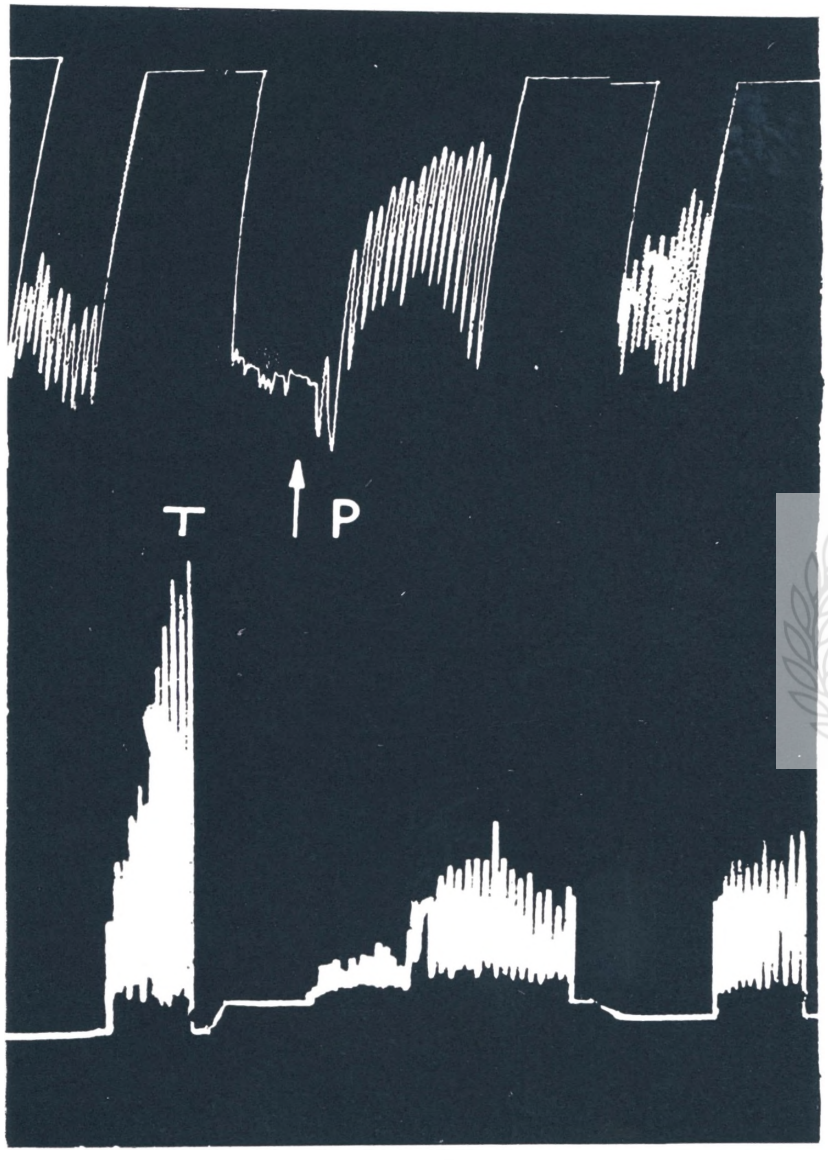


FIG. 7

The effect of SP on the peristaltic block produced by d-TC. Upper record: peristalsis. Lower record: contractions of longitudinal muscle. At **T**, 0,25 mg d-TC added to the bath. At **P**, 4 U. SP added to the bath. Bath volume: 15 ml.

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when acting on the mucous membrane of the intestine (Bülbring and Lin, 1957) and it can depress or abolish the peristaltic reflex when acting on the outside of the isolated intestine [Kosterlitz and Robinson (1957), Ginzel (1957)]. Considering this similarity of action of SP and 5-HT on the peristaltic reflex, experiments were carried out in order to see, whether SP can alter the 5-HT content of the gut. It was found that SP increased the amount of 5-HT both in the isolated guinea pig ileum and rat ileum (Table I). The same was true for the rat stomach. On the contrary, no change in 5-HT content of the rat spleen was observed after treatment of the animal by 100 U./kg intraperitoneally for three successive days. It is remarkable that SP increased the amount of 5-HT only in organs containing chromaffine tissue (Beleslin and Varagić, 1959b).

Interaction with other biologically active substances. — It was found in the isolated guinea pig ileum that SP potentiated the responses to nicotine and ACh and depressed the effect of 5-HT. The responses to histamine were unaffected by SP (Beleslin and Varagić, 1960). It has been known that blocking the ganglia by hexamethonium slightly reduced the response to ACh in a number of experiments on the isolated guinea pig ileum (Feldberg, 1951). Therefore, at least in this preparation, ganglion stimulation contributed to a certain extent to the ACh contraction of the intestine. In accordance with this view it was found that the potentiating effect of SP on the response to ACh was abolished by hexamethonium (Fig. 8). SP potentiated also the response of the guinea pig ileum to nicotine (Fig. 9). It is generally accepted that both ACh and nicotine depolarize the ganglion cells, but our experiments do not allow any conclusion regarding the mode of action of SP on the process of depolarization.

Similarly to SP, 5-HT was found to increase the longitudinal muscle contractions in response to ACh while the ganglionic effect of nicotine was first facilitated and then blocked (Bülbring and Crema, 1958). SP in large doses was also found to depress the response to nicotine (Fig. 9). On the other hand SP depressed the response to 5-HT, but it is not sure whether muscular or nervous 5-HT receptors were affected (Gaddum and Picarelli, 1957). Feldberg (1951) has shown that there was no evidence that the histamine (H) contractions of the guinea pig ileum were, in part, the result of stimulation of nerve endings. Beleslin and Varagić (1960) have found that SP did not change the response of the guinea pig ileum to H. It is therefore possible that SP potentiated the responses of the guinea pig ileum only to those biologically active substances which act completely or partly through the nervous structures.

It has also been shown that the response of the longitudinal muscle of rabbit and guinea pig intestine to SP is partly depressed by lowering the temperature of the bath. It is known that the nervous structures are more thermo-sensitive than the muscle fibres and this has also been taken as an evidence for the neurotropic effect of SP (Pernow,

1960). The previous evidence presented by Pernow (1953) suggested that SP stimulates the muscle fibres directly. To these findings the peripheral neurotropic effects of SP are now added.

Radmanović (1961b, in press) has found that *d*-TC and gallamine inhibit the longitudinal muscle contractions of the isolated guinea pig ileum caused by SP. The doses of *d*-TC and gallamine producing 50 per cent inhibition (ED_{50}) of the effect of SP were 0.0018 μg/ml and 0.035 μg/ml respectively. A dose-effect relationship was found in the inhibitory action of *d*-TC toward the SP. On the other hand, succinylcholine was found to potentiate slightly the effect of SP.

SP and the superior cervical ganglion. — While investigating peripheral neurotropic effects of SP some evidence was obtained indicating that block of peristalsis might be, at least partly, produced by the action of SP on the intestinal ganglia. It was therefore decided to investigate this problem in detail. It was found that the intraarterial injections of SP in doses from 10 to 30 units potentiated the response of the nictitating membrane of the cat to submaximal stimulation of the preganglionic sympathetic nerve (Fig. 10), while higher doses (30—100 U.) usually depressed the response (Fig. 11). The stimulating action of ACh on the superior cervical ganglion (as judged by the response of the nictitating membrane) was also potentiated by SP. It was found that that the responses of the nictitating membrane to adrenaline, noradrenaline and tyramine were potentiated by SP as well (Beleslin, Radmanović and Varagić, 1960). All these results were obtained on the superior cervical ganglion »in situ«.

Beleslin and Radmanović (unpublished results) repeated these experiments on the isolated superior cervical ganglion and found more frequently a depressive action of SP. It is possible that the excitatory action of SP on the ganglion »in situ« might be connected with the presence of blood. Some interaction of SP and blood constituents might take place before it acts on the ganglion cells. Beleslin and Radmanović carried out their experiments with a highly purified sample of SP, while the previous experiments were done with unpurified batches of SP. It is possible that in the raw material containing SP some other active substance might be present.

Summary

The ability of SP to stimulate smooth muscle has been described as far back as 1931 (Euler and Gaddum), but its more detailed pharmacological study was carried out only in 1953 (Pernow). This substance is, however, a normal constituent of brain tissue, and was found to exert a stimulating activity in aferrent neurons. It was, therefore, decided to study in more detail the peripheric neurotropic effects of SP.

SP strongly stimulated peristaltic movements in the isolated guinea pig ileum (Beleslin and Varagić, 1958b). So it was possible, e. g., to attain the resuming of peristalsis by intraluminal injections into pieces

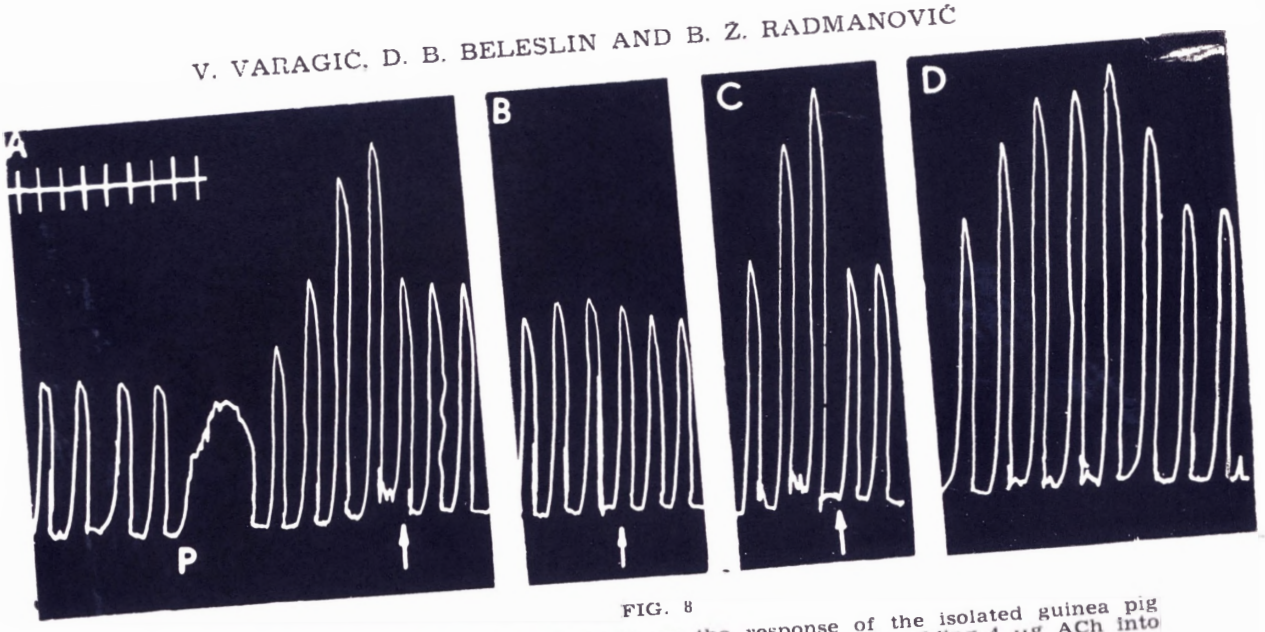


FIG. 8

The effect of SP and of hexamethonium on the response of the isolated guinea pig ileum to ACh. Contractions of the intestine were produced by adding 4 µg ACh into the bath every 4 min. At **P** in **A** and between **B** and **C** 3 units of SP added into the bath. At the arrows 5 µg hexamethonium added into the bath 2 min. before ACh. **B** was taken 40 min. after first addition of SP. **C** was taken 10 min. and **D** 30 min. after second addition of SP. Time, 1 min. (Beleslin and Varagić, 1960).

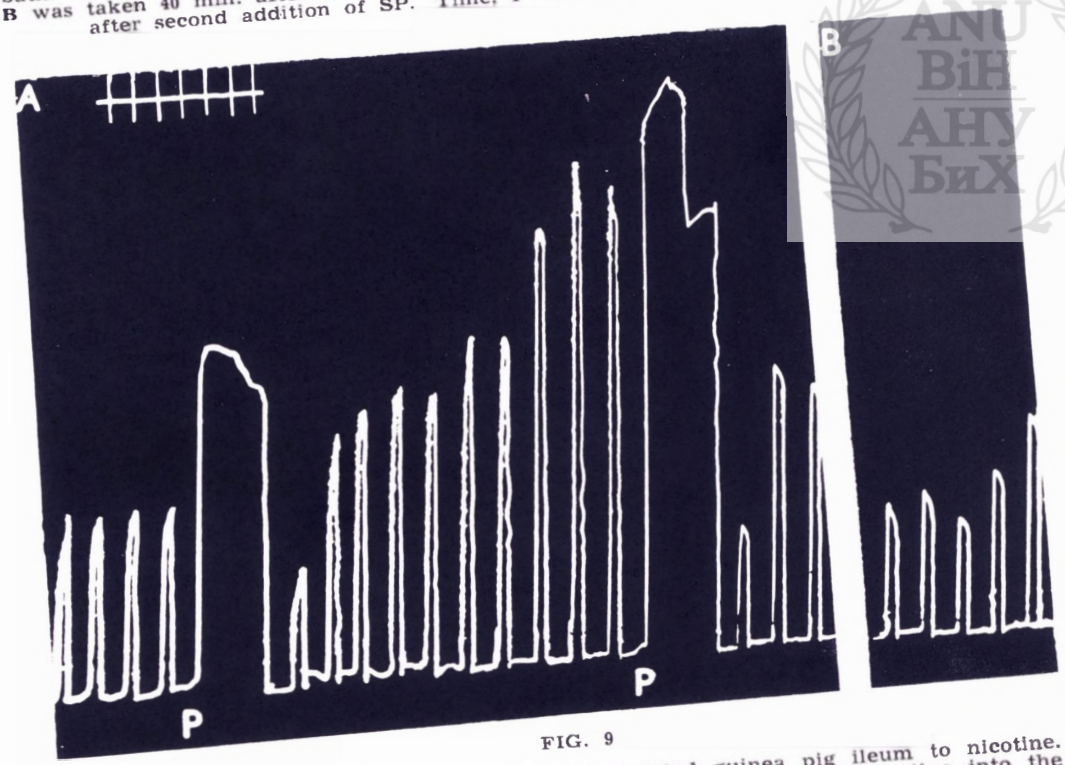


FIG. 9

The effect of SP on the response of the isolated guinea pig ileum to nicotine. Contractions of the intestine were produced by adding 1.5 µg nicotine into the bath every 10 min. At **P** 3 units and at P_1 30 units of SP into the bath. **B** was taken 30 min. after addition of 70 U. SP into the bath. Time, 1 min. (Beleslin and Varagić, 1960).

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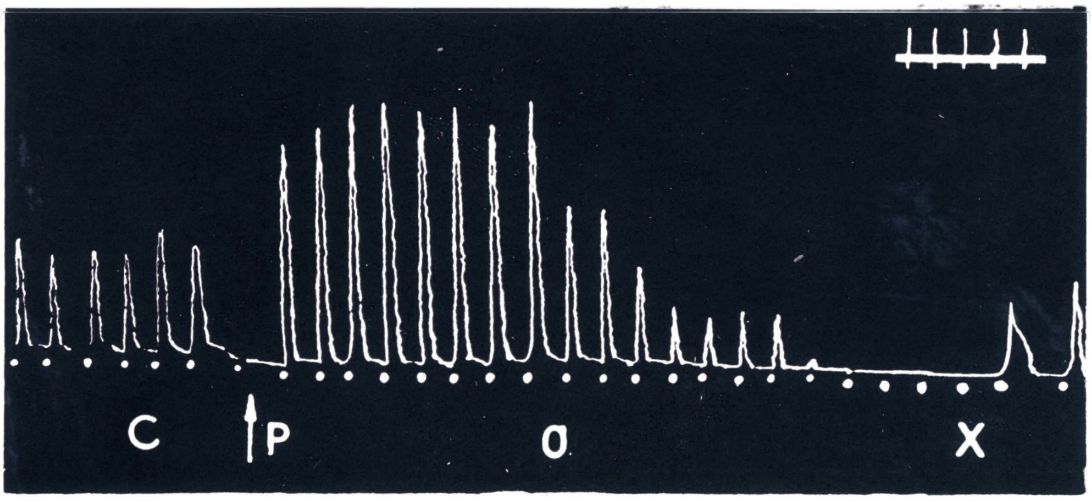


FIG. 10

The effect of SP on the superior cervical ganglion of the cat. Contractions of the nictitating membrane due to submaximal preganglionic stimulation of the cervical sympathetic nerve (5 pulses/sec., 0.8 msec., 7 mA, at dots) for 5 sec. every min. At **C** the external carotid artery was occluded. At **P**, 18 U. SP injected into the lingual artery. At **O** the external carotid artery was opened. At **X**, kymograph stopped for 15 min. Time, 1 min. (Beleslin, Radmanović and Varagić, 1960).

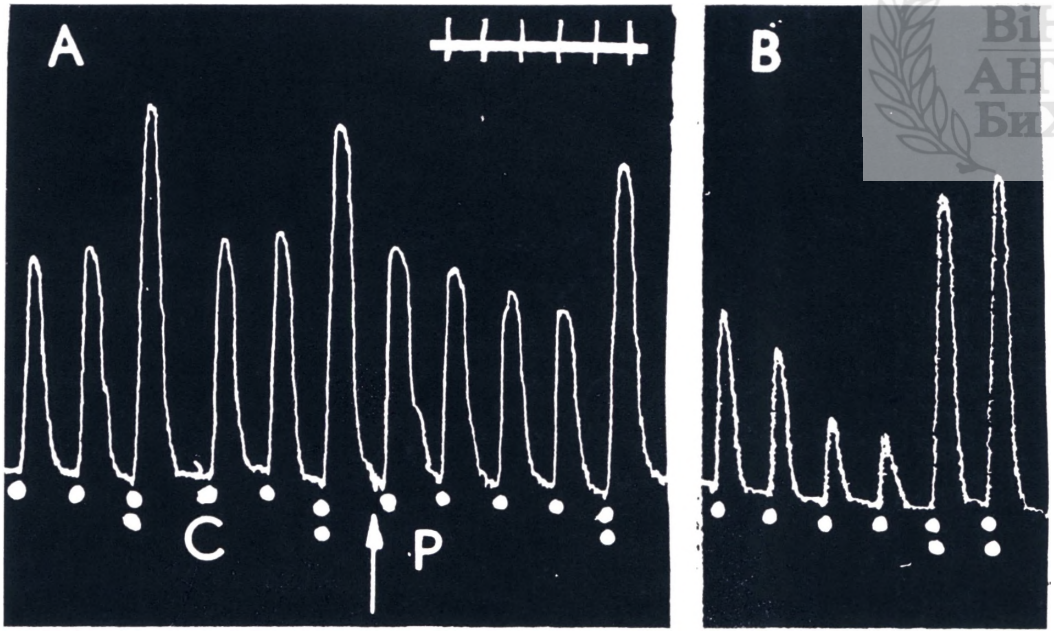


FIG. 11

Contractions of the nictitating membrane of the cat (at dots) due to submaximal preganglionic stimulation of the cervical sympathetic nerve (4 pulses/sec., 0.8 msec., 1.2 mA) for 10 sec. every 90 sec. At every two dots: submaximal postganglionic stimulation of the cervical sympathetic nerve (4 pulses/sec., 0.8 msec., 0.1 V) for 10 sec. every 90 sec. At **C** the external carotid artery was occluded. At **P** 100 U. SP injected into the lingual artery. **B** was taken 20 min. after injection of SP. Time, 1 min. (Beleslin, Radmanović and Varagić, 1960).

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of gut where peristaltic activity had been previously blocked by fatigue, action of 5-HT, or lowering of temperature. This effect of SP could not be suppressed by hexamethonium, hence it was concluded that its mechanism is a stimulation of afferent nerve elements in the peristaltic reflex arc.

The stimulating effect of SP on peristalsis was considerably weaker with outside treatment of the gut (Beleslin and Varagić, 1959a). In these experiments frequently even blocking of the peristaltic reflex was observed.

SP increases the 5-HT content in the isolated guinea pig ileum and in rat ileum. Intraperitoneal administration, in contrast, did not change the amount of 5-HT in the spleen (Beleslin and Varagić, 1959b). Strikingly, SP increases the 5-HT content in organs with chromaffine tissue only.

In the isolated gut SP potentiated the action of substances only, which partly or entirely act through nervous elements (e. g. ACh and nicotine), while it did not affect substances acting directly on smooth muscle (Beleslin and Varagić, 1960).

Intraarterial injections of SP potentiated the response of the nictitant membrane to submaximal stimulation of preganglionic fibres in the superior cervical ganglion of the cat in situ. Under the same conditions higher doses of SP depressed the response of the nictitant membrane (Beleslin, Radmanović and Varagić, 1960). In the isolated superior cervical ganglion of the cat Beleslin and Radmanović (This Symposium) found that SP exerts almost exclusively a depressive action.

NEKI PERIFERNI NEUROTROPNI EFEKTI SUPSTANCIJE P

Sposobnost supstancije P (SP) da stimuliše glatku muskulaturu opisana je još 1931. godine (Euler i Gaddum), a farmakološki je detaljnije proučavana tek 1953 (Pernow). Ova supstancija je, medutim, i normalni sastojak moždanog tkiva, a nadeno je da ima i nadražajno delovanje na aferentna nervna vlakna. Iz tog razloga je bilo odlučeno da se detaljnije prouče periferni neurotropni efekti SP.

Nadeno je da SP snažno stimulira peristaltiku na zamorčevom izolovanom ileumu (Beleslin i Varagić, 1958b). Tako je intraluminalnim ubrizgavanjem SP bilo mogućno izazvati peristaltiku ako je ona bila blokirana za morom, 5-HT-om ili hladenjem. Ovaj efekat je bilo mogućno isključiti heksametonijumom. Zbog toga je zaključeno da SP draži aferentne nervne elemente peristaltičkog refleksnog luka.

Znatno slabiji nadražajni efekat na peristaltiku imala je SP kad je aplikovana spolja na crevo (Beleslin i Varagić, 1959a). Naprotiv, posle spoljašnje aplikacije SP, češće je dolazilo do bloka peristaltičkog refleksa.

SP povećava količinu 5-HT u zamorčevom izolovanom ileumu i u ileumu pacova. Suprotno ovome, SP aplikovana intraperitonealno ne menja količinu 5-HT u slezini (Beleslin i Varagić, 1959b). Pada u oči da SP povećava količinu 5-HT samo u organima koji sadrže hromafino tkivo.

Na izolovanom crevu SP potencira delovanje samo onih supstancija koje deluju delimično ili potpuno putem nervnih elemenata (na primer, ACh i nikotin), dok ne menja efekte materija koje deluju direktno na glatku muskulaturu (Beleslin i Varagić, 1960).

Na gornjem cervikalnom ganglionu mačke in situ nadeno je da intraarterijsko ubrizgavanje SP potencira reakciju membrane niktitans na submaksimalno draženje preganglijskih simpatičkih vlakana. Veće doze SP u istim uslovima deluju depresivno i smanjuju reakciju membrane niktitans na preganglijsko draženje (Beleslin, Radmanović i Varagić, 1960). Beleslin i Radmanović (v. sledeći članak) našli su skoro isključivo depresivno delovanje SP na izolovanu gangliju mačke.

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DISCUSSION

LEMBECK: The effect of SP on the intestinal HT content raises the question whether it means an increased synthesis or a decreased release of HT. It would be interesting to know the influence on the intestinal content.