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THE EFFECTS OF SUBSTANCE P ON SMOOTH MUSCLE CELLS AND ON NEURO-EFFECTOR TRANSMISSION IN THE GUINEA-PIG ILEUM

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1 The effects of substance P (SP) on the membrane and contractile properties of the smooth muscle cell, or on neuro-effector transmission in the guinea-pig ileum were observed by means of microelectrodes, double sucrose gap and tension recording.

2 SP (10^{-13}–10^{-10} M) induced a phasic contraction of longitudinal muscle strips, but did not change the muscle tone of circular muscle strips, in concentrations up to 10^{-8} M.

3 SP (10^{-10}–10^{-8} M) evoked three different membrane responses in longitudinal muscle cells: (i) bursts of spike discharges with no significant change in the membrane potential and input membrane resistance; (ii) bursts of spike discharges with a small but clear depolarization of the membrane and increase in the input membrane resistance; (iii) slow waves with no change in the membrane potential.

4 In the circular muscle cells, low concentrations of SP ($< 10^{-8}$ M) did not affect the membrane potential or the spikes, but SP (10^{-7} M) increased the spike discharges with no significant change in the membrane potential.

5 SP (10^{-10} M) reduced the threshold depolarization required for the generation of action potentials with no change in membrane potential of the longitudinal muscle cells.

6 Pretreatment with atropine (5×10^{-6} M), tetrodotoxin (TTX 10^{-6} M) or baclofen (4.7×10^{-6} M) had no effect on the excitatory actions of SP on the smooth muscle cells of longitudinal and circular muscle strips.

7 Excitatory actions of SP on the membrane potential or spike activities of longitudinal muscle cells were preserved in NaCl but not in Ca-deficient solution.

8 SP (10^{-10}–10^{-9} M) enhanced the amplitude of the excitatory junction potentials (e.j.ps) evoked by electrical field stimulation in longitudinal muscle cells with no change in the membrane potential and input resistance. SP (10^{-10}–10^{-9} M), however, did not change the amplitude of inhibitory junction potentials (i.j.ps) recorded from the circular muscle cells.

9 These results indicate that SP in relatively low concentrations acts on both smooth muscle cells and on excitatory neuro-effector transmission in the longitudinal muscle; the main site of the action of SP is probably the muscle membrane.

Introduction

In the mammalian gut, substance P (SP) is localized in nerve fibres around ganglionic cell bodies or in the smooth muscle layers (Euler & Gaddum, 1931; Gaddum & Schild, 1935; Nilsson, Larsson, Håkanson, Brodin, Pernow & Sundler, 1975; Pearse & Polak, 1975; Nilsson & Brodin, 1977; Sundler, Håkanson, Larsson, Brodin & Nilsson, 1977; Franco, Costa & Furness, 1979), and may be released from the enteric plexus (Franco, *et al.*, 1979).

SP contracts smooth muscle in the gut by a direct action (Bass & Bennett, 1968). However, SP also possesses a prejunctional stimulating action on cholinergic transmission (Hedqvist & Euler 1975), which is resistant to atropine, tetrodotoxin, and baclofen (Bury & Mashford, 1977; Yau, 1978; Schrauwen & Houvenaghel, 1980).

In the present work, the effects of SP on the electrical and mechanical properties of smooth muscle and on neuro-effector transmission in the guinea-pig ileum have been investigated by means of microelectrodes, double sucrose gap and tension recording.

Methods

Guinea-pigs of either sex (300–350 g) were stunned and exsanguinated. About 15 cm of the terminal ileum was dissected out, and the lumen flushed with Krebs solution. Strips of longitudinal muscle together with the myenteric plexus (about 10 mm in length and 1.0–4.0 mm in width) and strips of circular mus-

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cle together with the myenteric plexus (about 10 mm in length and 1.0–4.0 mm in width) were prepared for recording by microelectrodes and the double sucrose gap method, according to the procedures described by Ito & Tajima (1980).

Modified Krebs solution of the following ionic concentration was used (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2, and pH was maintained at 7.2–7.3. Polypropylene baths were used instead of glass baths as synthetic SP, dissolved in oxygenated Krebs solution is rapidly inactivated by contact with glass (Franco *et al.*, 1979).

To prepare Na-deficient or NaCl-free solution, NaCl was replaced with choline chloride or sucrose, isotonically. Solutions containing excess $[K]_o$ were prepared by replacing NaCl with equivalent amounts of KCl up to 20.2 mM; Ca-deficient or K-deficient solutions were prepared by replacing $CaCl_2$ or KCl with NaCl, isotonically.

To record the membrane potential of single cells of the longitudinal or circular muscle layer, a conventional micro-electrode filled with 3 M KCl was inserted from either the serosal or mucosal side. The membrane potential was expressed as mean ± s.d. Field stimulation (50–100 μs in duration) was used to stimulate the nerve terminals. To measure the current-voltage relationship of muscle membranes, the partition stimulating method was used (Abe & Tomita, 1968). The chamber for microelectrode recording had a volume of 2 ml and was superfused with Krebs solution at a rate of 6 ml/min.

The double sucrose gap, used to record membrane potential and tension development included the tissue chamber described by Ito & Tajima (1979). To produce excitatory and inhibitory junction potentials, field stimulation was applied by a pair of electrodes placed in the centre pool of the apparatus. The stimulating electrodes consisted of two silver wires (0.2 mm in diameter) separated by 2 mm, placed so that a current pulse would pass tranversely across the tissue. Both single and repetitive stimulation were applied, using a current pulse of 50–100 μs at approx. 10–20 V.

To investigate only the mechanical responses, the tissue was mounted in a 0.5 ml organ bath through which the test solution, at a temperature of 35°C, flowed continuously (0.2 ml/s). The preparation was placed vertically in the bath and each end tied with silk thread, one to a mechanotransducer (Nihon-Kohden Ltd. RCA 5334) and the other end to a hook at the bottom of the bath.

The bathing solution was kept at room temperature (23–27°C), as under these conditions the frequency of the spontaneous spike discharges was relatively low, and the effects of SP on the membrane potential could be readily observed.

The following drugs were used; atropine sulphate (Daiichi), tetrodotoxin (Sankyo), deca-substance P (Protein Research Foundation, Osaka, Japan) and baclofen (CIBA-Geigy).

Results

Effects of substance P on the mechanical properties of guinea-pig ileum

In concentrations up to 10^{-8} M, SP did not alter the resting tension of circular muscle strips of ileum. On the other hand, in the longitudinal muscle strips, a low concentration of SP (10^{-13} M) evoked a phasic contracture and enhanced the amplitude of the phasic contracture in a dose-related manner (Figure 1b). However, the contracture was transient and gradually disappeared, even in the presence of SP.

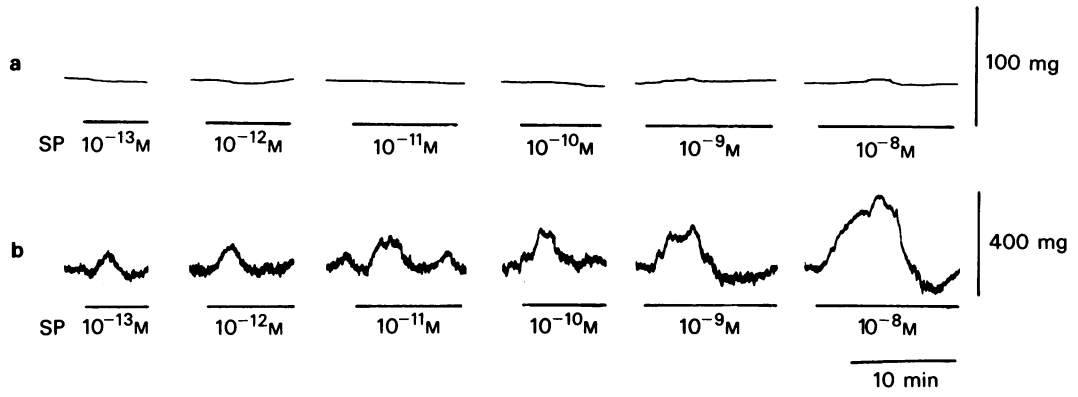

Figure 1 The effects of substance P (SP, 10^{-13}–10^{-8} M) on the mechanical properties of strips of circular (a) and longitudinal muscle (b) of guinea-pig ileum. Bars indicate the duration of application of SP.

Effects of substance P on the membrane potential of smooth muscle cells

SP (10^{-10}–10^{-7} M) did not affect membrane potential of the circular muscle cells. The membrane potential was -50.3 ± 1.3 mV ($n = 50$, ± s.d.) in Krebs solution, and -49.0 ± 0.9 mV ($n = 50$), in 10^{-7} M SP. Between 10^{-13} and 10^{-9} M, SP did not change the membrane potential of longitudinal muscle cells but at 10^{-8} or 10^{-7} M, SP significantly depolarized the membrane from -50.2 ± 1.1 mV ($n = 50$) to -47.4 ± 1.6 mV ($n = 30$) or to -45.7 ± 1.8 mV ($n = 30$), respectively ($P < 0.01$).

Effects of substance P on the electrical membrane properties of the smooth muscle cells

Under resting conditions, longitudinal muscle cells generated low frequency (0.05–0.1 Hz) spontaneous spike discharges but the circular muscle cells were electrically quiescent. In circular muscle cells, low concentrations of SP ($< 10^{-8}$ M) did not alter the membrane potential; increased concentrations of SP (10^{-7} M) generated spontaneous spike discharges with no significant change in the resting membrane potential between the periods of spiking activity (Figure 2). In longitudinal muscle cells, however, a low concentration of SP (10^{-10} M) increased the frequency of spontaneous spike discharges. Increased concentrations of SP (10^{-8} M) depolarized the membrane (by about 4 mV), and markedly increased the frequency of the spontaneous spike discharge (Figure 2c).

Some longitudinal muscle cells showed bursts of slow waves alternating with silent periods (Figure 3a) in the presence of atropine (1.4×10^{-6} M). The amplitude of the slow waves gradually increased and when these potential changes exceeded the threshold depolarization, the spike was generated. By application of SP (10^{-10}–10^{-8} M) and atropine (1.4×10^{-6} M), the frequency and the amplitude of slow waves were enhanced markedly, so that a pattern of regular slow wave activity developed (Figure 3c). The effects of SP on the slow waves were transient, and after the initial increase in the frequency and amplitude of slow waves, a gradual reduction in the excitatory actions occurred, even in the presence of SP (Figure 3b–d). However, the cumulative application of SP (10^{-8} M) and atropine (1.4×10^{-6} M) again increased the amplitude and frequency of the slow waves (Figure 3e). To investigate the gradual reduction in the frequency and amplitude of the slow waves in the presence of SP, the membrane was hyperpolarized or depolarized by extracellulary applied inward and outward current pulses for about 15 min after the application of SP (10^{-8} M) and atropine (1.4×10^{-6} M). Membrane depolarization (about 3 mV) induced by outward current pulses (25 s in duration) but not hyperpolarization (5–10 mV) evoked slow waves which were not observed in the absence of SP (Figure 3h). This means that the reduction in the frequency of slow waves induced by SP was not due to a block in the depolarization, but rather to a repolarization of the small membrane depolarization induced by 10^{-8} M SP. If

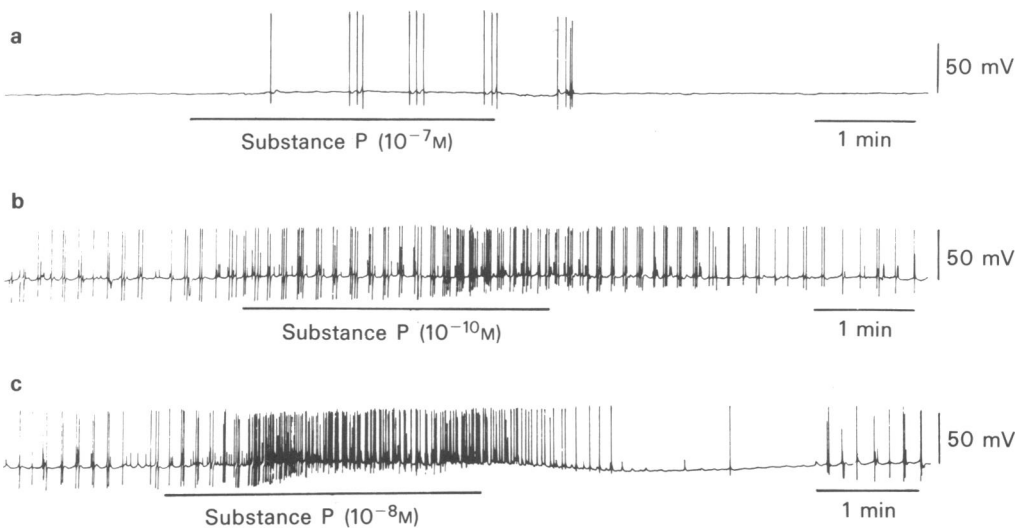

Figure 2 The effects of substance P (SP, 10^{-10}–10^{-7} M) on the membrane potential and spike activities of the circular (a) and longitudinal (b and c) muscle cells of guinea-pig ileum. Bars indicate the duration of application of SP.

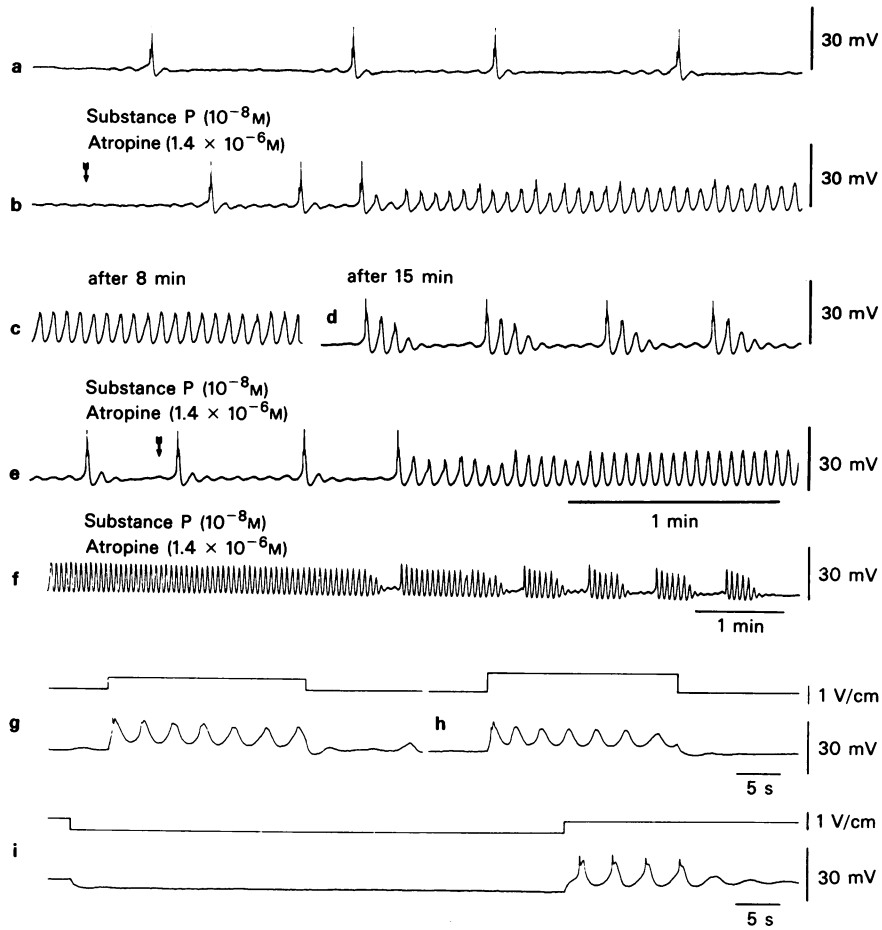

Figure 3 The effects of substance P (SP, 10^{-8} M) on the spontaneous slow waves observed in the longitudinal muscle cells of guinea-pig ileum in the presence of atropine (1.4×10^{-6} M). (a) Control; (b–d) effects of SP and atropine with time. During the continous application of SP and atropine, between arrows, the frequency of slow waves gradually reduced (d). Times between (b) and (c), (b) and (d) were about 8 and 15 min respectively. Arrows in (b) indicate the continuous application of SP and atropine up to (e). (e) and (f), Effects of cumulative application of newly prepared SP with atropine. Time between (d) and (f) was about 2 min; (e) and (f) were continuous recordings. (g)–(i) Effects of depolarizing (upward, g and h) or hyperpolarizing (i) current pulses on the slow waves in the presence of SP and atropine. Times between (f) and (g), (g) and (h), and (h) and (i) were 5, 1 and 1 min respectively. The microelectrode was inserted into the same cell throughout the experiment from (a) to (i).

so, then the reduction in the frequency and amplitude of SP is not due to a fade or to a desensitization of specific receptors for SP. These excitatory actions of SP were also observed in the presence of tetrodotoxin (10^{-6} M).

The effects of SP on the input membrane resistance of longitudinal muscle cells were also observed using microelectrodes. As shown in Figure 4, 10^{-8} M SP depolarized the membrane (4 mV), induced spontaneous spike disharges and increased the amplitude of the electrotonic potentials. Lower concentrations were ineffective. Current-voltage relationships were determined before and during application of SP. Throughout the experiment, the microelectrode was inserted into the cell at a distance of 0.1 mm from the stimulating electrode. Application of SP (10^{-8} M) depolarized the membrane (3 mV), and the observed current-voltage relationship was steeper during application of SP than that in the control solution (Figure 5). When the membrane was repolarized to the level of the control membrane potential by application of an inward d.c. current in the presence of SP, the slope of the current-voltage relationship in the presence of SP was also consistently larger than that

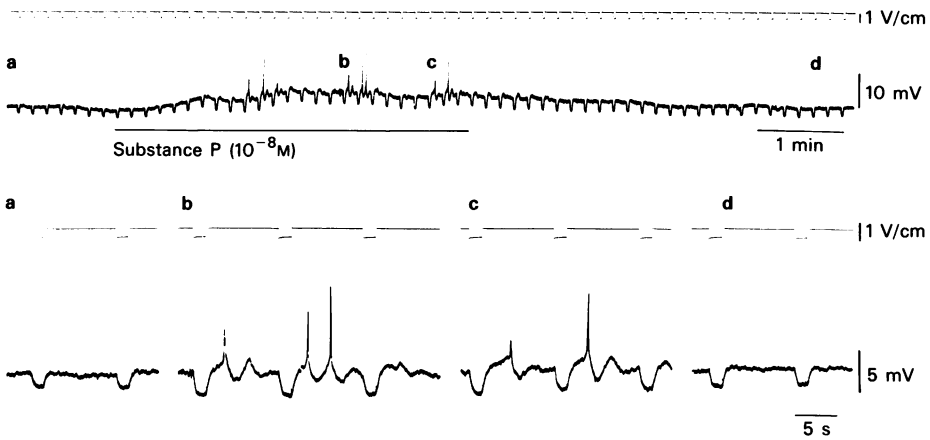

Figure 4 Effects of application for the period of time indicated by the bar of substance P (SP, 10^{-8} M) on the membrane potential and electrotonic potentials evoked by extracellulary applied inward current pulses (2 s in duration) in the longitudinal muscle of the guinea-pig ileum. Upper panel, continuous recording. Lower panel, faster and enlarged recordings of upper panel; (a) control; (b and c) during application of SP; (d) after washout of SP.

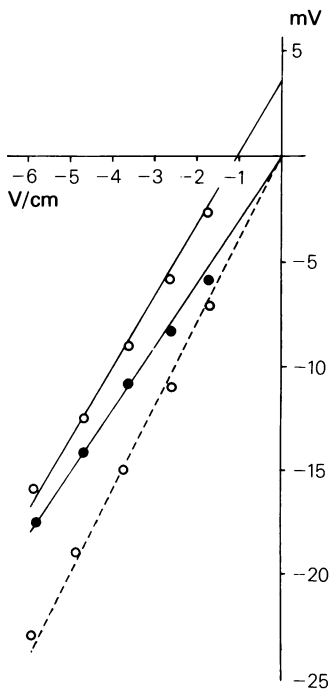

Figure 5 Current voltage relationships from a cell of the longitudinal muscle layer of the guinea-pig ileum in the presence and absence of substance P (SP, 10^{-8} M). Filled and open circles indicate the absence and presence of SP, respectively. The dotted line shows that the membrane depolarization induced by SP was eliminated by injecting an inward d.c. current.

observed in Krebs solution. The input membrane resistance was increased to 1.4 ± 0.1 times the control value ($n = 3$). The input membrane resistance (R_{in}) could be defined from the following equation (Bolton 1971):

$$R_{in} = \left(\frac{\text{Amplitude of electrotonic potential in test solution}}{\text{Amplitude of electrotonic potential in Krebs solution}} \right)^2,$$

under our experimental conditions where the distance between the stimulating and recording electrode was small compared to the space constant.

The double sucrose gap method was used to measure the effects of SP on the threshold depolarization required for gereration of the action potential. As shown in Figure 6c, 10^{-10} M SP in the presence of atropine (5×10^{-6} M) induced a train of repetitive spikes with no additional significant change in membrane potential. After the cessation of a train of repetitive discharges, outward current pulses of various intensities were applied in a stepwise manner to evoke electrotonic potentials with or without action potentials. The extent of membrane depolarization just below that at which the action potential was induced, was defined as the threshold membrane depolarization. The threshold depolarizations before and during application of 10^{-10} M SP were 15 ± 2 mV ($n = 5$) and 10 ± 2 mV ($n = 5$) indicating that SP reduced the threshold depolarization for action potentials (Figure 6a, b). The input membrane resistance measured from the amplitude of electrotonic potentials evoked by inward current pulses was not affected by a low concentration of SP (10^{-10} M).

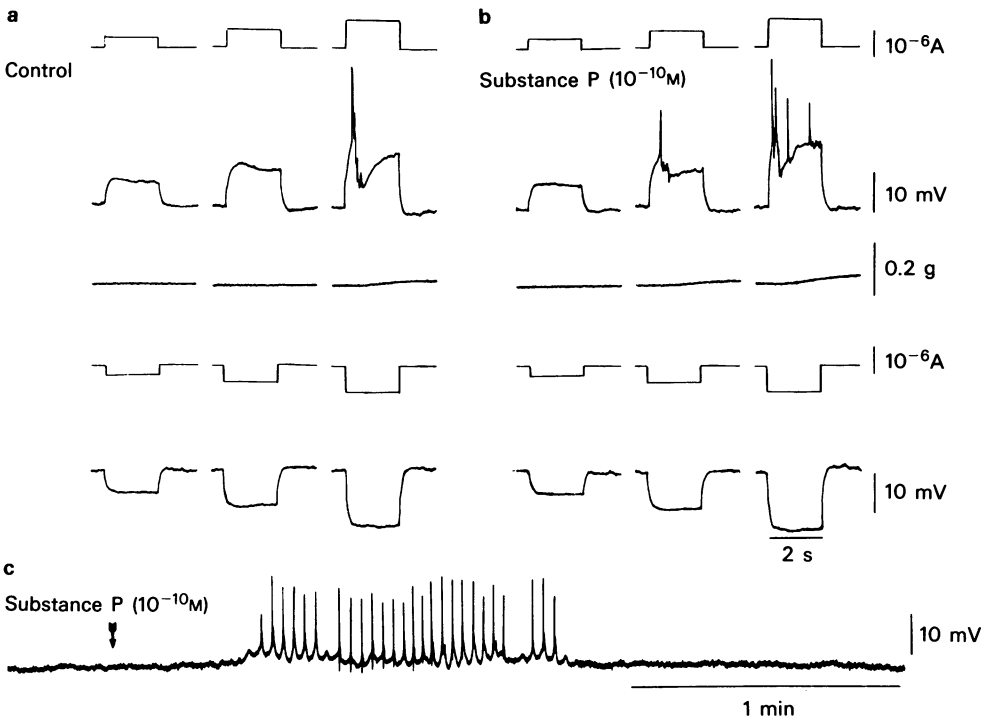

Figure 6 The effects of substance P (SP, 10^{-10} M) on the membrane potential, input membrane resistance and threshold depolarization required for the generation of action potentials measured by the double sucrose gap method. Outward (upward) and inward current pulses (2 s in duration) at three different intensities were applied before (a) and during application of SP (b). (c), Effects of SP on the membrane potential and spike activities of the longitudinal muscle cells.

Effects of substance P in various ionic environments

To study the ionic mechanisms involved in the action of SP, experiments were carried out in various ionic environments in longitudinal smooth muscle cells. When normal Krebs solution was replaced with sodium-free (choline) solution, the membrane potential was slightly depolarized from -50.1 ± 0.5 mV ($n = 25$) to -49.1 ± 1.0 mV ($n = 25$), and slow waves were generated, (20–30 mV in amplitude and at about 10–20 c/min) with or without the appearance of action potentials (Figure 7a). These potential changes were completely blocked by atropine (1.4×10^{-6} M). In the presence of atropine, however, a train of slow (1–5 s in duration) small potential changes (1–5 mV in amplitude) was generated spontaneously. During the repetitive discharges, the amplitude of these potential changes gradually increased and evoked action potentials (Figure 7b). SP (10^{-10}–10^{-7} M) enhanced the frequency and amplitude of these potential changes and triggered a more prolonged burst of repetitive spike discharges without affecting the resting membrane potential in the absence of discharges (Figure 7c, d). However, the effect of SP was transient, as observed in normal Krebs solution, i.e. even in the presence of SP, the frequency of repetitive spike discharge decreased gradually. In NaCl-free (sucrose) Krebs solution, slow small potential changes were also generated; SP (10^{-10}–10^{-8} M) increased their frequency and amplitude and triggered repetitive spike discharges (Figure 7e, f).

When the external concentration of K ion was increased to 15.4 mM, the membrane was depolarized from -51.0 ± 2.5 mV ($n = 30$) to -40.6 ± 2.4 mV ($n = 25$) and, after an initial train of spike discharges, became electrically quiescent. By application of SP (10^{-8} M), the membrane was further depolarized by about 4 mV and the membrane resistance increased (Figure 8c, d). The mean increase in membrane resistance was 1.6 ± 0.3 ($n = 3$) times the value before the treatment with SP. Furthermore, slow waves were still generated (2–5 mV in amplitude and at 5–20 c/min) in excess K-solution (20.2 mM), as shown in Figure 8e. When the depolarization induced by SP was restored by application of

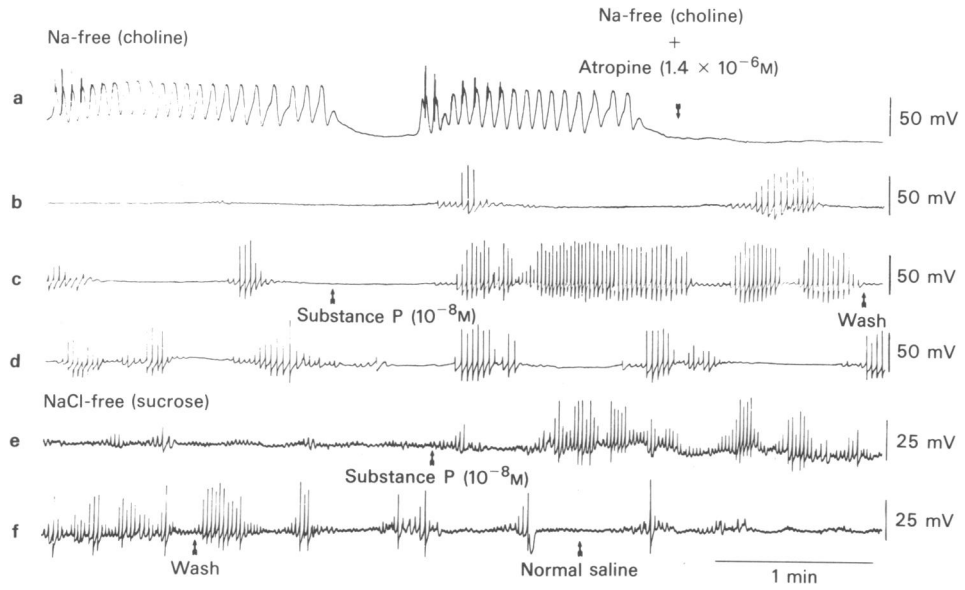

Figure 7 The effects of substance P (SP) on the electrical membrane properties of longitudinal muscle cells in Na-deficient and NaCl-deficient solution: (a), when Na ions in the Krebs solution were replaced with choline, the slow waves (10–20 mV in amplitude) were suppressed by atropine (1.4×10^{-6} M). (b), In the presence of atropine, small slow potential changes with or without action potentials were observed, and SP (10^{-8} M at arrow) enhanced the frequency and amplitude of these potential changes (c and d). (a), (b), (c) and (d) are continous recordings. Arrows indicate the continuous application of Na-free (choline) Krebs solution with or without SP, or wash out of SP. (e) and (f) In NaCl-free (sucrose) solution, slow small potential changes in membrane potential were also observed. SP (10^{-8} M at arrow) increased their amplitude. (e) and (f) are continuous recordings from another cell. Arrows indicate the continuous application of SP or washout of Sp.

hyperpolarizing d.c. current, to the level where 20.2 mM K induced depolarization, generation of slow waves was still observed (Figure 8f). This means that the generation of slow waves was not solely due to the membrane depolarization induced by SP. In K-deficient solution (1.7 mM), similar effects of SP on the membrane potential and resistance were observed, i.e. the membrane was depolarized from -50.5 ± 1.0 mV ($n = 25$) to -45.0 ± 1.0 mV ($n = 25$) and the input membrane resistance was increased to 1.3 times that of the control value. Slow small potential changes were also generated in K-deficient solution.

When the external concentration of calcium was reduced to below 0.25 mM, spontaneous spikes and slow waves were absent. SP (10^{-8} M) slightly depolarized the membrane (2–3 mV), but evoked no spikes or slow potentials in 0.25 mM $[Ca]_o$ (Figure 9).

Effects of substance P in the presence of baclofen

Baclofen is a putative SP antagonist in several parts of the central nervous system (Saito, Konishi & Otsuka, 1975; Otsuka & Yanagisawa, 1980; Ogata & Abe, 1981). Therefore, the effects of SP were observed after pretreatment with baclofen. Baclofen itself (4.7×10^{-6} M) did not alter the resting membrane potential or the amplitude or frequency of the spontaneous spike discharges; in its presence as in its absence, SP (10^{-8} M) depolarized the membrane and increased the rate of spontaneous spike discharges. Thus, baclofen had no effect on the excitatory action of SP.

Effects of substance P on excitatory junction potentials

To investigate the effects of SP on the e.j.p., e.j.ps were recorded by application of brief stimulations (50 μs in duration) at a constant stimulus frequency (20 Hz) and intensity, by the double sucrose gap method. The amplitude of the e.j.p. was kept below the threshold depolarization required for muscle action potentials.

Figure 10 (a) and (b) shows an example of a typical experiment. The amplitude of the e.j.p. increased in proportion to the number of stimuli at a constant stimulus frequency (20 Hz), and generated action potentials when the threshold depolarization was reached. Application of 10^{-9} M SP induced a membrane depolarization (about 4 mV) and a train of

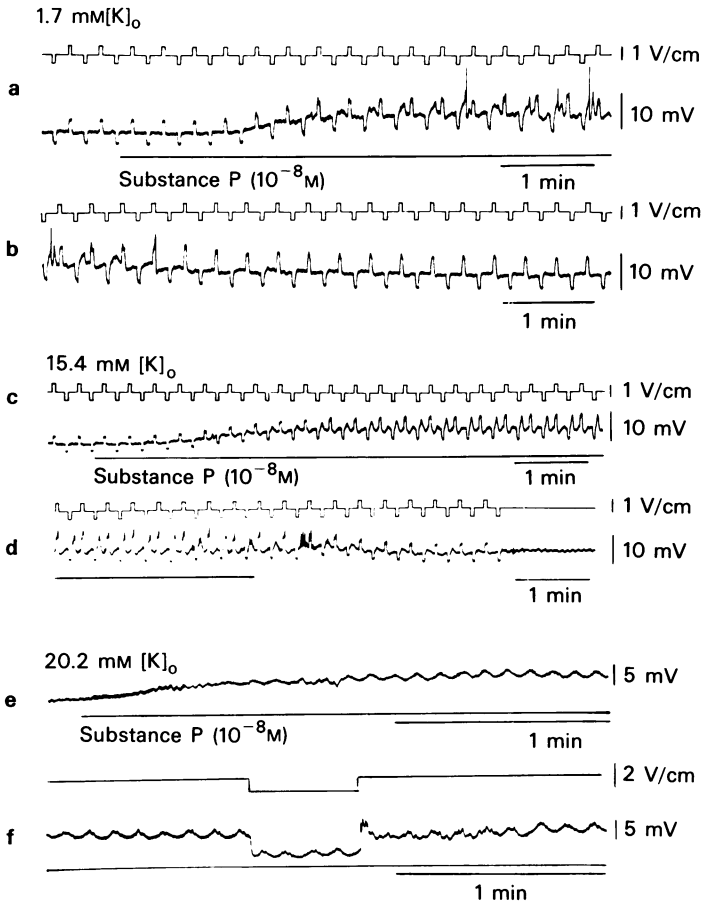

Figure 8 The effects of substance P (SP, 10^{-8} M) on the membrane potential and input membrane resistance of the longitudinal smooth muscle cells of the guinea-pig ileum in various $[K]_o$ concentrations, measured with microelectrodes. (a) and (b), (c) and (d), and (e) and (f), are continuous recordings and show the effects of SP in 1.7 mM, 15.4 mM and 20.2 mM $[K]_o$ respectively. Bars indicate the duration of application of SP (10^{-8} M).

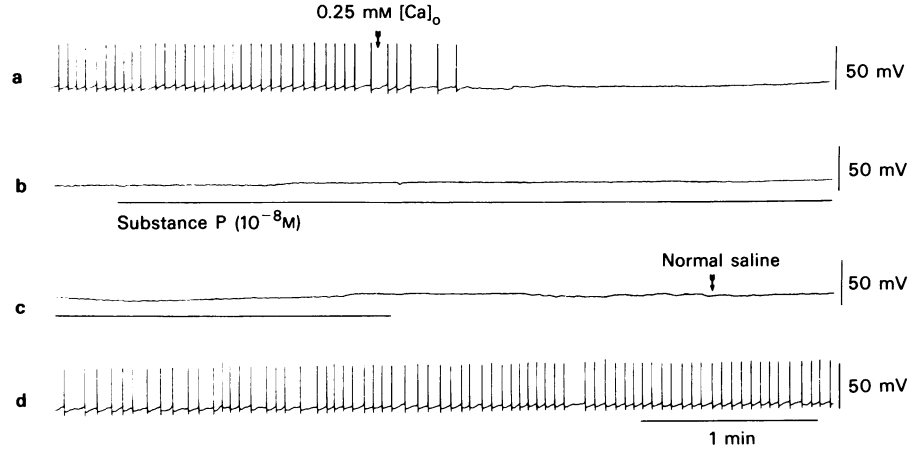

Figure 9 The effects of substance P (SP, 10^{-8} M) on the membrane potential and action potential of longitudinal muscle cells of the guinea-pig ileum in Ca-deficient (0.25 mM) solution: (a) and (b), and (c) and (d) are continuous recordings. Arrows indicate the application of Ca-deficient solution and the return to normal saline, and bars indicate the duration of treatment with SP (10^{-8} M).

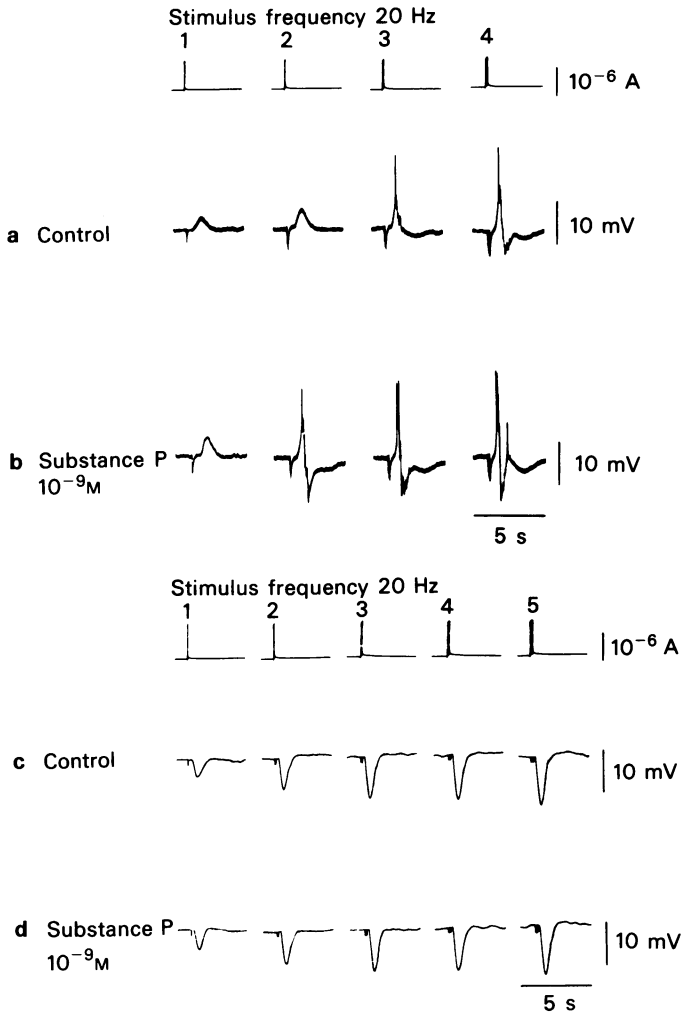

Figure 10 The effects of substance P (SP, 10^{-8} M) on the amplitude of the e.j.ps or the i.j.ps evoked by brief (1–5 Hz) periods of stimulation (pulse duration 50 μs) at constant stimulus intensity and frequency (20 Hz) and recorded from smooth muscle cells in the longitudinal or circular muscle coat of the guinea-pig ileum by the double sucrose gap method. (a) Control e.j.ps; (b) effects of SP (10^{-9} M) on e.j.ps; (e) control i.j.ps; (d) effects of SP on i.j.ps.

spike discharges followed by repolarization of the membrane. In the absence of spikes the amplitude of the e.j.p. evoked by a single stimulation was increased to 140% of the control value, in this particular example. In concentrations over 10^{-10} M, SP enhanced the amplitude of e.j.p.. For instance, the amplitude of e.j.p. was increased to $124 \pm 9\%$ ($n = 5$) $131 \pm 10\%$ ($n = 6$) and to $165 \pm 12\%$ ($n = 5$) of the control value by the application of 10^{-10}, 10^{-9}, 10^{-8} M SP respectively.

In the presence of 10^{-10}–10^{-9} M SP, neither the resting membrane potential nor the input membrane resistance, measured from the amplitude of the electrotonic potentials, was affected, indicating that the stimulatory effects of SP on the amplitude of e.j.p. are probably due to a presynaptic action.

Effects of substance P on the inhibitory junction-potentials of circular muscles of the guinea-pig ileum

In the longitudinal muscle, field stimulation evoked mainly e.j.ps, but in the circular muscle it evoked both e.j.ps and inhibitory junction potentials (i.j.ps). To observe the effects of SP on the i.j.p., the circular muscle was used. The amplitude of the i.j.p. was increased in proportion to the number of stimuli at a constant stimulus frequency (20 Hz), as shown in Figure 10 (c) and (d). The amplitude of i.j.ps, how-

ever, was not affected by SP (10^{-9} M), under any condition of stimulation.

Discussion

From the present experiments, the effects of SP on the smooth muscle of guinea-pig ileum can be summarized as follows: (i) low concentrations of SP (10^{-13}–10^{-8} M) induce phasic contractions in the longitudinal but not in the circular muscle; (ii) SP (10^{-13}–10^{-8} M) evoked two different membrane responses in longitudinal muscle cells, bursts of repetitive spikes or regular slow waves without change of membrane potential or membrane input resistance between the periods of spiking activity; (iii) increased concentrations of SP ($>10^{-8}$ M) induced a small but clear membrane depolarization with an increase in the input resistance; (iv) the excitatory actions of SP were unaffected by pretreatment with atropine, tetrodotoxin or baclofen; (v) SP reduced the threshold membrane depolarization required for the generation of action potentials.

Similar excitatory actions of SP have been described for peripheral (Katayama, North & Williams 1979) and central neurones (see for example, Euler & Pernow 1977). In the myenteric plexus of the guinea-pig, SP caused a dose-dependent membrane depolarization which was unaffected by hexamethonium, hyoscine, naloxone or baclofen. The membrane depolarization observed was associated with an increase in the membrane resistance, augmented by membrane depolarization and reduced by membrane hyperpolarization induced by the application of outward and inward d.c. currents respectively. Furthermore, the relation between the reversal potential for SP and $[K]_O$ plotted on a logarithmic scale was linear, indicating that SP inactivates the resting potassium conductance of myenteric neurones (Katayama *et al.*, 1979).

Since the membrane depolarization induced by SP (10^{-8} M) in the longitudinal smooth muscle cells was slow and small (4 mV) compared to that observed in the myenteric neurones (20 mV), quantitative studies were not feasible in the present experiments. However, SP (10^{-8} M) increased the input membrane resistance to a greater extent (1.6 times) in the excess K (15.4 mM) solution than in the K-deficient solution (1.7 mM) (1.3 times). These findings indicate that SP inactivates the resting potassium conductance and depolarizes the membrane of the smooth muscle cells. However, the contribution of sodium and Cl ions has to be considered, since in the NaCl-deficient solution, SP did not depolarize the membrane.

In longitudinal muscle cells of the guinea-pig ileum, the generation of slow waves with or without action potentials was also noted when the muscarinic receptor was activated by acetylcholine or carbachol (Kuriyama, Oso & Toida, 1967; Bolton, 1971; 1972). With the voltage clamp technique, it was found that when muscarinic receptors of the ileum were stimulated, inward current and negative resistance appeared in the potential range between −10 and −40 mV whereas only an outward current existed in the control condition (Bolton, 1975). Thus, the activation of muscarinic receptors may open ionic channels, the conductance of which increases in the potential range of −40 to −10 mV, so that depolarization causes an increase in the inward and largely sodium current (Bolton, 1971; 1972), creating regenerative slow waves. In the present experiments, the slow waves or repetitive spike discharges evoked by SP could be observed in Na-free but not Ca-free solution. On the assumption that SP acts through specific receptors on the muscle membrane, the receptor-operated ionic channels seem to be different from those related to the muscarinic acetylcholine receptors.

The present results also show the topical difference in sensitivity to SP, since SP ($<10^{-8}$ M) had no effect on the mechanical and electrical properties of the circular muscle cells of guinea-pig intestine.

One of the striking features of the action of low concentrations of SP on the electrical membrane properties is the reduction in the threshold membrane depolarization for the generation of muscle action potentials without any significant effect on the resting membrane potential or the input membrane resistance. The importance of the C-terminal amide for the action of SP had been suggested from comparative studies on SP, substance P pentapeptide and substance P free acid (Yanaihara, Yanaihara, Hirohashi, Sato, Iizuka, Hashimoto & Sakagami, 1977; Otsuka & Konishi, 1977; Katayama *et al.*, 1979) and the binding of this group to receptor sites on the muscle membrane could be responsible for the reduction in threshold depolarization.

Experimental evidence to support the idea that SP possesses a stimulatory effect on the release of acetylcholine has been provided by Hedqvist & Euler, (1975), Milenov, Nieber & Oehme (1978), Holzer & Lembeck (1980) and Holzer, Lembeck & Donnerer (1980). One possible explanation for the stimulatory effects of SP on the release of acetylcholine is a direct excitatory action of SP on the soma of cholinergic motoneurones or on fibres impinging on these cholinergic neurones, since recent electrophysiological evidence has shown that some neurones within the myenteric plexus are sensitive to SP (Katayama *et al.*, 1979). In the present experiments, low concentrations of SP (10^{-10}–10^{-9} M) enhanced the amplitude of e.j.ps with no change in membrane potential and membrane resistance. SP is present in intrinsic neurones of the gut (Schultzberg, Dreyfus, Gershon,

Hökfelt, Elde, Nilsson, Said & Golstein, 1978), and a considerable number of these fibres seems to innervate the muscle layer of the intestine (Costa, Cuello, Furness & Franco, 1980). Thus, the possibility that endogenous SP acts on the cholinergic nerve terminals to enhance the transmitter release cannot be ruled out. However, SP in concentrations between 10^{-10} to 10^{-9} M had no effect on the amplitude of nonadrenergic inhibitory potentials, indicating the difference in the distribution of the receptive sites for SP in the cholinergic and nonadrenergic nerve terminals.

The actions of SP on neurones of the myenteric plexus, cholinergic nerve terminals in the muscle layer and on smooth muscle cells may explain the potent spasmogenic effects observed in the smooth muscle tissues in the digestive tract.

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