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The influence of substance P on the response of guinea-pig isolated ileum to periarterial nerve stimulation

Leposava Grbović & B.Ž. Radmanović

Department of Pharmacology, Medical faculty, 11000 Belgrade, Yugoslavia POB 662

1 The effects of substance P (SP) on the responses of the guinea-pig isolated ileum to periarterial nerve stimulation were studied. Electrical stimulation (2–50 Hz) of mesenteric periarterial nerves resulted in contraction of preparations pretreated with guanethidine. The responses were abolished by atropine and morphine, but unaffected by hexamethonium.

2 SP in a high concentration (0.65 μmol) inhibited reversibly, by about 40%, the responses of the guinea-pig isolated ileum to periarterial nerve stimulation.

3 SP in a very low concentration (0.18 pmol) potentiated (by about 20%) the responses of the guinea-pig isolated ileum to periarterial nerve stimulation.

4 In the presence of low concentrations (0.06–0.32 pmol) of SP, morphine (2.65 μmol) produced less inhibition of the responses to periarterial nerve stimulation, and recovery from morphine inhibition of contraction was accelerated.

5 These results indicate that SP may act as a modulator on prejunctional acetylcholine release in the guinea-pig ileum.

Introduction

Substance P (SP) was identified first by its stimulant action on intestinal smooth muscle and by its vasodilator effect (Euler & Gaddum, 1931). Although both intestinal contraction and vasodilatation can be explained by a direct effect on smooth muscle cells, an action on autonomic neuroeffector transmission in these tissues cannot be excluded. Early experiments showed that SP acts, not only directly on intestinal smooth muscle but also by stimulating ganglion cells and by exciting afferent neurones (Beleslin, Radmanović & Varagić, 1961). These findings were confirmed and extended using synthetic SP by Euler & Hedqvist (1974) who found that SP increased the twitch response of the guinea-pig isolated vas deferens to transmural stimulation by a prejunctional influence on postganglionic nerves; the same result has been found for the ileum (Hedqvist & Euler, 1977). Moreover, immunohistochemical investigation has shown that SP is present in several nerve tissues including the myenteric plexus (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygards & Pernow, 1977), and may play a transmitter role in primary sensory nerves (Lembeck, 1953; Otsuka & Konishi, 1977).

Recently Szolcsányi & Barthó (1978) proposed that nerve fibres in a periarterial plexus (specifically sensitive to capsaicin), presumably originating from sensory neurones, excited cholinergic neurones of the myenteric plexus. The hypothesis presumes that, in the gut, capsaicin-sensitive sensory nerve endings release a transmitter when electrically stimulated. This transmitter at the myenteric plexus excites cholinergic neurones or their terminals through nonnicotinic receptors. The present paper describes experiments designed to determine a possible role for SP in contractions of the guinea-pig ileum during periarterial nerve stimulation.

Methods

Adult guinea-pigs of either sex were killed by a blow to the head and bled. The abdomen was opened and a 3 cm segment of ileum with attached mesenteric vessels and nerves was excised and placed in an organ bath containing 20 ml of Krebs solution at 37°C (Szolcsanyi & Barthó, 1978), bubbled with 5% CO_2 in O_2. The mesenteric vessels and nerves were pulled through a pair of ring platinum electrodes.

A square wave stimulator was used. Stimulation parameters were: 10 V, 0.4 ms, 2–50 Hz. The total duration of the stimulation cycle was 5 min; stimulation was carried out for 15 s at 1 min intervals. A 15 min resting period, during which the preparation was washed three times, was allowed after each cycle (Szolcsanyi & Bartho, 1978). Isometric responses of

the ileum were measured with a force displacement transducer connected to a recording microdynamometer 7050 (Ugo Basile).

Drugs were added to the organ bath in 100–200 μl of distilled water except for SP which was dissolved in 0.01 M acetic acid. The following drugs were used: acetylcholine chloride, atropine sulphate, hexamethonium bromide, guanethidine sulphate (CIBA), morphine hydrochloride and substance P (Sigma). The molar concentrations of drugs given in the text refer to the final bath concentrations.

Results

In the presence of guanethidine (10 μmol for 30 min, then 2 μmol for the rest of the experiment) to eliminate the effects of sympathetic nerve stimulation, periarterial nerve stimulation invariably produced contraction, the height of which depended on the stimulus frequency. At frequencies up to 10–20 Hz there was an increase in the height of the contractions with increase in frequency. Maximal contractions obtained at 20 Hz reached a level of 85–90% of the maximal effect evoked by 0.55 μmol of acetylcholine. The contractions were reproducible for 3 h or longer if the bath was washed out and a 5–15 min resting period interposed between each (5 min) cycle of stimulation. We also confirmed the finding of Szolcsanyi & Bartho (1978) that atropine (0.14 μmol) and morphine (2.65 μmol) abolished the effect of periarterial nerve stimulation, whereas hexamethonium (0.27 mmol) did not.

High doses of SP desensitize SP receptors on the smooth muscle of the guinea-pig ileum (Gaddum, 1953). To desensitize the receptors, SP was added to the organ bath in a concentration of 0.65 μmol. After a 3–5 min contact period when the response to SP had disappeared, the contractions produced by periarterial nerve stimulation were inhibited by about 40%. Inhibition occurred at all frequencies between 5 and 50 Hz, the differencies being statistically significant (Figure 1). The blockade was reversed by repeated washing over a period of 20 min.

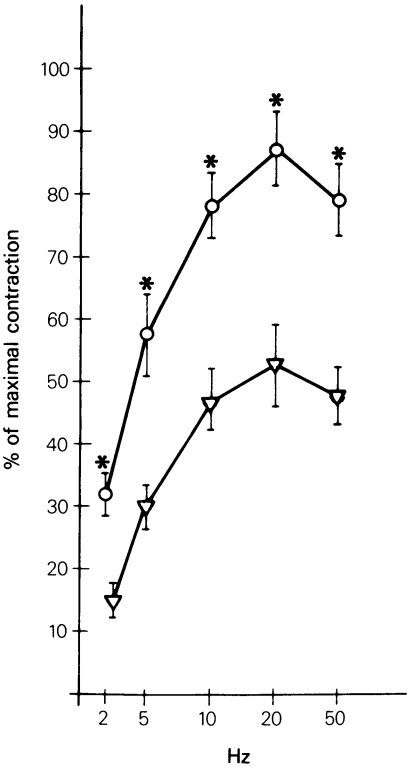

Figure 1 The effect of a high concentration of substance P (SP, 0.65 μmol) on the responses of the guinea-pig ileum to periarterial nerve stimulation. Preparations were pretreated with guanethidine (10 μmol). (○) Control responses to periarterial nerve stimulation; (▽) responses of the same segments after incubation with a high SP concentration (0.65 μmol). The height of the contractions is expressed as a percentage of the maximal contraction evoked by 0.55 μmol acetylcholine. Each point represents the mean of 12 experiments; vertical lines are s.e.mean. Inhibition caused by the high concentration of SP was significant at all frequencies of stimulation ($P<0.005$, Student's t test).

In the second series of experiments the effects of small concentrations of SP (0.06–0.32 pmol) on the contractions produced by periarterial nerve stimulation were studied. Small concentrations of SP produced either a slight increase in the tone of the longitudinal smooth muscle, or were ineffective. In the presence of these concentrations of SP, the contractions produced by periarterial nerve stimulation were potentiated by about 20%. Nerve stimulation began 30 s after the addition of SP to the organ bath. The potentiation by SP was statistically significant at 5, 20 and 50 Hz (Figure 2).

Morphine inhibits the release of acetylcholine from cholinergic nerve endings in the longitudinal muscle of the guinea-pig isolated ileum (Paton, 1957). In the presence of morphine (2.65 μmol) contractions to periarterial nerve stimulation were almost completely abolished (up to 85%; Figure 3). After washing out of morphine, 30 min was required for the recovery of contractions to periarterial nerve stimulation. In the presence of subthreshold concentrations of SP which potentiated the contractions to periarterial nerve stimulation, morphine produced significantly less inhibition of contractions (Figure 3). Furthermore, small concentrations of SP reduced the duration of morphine inhibition. Complete recovery

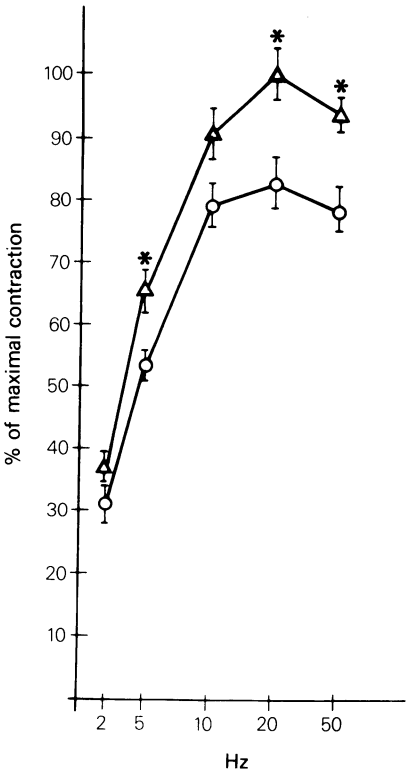

Figure 2 The effect of a low concentration of substance P (SP 0.2 pmol) on the responses of the guinea-pig ileum to periarterial nerve stimulation. All preparations were pretreated with guanethidine 10 μmol. (○) Control response; (△) responses of the same segments in the presence of the low concentration of SP. The height of the contractions is expressed as a percentage of the maximal contraction evoked by 0.55 μmol acetylcholine. Each point represents the mean of 12 experiments; s.e.mean shown by vertical lines. Significant potentiation of the stimulation indicated by asterisk ($P<0.05$, Student's t test) occurred at 5, 20 and 50 Hz.

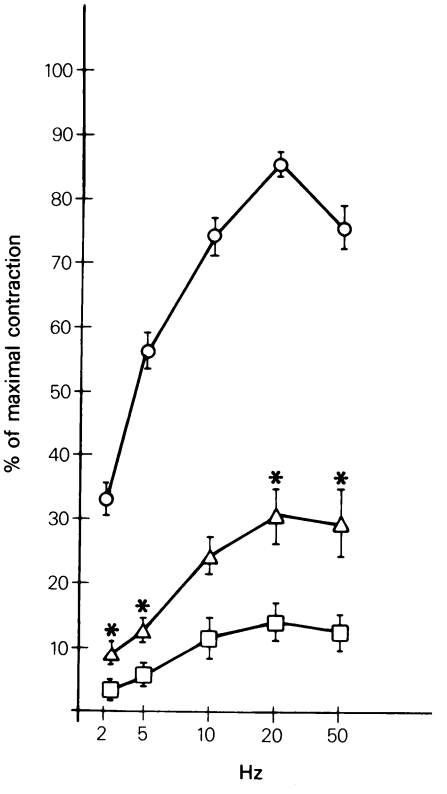

Figure 3 The effect of morphine (2.65 μmol) alone and in the presence of a low concentration of substance P (SP, 0.2 pmol) on the responses of the guinea-pig ileum to periarterial nerve stimulation. All segments were pretreated with guanethidine 10 μmol. (○) Control responses to periarterial nerve stimulation; (□) responses after incubation with morphine; (△) responses after incubation with morphine and in the presence of SP (0.2 pmol). The height of contractions is expressed as a percentage of maximal contraction evoked by 0.55 μmol acetylcholine. Each point represents the mean of 12 experiments; s.e.mean shown by vertical lines. Significant potentiation at a given stimulation frequency is indicated by asterisk ($P<0.05$, Student's t test).

was achieved after 20 min, and that difference was significant (Figure 4).

Discussion

Electrical stimulation (2–50 Hz) of the mesenteric nerves of the guinea-pig isolated ileum, pretreated with guanethidine, resulted in contraction of the ileum. The findings of Szolcsanyi & Bartho (1978) that contractions were abolished by atropine and morphine, but not by hexamethonium were confirmed. These authors found that after incubation with physostigmine, the responses to nerve stimulation were potentiated but unaffected by desensitization of the gut to 5-hydroxytryptamine. Capsaicin, which blocks sensory nerves, inhibited or abolished irreversibly the contractions elicited by stimulation of mesenteric periarterial nerves. On the basis of these results it has been proposed that sensory nerve fibres sensitive to capsaicin, excite cholinergic neurones of the myenteric plexus through the release of an unknown transmitter (Szolcsanyi & Bartho, 1978). We do not know whether the nerves involved in the present work are 'capsaicin-sensitive'. The present results show only that periarterial mesenteric nerves

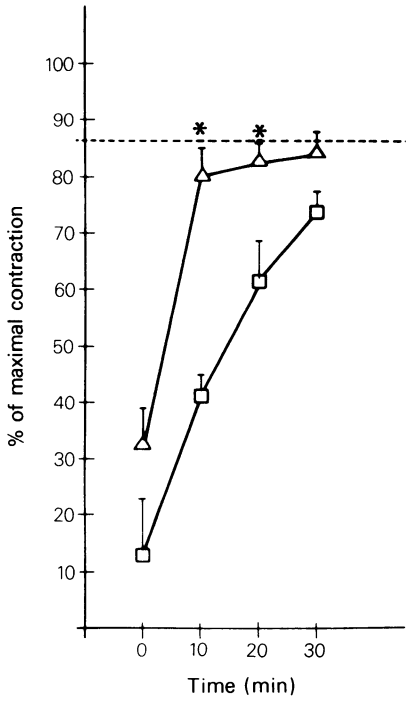

Figure 4 Recovery time after morphine inhibition of the responses of the guinea-pig ileum to periarterial nerve stimulation, in the presence of a low concentration of substance P (SP). Abscissa scale: time in min. Ordinate scale: percentage of the maximal contraction evoked by 0.55 μmol acetycholine. (□) Responses of the ileum in the presence of morphine alone; (△) responses of the ileum in the presence of morphine and low concentration of SP. Each point represents the mean responses evoked by 20 Hz stimulation in 12 experiments; s.e.mean shown by vertical lines. Dotted horizontal line shows control response of the ileum to 20 Hz stimulation. Significant difference is indicated by asterisk ($P < 0.05$, Student's t test).

are, to some extent, sensitive to SP since in the presence of a desensitizing concentration of SP, the contractions to periarterial nerve stimulation were inhibited. Holtzer, Lembeck & Donnerer (1980) found that tachyphylaxis to SP appeared to be rather specific for SP. It therefore seems likely that periarterial nerve fibres with dual (afferent and efferent) functions may contain some SP-sensitive fibres.

The present finding that extremely low concentrations of SP clearly potentiated the contractions to periarterial nerve stimulation, together with the finding of Hedqvist & Euler (1977) that small concentrations of SP potentiated the response to field stimulation of the ileum, while leaving contractions induced by acetylcholine unchanged, indicates a prejunctional stimulant effect of SP on acetylcholine release. The stimulant effect of very low concentrations of SP may be explained, therefore, by its excitatory effect on cholinergic nerve terminals which innervate the muscle layer of the intestine, and/or by the excitatory effect on the neurones in the myenteric plexus. This explanation is supported by the present finding that small concentrations of SP reversed, to some extent, the inhibitory effect of morphine on the responses to periarterial nerve stimulation. Morphine inhibits the release of acetylcholine from cholinergic nerve endings in the gut (Paton, 1957; Schaumann, 1957). Interestingly, small concentrations of SP potentiated to the same extent (by about 20%) the contractions evoked by periarterial nerve stimulation, and those contractions which had been previously inhibited by morphine. In addition, small concentrations of SP reduced the duration of morphine inhibition of contractions.

In conclusion, SP in desensitizing concentrations (0.65 μmol) inhibited the contractions of the guinea-pig ileum elicited by periarterial nerve stimulation. On the other hand, very low concentrations of SP (0.06–0.32 pmol) potentiated the contractions and were able to counteract, to some extent, the inhibitory effect of morphine and to reduce the duration of morphine inhibition of contractions. In addition to its direct excitatory effect on the smooth muscle of the gut, SP may act as a modulator of transmission on prejunctional acetylcholine release.

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