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THE ELECTROPHYSIOLOGICAL EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE IN THE GUINEA-PIG INFERIOR MESENTERIC GANGLION

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SUMMARY

1. The effects of vasoactive intestinal polypeptide (VIP) on the inferior mesenteric ganglion of the guinea-pig were studied *in vitro*.

2. In 67% of the neurones tested, application of VIP ($1–7{\cdot}5 \times 10^{-5}$ M) by pressure ejection caused a depolarization of the membrane potential which averaged $8{\cdot}6 \pm 0{\cdot}4$ mV.

3. In 52% of the cells that were responsive to VIP, the membrane depolarization was accompanied by a decrease in membrane input resistance. In another 48% of the cells tested, there was an increase in membrane input resistance.

4. Membrane depolarization caused by VIP enhanced the excitability of postganglionic neurones and converted subthreshold electrotonic and subthreshold synaptic potentials to action potentials.

5. The effects of VIP persisted during nicotinic and muscarinic synaptic blockade. The effects of VIP also persisted in a low-Ca^{2+}, high-Mg^{2+} solution. Thus, the site of action of VIP was on the postsynaptic membrane.

6. Electrical stimulation of the lumbar colonic nerves evoked a slow non-cholinergic depolarization of the membrane potential.

7. VIP appeared to be one of the transmitters involved in the electrically evoked e.p.s.p. because both prior desensitization with exogenous VIP and VIP antiserum reduced the amplitude of the slow, non-cholinergic e.p.s.p.

8. Radial distension of a segment of colon attached to the inferior mesenteric ganglion (i.m.g.) evoked a non-cholinergic depolarization of the membrane potential in neurones in the i.m.g.

9. The distension-induced non-cholinergic depolarization was reduced by VIP antiserum.

10. The data support the hypothesis that a population of the mechanosensory afferent nerves running between the colon and the i.m.g. utilize VIP or a VIP-like peptide as a transmitter to modulate reflex activity between the colon and the i.m.g.

INTRODUCTION

The inferior mesenteric ganglion (i.m.g.) is an important site of peripheral control of colonic motility. It integrates synaptic input from the central nervous system with

afferent mechanoreceptor input from the distal colon and thereby determines the post-ganglionic sympathetic outflow to the distal colon (Szurszewski, 1981). In addition to fast cholinergic excitatory postsynaptic potentials (e.p.s.p.s), neurones of the i.m.g. exhibit slow, long-lasting, non-cholinergic e.p.s.p.s following either repetitive nerve stimulation (Neild, 1978) or radial distension of the colon (Peters & Kreulen, 1986). Immunohistochemical studies have revealed a prominent collection of peptidergic nerve terminals containing enkephalin-, substance P- and vasoactive intestinal polypeptide (VIP)-like immunoreactivity (Dalsgaard, Hökfelt, Schultzberg, Lundberg, Terenius, Dockray & Goldstein, 1983*a*). Each of these peptides may function as either a neurotransmitter or a neuromodulator in the i.m.g. Thus, it has been suggested that the enkephalins liberated from preganglionic fibres of central origin act as an inhibitory neuromodulator regulating the release of acetylcholine from central, preganglionic terminals (Konishi, Tsunoo & Otsuka, 1979*a*) and from peripheral cholinergic terminals of colonic mechanoreceptors (Shu, Love & Szurszewski, 1986). Substance P, also contained in preganglionic terminals of central origin (Matthews & Cuello, 1984), has been implicated as a neurotransmitter responsible for the slow non-cholinergic e.p.s.p. evoked by electrical stimulation of peripheral nerves (Konishi, Tsunoo & Otsuka, 1979*b*) and by radial distension of a segment of the distal colon (Peters & Kreulen, 1986). A particularly dense network of VIP-like immunoreactive nerve fibres also exists in the i.m.g. These fibres, which have their cell bodies in the wall of the distal colon, travel in the lumbar colonic nerves to form a dense pericellular network around principal ganglion cells of the i.m.g. (Hökfelt, Elfvin, Schultzberg, Fuxe, Said, Mutt & Goldstein, 1977; Costa & Furness, 1983; Dalsgaard *et al.* 1983*a*). While the electrophysiological effects of VIP on a variety of central and peripheral neurones have been studied, only one brief report of the effects of this peptide on prevertebral ganglia has appeared (Mo & Dun, 1984).

The purposes of this study were to examine the effects of VIP on the electrical properties of the neurones of the i.m.g. and to determine whether VIP has any role in slow, non-cholinergic synaptic potentials. It will be shown that in a population of neurones in the i.m.g., VIP depolarizes the neurones by an action on the postsynaptic membrane thereby increasing the efficiency of spatiotemporal summation of fast e.p.s.p.s. It will also be shown that VIP, or a closely related peptide, may be one of the peptidergic transmitters for the non-cholinergic depolarization evoked by electrical stimulation of colonic afferent fibres and by radial distension of a segment of attached colon.

Some of the results have been previously communicated (Love & Szurszewski, 1985*a*, *b*).

METHODS

Male guinea-pigs weighing 400–800 g were killed by a blow to the head and bled. The i.m.g. and attached nerve trunks were removed with or without the distal 6–8 cm of the colon attached. In experiments requiring spontaneous mechanoreceptor input from the colon, an i.m.g.–colon preparation was pinned out in a two-compartment organ bath. The mesentery containing the lumbar colonic nerves was draped over the wall separating the two compartments and covered with moist strips of tissue-paper to prevent dessication. Both chambers were perfused separately with

a modified Krebs physiological solution containing (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; glucose, 11·5; equilibrated with 97% O_2 and 3% CO_2 and maintained at 35–37 °C. In experiments where colonic mechanoreceptor synaptic input was undesirable, the colon was dissected away leaving the i.m.g. and its attached peripheral nerve trunks.

The methods for measuring intraluminal colonic pressure have been described in detail previously (Szurszewski & Weems, 1976). Briefly, the proximal end of the colon was tied off and the distal end cannulated. The catheter was then attached to a Y-fitting, one end of which was connected to a Statham pressure transducer and the other to a calibrated cylindrical reservoir and a 20 ml syringe. The intraluminal pressure was altered by adding or removing Krebs solution from the syringe thereby changing the height of solution in the reservoir and the pressure in the colonic segment. This system allowed the colon to change its intraluminal volume during contraction. Zero pressure was that pressure at the catheter tip before it was inserted into the colonic lumen. Basal pressure was then defined as the difference in pressure observed when the catheter was connected to the fluid-filled colon at rest.

Intracellular recordings were made with 3 M-KCl-filled glass microelectrodes (40–100 MΩ resistance) connected to an electrometer with an active bridge circuit which allowed the passage of depolarizing or hyperpolarizing current through the recording electrode. The membrane potential, electrotonic stimulus current, and intraluminal pressure were displayed on an oscilloscope (Tektronix 5113) and permanent records were made on a chart recorder (Gould Brush 220) and an FM tape-recorder (Hewlett–Packard 3968A). Impalement of a neurone in the i.m.g. was judged satisfactory if the recorded potential showed an abrupt deflection in excess of −40 mV which was maintained and the neurone exhibited action potentials overshooting zero potential.

Peripheral nerve trunks were stimulated using bipolar silver–silver chloride electrodes connected to a stimulator (Grass S88) and stimulus isolation unit (Grass SIU5A). Supramaximal stimuli with pulse durations of 100–500 μs were employed to evoke synaptic potentials.

The following drugs and peptide were used: atropine sulphate (Sigma), hexamethonium bromide (Sigma) and natural porcine VIP. The concentrations of atropine sulphate and hexamethonium bromide given in the text are those of the final concentration reaching the tissue by superfusion. VIP was dissolved in Krebs solution in final concentrations ranging from 1 to $7·5 \times 10^{-5}$ M. Glass micropipettes were filled with VIP-containing Krebs solution and brought into close proximity to the neurone from which the recording was being made. VIP was applied in the vicinity of the neurone by pressure ejection using a Picospritzer. The concentration of VIP given in the Results is the concentration of VIP in the micropipette.

In some experiments, a Ca^{2+}-deficient solution was used. This solution was prepared by reduction of the Ca^{2+} concentration to 0·1 mM and elevation of the Mg^{2+} concentration to 15 mM.

Rabbit antiporcine VIP antiserum was used. VIP antiserum No. 4823 was raised in a rabbit immunized with a conjugate of natural porcine VIP and bovine serum albumin. The binding capacity of the undiluted antiserum was calculated to be 2·4 μg VIP per millilitre antiserum. In a radioimmunoassay system for VIP, the antiserum used in this study showed no cross-reactivity with substance P, glucagon, secretin, neurotensin, gastric inhibitory polypeptide, porcine pancreatic polypeptide, somatostatin, bombesin, cholecystokinin or gastrin. The VIP antiserum was previously demonstrated in *in vitro* experiments to specifically antagonize the effects of neurally released VIP and the effects of exogenously added VIP (Angel, Go, Schmalz & Szurszewski, 1983; Angel, Go & Szurszewski, 1984). Furthermore, the VIP antiserum did not alter the response to neurally released and exogenously added substance P (Angel *et al.* 1984). Thus the VIP antiserum used in this study was selective for VIP-like peptide. In some experiments, non-immune serum from a non-immunized rabbit was used as a control serum.

The data were expressed as the mean ± the standard error of the mean. Student's paired *t* test was used to test for significance of differences between paired groups of data.

RESULTS

Effects of VIP on membrane potential and input resistance

In 118 neurones from thirty-four ganglia, the mean resting membrane potential was 53 ± 1 mV and was consistent with values reported previously for neurones in

this ganglion (Crowcroft & Szurszewski, 1971). When VIP ($1–7{\cdot}5 \times 10^{-5}$ M) was applied by a series of 900 ms pressure pulses, a depolarization of the ganglion cell membrane occurred in 67% (seventy-nine cells) of the cells tested. The VIP-induced depolarization had a range of 2–22 mV and a mean amplitude of $8{\cdot}6 \pm 0{\cdot}4$ mV when the pipette contained 5×10^{-5} M-VIP. As illustrated in Fig. 1, the depolarization

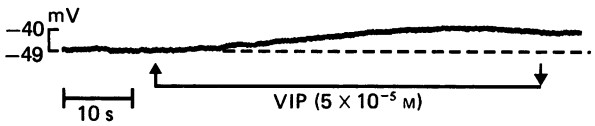

Fig. 1. Effect of VIP on the membrane potential of a neurone in the guinea-pig i.m.g. VIP (5×10^{-5} M), which was applied by a series of pressure ejections (900 ms; 20 lbf/in²; 0·15 Hz), caused a slowly developing membrane depolarization of 10 mV. Three minutes after stopping the application of VIP the membrane potential repolarized to the control value (−49 mV).

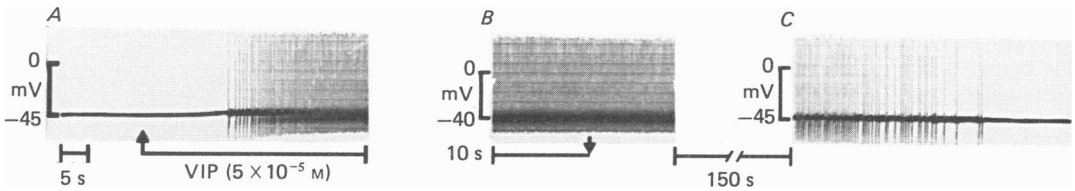

Fig. 2. Spontaneous action potentials initiated by VIP-induced membrane depolarization. VIP (5×10^{-5} M) was applied by a series of pressure ejections (900 ms; 20 lb-f/in²; 0·15 Hz). In *A*, spontaneous action potentials were first observed when the membrane potential was −42 mV. In *B*, the firing frequency of action potentials increased rapidly as the membrane depolarization peaked at −40 mV. In *C*, when VIP application was stopped the membrane potential slowly repolarized and the firing frequency decreased and ceased when the resting membrane potential returned to −45 mV.

required several seconds to appear and was slow to reach maximum (55 ± 3 s in fifty-five cells). Return from the maximum VIP-induced depolarization to the membrane potential recorded before application of VIP required an average of 112 ± 7 s. In some cells, the VIP-induced depolarization reached threshold for firing either single action potentials or a high-frequency train of action potentials (Fig. 2). In addition, electrotonic potentials which were subthreshold in normal Krebs solution reached threshold for firing action potentials during pressure application of VIP. Action potentials were never elicited by VIP in the absence of membrane depolarization.

The effect of VIP on membrane input resistance was studied in twenty-one neurones from eight ganglia. In these experiments, the current–voltage relationship was determined by injecting a series of hyperpolarizing and subthreshold depolarizing current pulses through the microelectrode and measuring the amplitude of the steady-state electrotonic potentials they produced. Input resistance was calculated from the slope of the current–voltage relationship. The current–voltage relationship was obtained during pressure application of normal Krebs solution and compared in the same neurone with the current–voltage relationship obtained during application of VIP. Two different effects on slope input resistance were observed (Fig. 3). In eleven cells, a significant ($P \leqslant 0{\cdot}005$) increase in slope resistance from

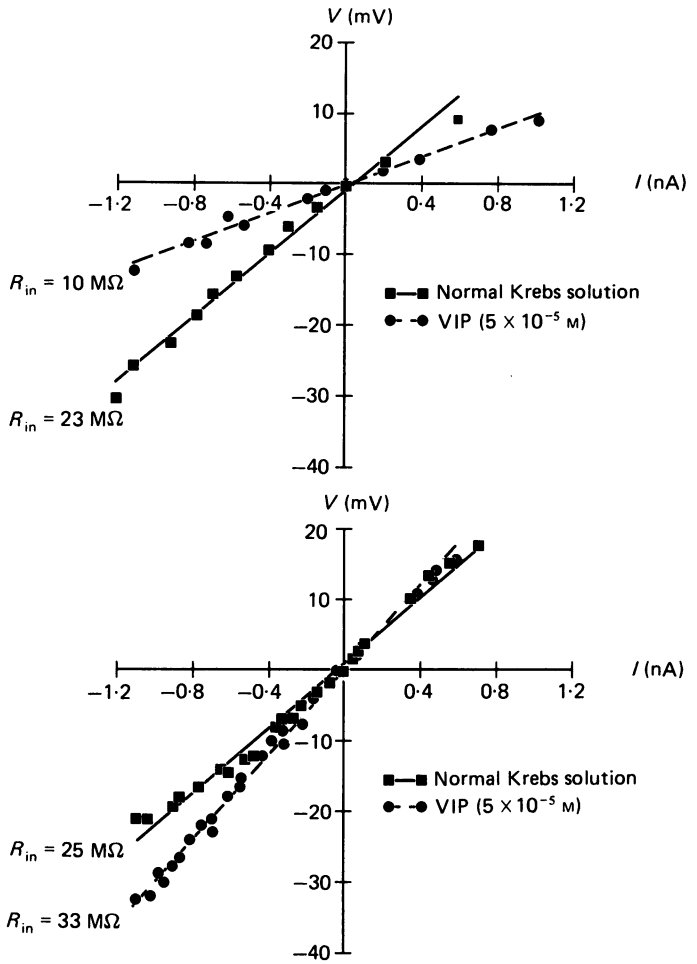

Fig. 3. Voltage–current relationship of two neurones in the i.m.g. during pressure application of normal Krebs solution (■——■) and during pressure application of VIP-containing Krebs solution (●---●). Pressure pipette contained 5×10^{-5} M-VIP-containing Krebs solution. All measurements made during application of VIP were obtained during the steady-state effect of VIP.

29 ± 5 to 34 ± 5 MΩ occurred during VIP application. In the other ten cells, a significant ($P \leqslant 0{\cdot}005$) decrease in slope resistance from 43 ± 6 to 26 ± 4 MΩ occurred during VIP application. Both types of responses could be observed in neurones of the same ganglion. The only apparent difference between the two populations of responding neurones was the membrane input resistance obtained before applying VIP. The population of neurones which responded to VIP with an increase in slope resistance had a lower resting membrane slope resistance (29 ± 5 MΩ) than the population which responded with a decrease in slope resistance (43 ± 6 MΩ). This difference in resting input resistance was statistically significant ($P < 0{\cdot}005$). There was no significant difference ($P > 0{\cdot}01$) between the magnitude of the VIP-induced

depolarization in the cells whose slope resistance increased (8 ± 1 mV) compared to the cells whose slope resistance decreased (10 ± 2 mV).

Site of action of VIP

To determine if the effects of VIP were due to an action of VIP on presynaptic terminals to release a transmitter, the VIP-induced depolarization was studied

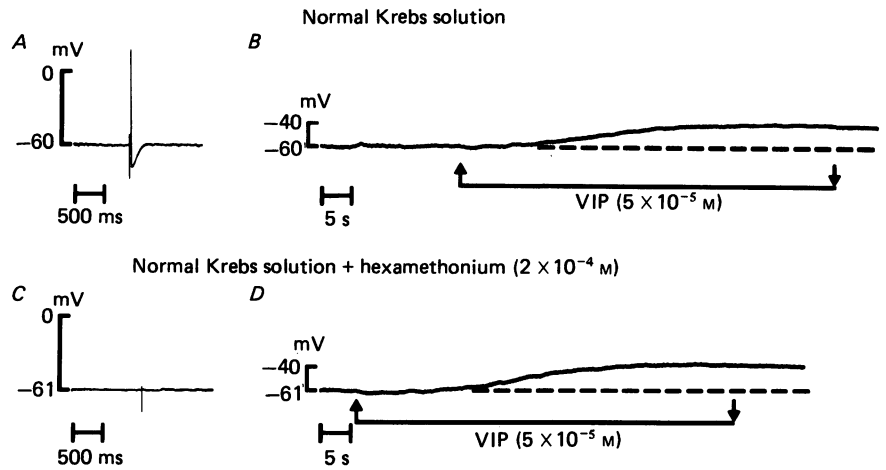

Fig. 4. Effect of blockade of nicotinic cholinergic transmission on VIP-induced membrane depolarization. *A*, synaptic action potential evoked by electrical stimulation of the lumbar colonic nerves. *B*, VIP (5×10^{-5} M) applied by pressure ejection (900 ms; 20 lbf/in^2; 0·15 Hz) caused a 19 mV membrane depolarization. *C*, in the presence of hexamethonium (2×10^{-4} M) the synaptic action potential was blocked but the VIP-induced membrane depolarization was unaffected (*D*). All recordings made from the same neurone.

before and after interrupting synaptic transmission either by receptor blockade or by preventing release of transmitter by a low-Ca^{2+}, high-Mg^{2+} solution. To determine if VIP acted on cholinergic, presynaptic terminals, the effect of VIP was studied before and during nicotinic receptor blockade. In five of five cells from four ganglia, hexamethonium ($1–2 \times 10^{-4}$ M) blocked orthodromically evoked action potentials but had no effect on the maximum VIP-induced depolarization when compared to control (Fig. 4). In normal Krebs solution, the VIP-induced depolarization averaged 11 ± 2 mV; in the presence of hexamethonium, it was 11 ± 3 mV. Thus, the effect of VIP on membrane potential was not attributable to release of acetylcholine from presynaptic nerve terminals. To determine whether VIP released one of the putative peptidergic transmitters, we studied the effect of VIP before and after synaptic transmission was interrupted with a Krebs solution containing a reduced concentration of Ca^{2+} (0·1 mM) and an elevated concentration of Mg^{2+} (15 mM). Such a Ca^{2+}-deficient solution has been demonstrated to be effective in blocking transmitter release in the i.m.g. (Crowcroft & Szurszewski, 1971). In six of six neurones from four ganglia, the Ca^{2+}-deficient, high-Mg^{2+} solution blocked the synaptically evoked action potential but had no effect on the VIP-induced membrane depolarization (Fig. 5). In normal Krebs solution, the VIP-induced depolarization averaged

8 ± 1 mV. In the Ca^{2+}-deficient solution, the average was 9 ± 1 mV. It was considered therefore likely that the VIP-induced depolarization was due to a direct action on the postsynaptic cell membrane.

Effects of VIP on evoked fast e.p.s.p.s and mechanoreceptor afferent input

The effect of pressure application of VIP on fast cholinergic e.p.s.p.s was studied. As seen in Fig. 6, synaptic responses that were below threshold in normal Krebs

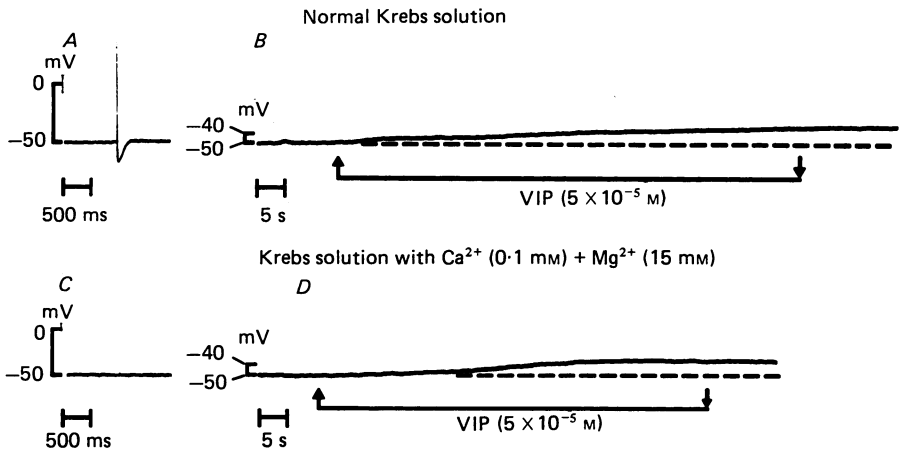

Fig. 5. Effect of low Ca^{2+}, elevated Mg^{2+} on VIP-induced membrane depolarization. *A*, synaptic action potential evoked by electrical stimulation of the lumbar colonic nerves. *B*, VIP (5×10^{-5} M) applied by pressure ejection (900 ms; 20 lbf/in^2; 0·15 Hz) caused an 8 mV membrane depolarization. *C*, superfusion of the i.m.g. with a Krebs solution containing a low concentration (0·1 mM) of Ca^{2+} and an elevated concentration (15 mM) of Mg^{2+} abolished the synaptic action potential. *D*, in the low-Ca^{2+}, elevated-Mg^{2+} solution, the VIP-induced membrane depolarization (9 mV) persisted. All recordings made from the same neurone.

solution reached threshold for initiation of an action potential during VIP-induced membrane depolarization. In cells not responding to VIP with membrane depolarization, no such increase in synaptic efficiency was observed. In ganglia isolated from the colon, VIP failed to evoke spontaneous nicotinic e.p.s.p.s in any of the cells tested. This latter observation is additional evidence supporting the notion that VIP did not act presynaptically on cholinergic nerve fibres.

The effect of VIP on fast, nicotinic cholinergic e.p.s.p.s due to afferent input from colonic mechanoreceptors was also studied. As seen in Fig. 7, subthreshold synaptic input from colonic mechanoreceptors was converted to action potentials by VIP-induced depolarization. In cells where action potentials were present due to strong mechanoreceptor input, there was an increase in the firing frequency of the neurone during VIP-induced depolarization. Thus, VIP-induced depolarization enhanced reflex activity between the distal colon and the i.m.g. In those neurones which failed to respond to VIP with a depolarization, VIP had no effect on either evoked e.p.s.p.s or e.p.s.p.s due to mechanoreceptor input.

Role of VIP in non-cholinergic input from the colon

Effect of VIP antiserum on slow depolarization due to electrical nerve stimulation. Since the effects of VIP-induced depolarization were similar in appearance to the slow, non-cholinergic depolarization evoked by repetitive stimulation of the peripheral nerves innervating the i.m.g. (Neild, 1978), we examined whether or not

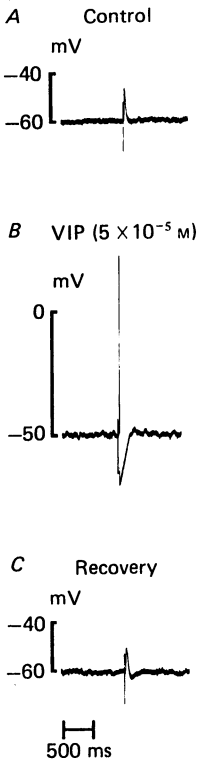

Fig. 6. Effect of VIP on a subthreshold fast, nicotinic transmission. *A*, in normal Krebs solution, a subthreshold, fast e.p.s.p. was evoked by electrical stimulation of the right hypogastric nerve. *B*, during the VIP-induced depolarization, the e.p.s.p. reached threshold for firing an action potential. VIP (5×10^{-5} M) was applied by pressure ejection. *C*, 2 min after ending the application of VIP the membrane potential repolarized to the control level and the e.p.s.p. was once again subthreshold for firing an action potential. All recordings made from the same neurone.

release of endogenous VIP had a role in mediating this synaptic potential. The lumbar colonic nerves were selected for electrical stimulation because they contain most of the VIP-reactive nerve fibres reaching the i.m.g. (Dalsgaard *et al.* 1983*a*). Since no specific receptor antagonists for VIP exist, we attempted to antagonize the effects of any endogenous VIP released by nerve stimulation by either prior desensitization to exogenous VIP or by application of rabbit antiserum raised to porcine VIP. We reasoned that if VIP or a VIP-like peptide were involved in the slow non-cholinergic depolarization evoked by electrical nerve stimulation, then desensitization by prolonged application of VIP should abolish or reduce the amplitude

of the slow non-cholinergic depolarization. Accordingly, VIP (5×10^{-5} M) was applied by a continuous series of pressure pulses. Hexamethonium (2×10^{-4} M) and atropine (2×10^{-6} M) were present in the bathing solution to block any cholinergic potentials resulting from nerve stimulation. It was found that desensitization to exogenous VIP was difficult to achieve in the majority of cells tested. Often during constant

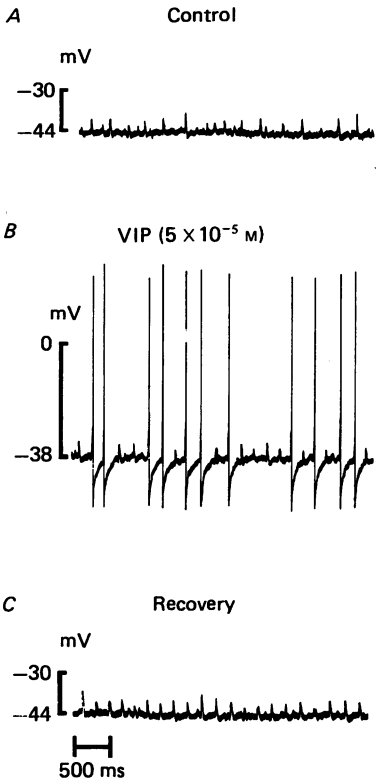

Fig. 7. Effect of VIP-induced depolarization on on-going colonic mechanoreceptor synaptic input to a neurone in the i.m.g. In this experiment a segment of the distal colon remained attached to the i.m.g. via the lumbar colonic nerves. *A*, e.p.s.p.s in a neurone receiving colonic mechanoreceptor input in normal Krebs solution. *B*, during application of VIP (5×10^{-5} M) by pressure ejection there was a 4 mV membrane depolarization and conversion of e.p.s.p.s to action potentials and an increase in the firing rate of the post-ganglionic neurone. *C*, 90 s after stopping the application of VIP the membrane potential and the firing rate returned to control levels. All recordings from the same neurone.

application of VIP, cells failed to repolarize completely back to the resting potential recorded before application of VIP. These cells were not studied further. However, in two of three neurones where desensitization was complete, the slow synaptic potential evoked by stimulation of the lumbar colonic nerves in cells completely desensitized to VIP was $2{\cdot}0 \pm 0{\cdot}7$ mV compared to $4{\cdot}0 \pm 0{\cdot}7$ mV obtained before VIP desensitization. The slow non-cholinergic synaptic potential before and during desensitization to VIP is shown in Fig. 8. No change in the synaptic potential was observed in the third cell.

Rabbit VIP antiserum was also used to study the possible role of VIP in the non-cholinergic slow e.p.s.p. We tested for the effects of either non-immune rabbit serum or rabbit VIP antiserum or, when possible, both sera on the same neurone, on the neurally evoked slow e.p.s.p. In these experiments, hexamethonium (2×10^{-4} M) and atropine (2×10^{-6} M) were present to block cholinergic inputs. In three of seven

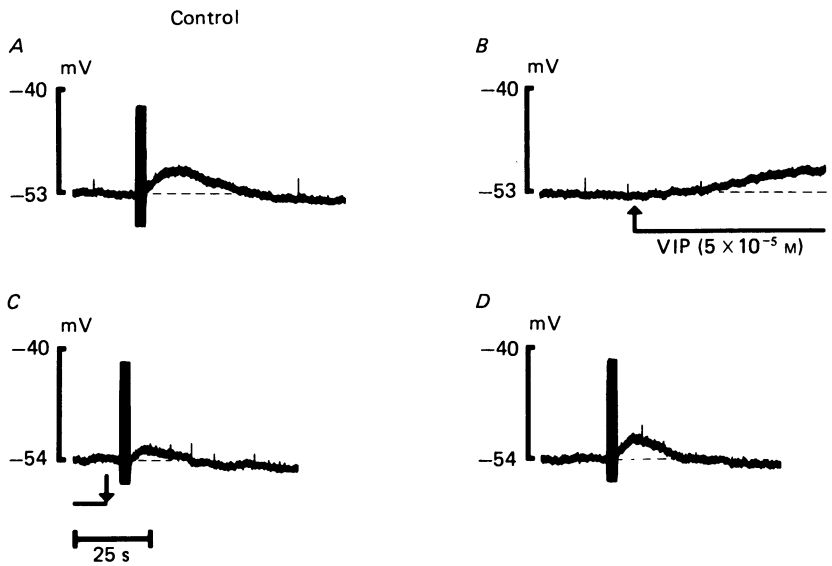

Fig. 8. Effect of VIP desensitization on the slow e.p.s.p. evoked by lumbar colonic nerve stimulation. *A*, in normal Krebs solution a 4 mV slow e.p.s.p. was evoked by supramaximal (20 Hz, 4 s) lumbar colonic nerve stimulation. *B*, VIP (5×10^{-5} M) applied by pressure ejection (900 ms; 20 lbf/in^2; 0·15 Hz) evoked a 4 mV membrane depolarization (*C*). During continuous application of VIP the depolarizing response to VIP faded and the electrically evoked slow e.p.s.p. was reduced to 1 mV. The slight hyperpolarization following the attenuated slow e.p.s.p. was seen only in this neurone. *D*, 2 min after stopping application of VIP lumbar colonic nerve stimulation (20 Hz, 4 s) evoked a train of spikes which was followed by a slow e.p.s.p. whose amplitude was the *same* as that shown in *A*. All recordings from the same neurone.

neurones tested with VIP antiserum only, the amplitude of the slow e.p.s.p. was $3{\cdot}6 \pm 1{\cdot}0$ mV. In these same neurones, the amplitude of the slow e.p.s.p. without the VIP antibody was $5{\cdot}8 \pm 1{\cdot}8$ mV. The decrease in amplitude observed in the presence of the VIP antibody was significant ($P < 0{\cdot}02$). In the other four neurones tested, the VIP antiserum had no effect. In three other neurones, we were able to study the effects of both VIP antiserum and non-immune serum applied separately. In one neurone, there was a decrease in the amplitude of the slow non-cholinergic e.p.s.p. during the presence of VIP antiserum but not during the presence of non-immune serum (Fig. 9). A second neurone responded to both sera with a decrease in the slow e.p.s.p. from 12 to 7 mV. The third neurone showed no change in response to either serum. When all treatment groups described above were combined, four of ten neurones exhibited a decrease in slow e.p.s.p. amplitude when exposed to VIP

antiserum while only one of six cells exposed to non-immune serum had a decreased slow e.p.s.p. amplitude. These results suggest the possibility that in a population of cells in the i.m.g., VIP may contribute to the slow non-cholinergic e.p.s.p. evoked by electrical stimulation of the lumbar colonic nerve. Since colonic mechanosensory afferent fibres travel in the lumbar colonic nerve to reach the i.m.g., we hypothesized

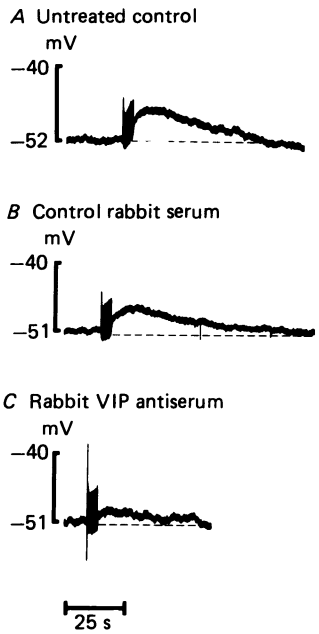

Fig. 9. Effect of VIP antiserum on the slow non-cholinergic e.p.s.p. evoked by lumbar colonic nerve stimulation. In this experiment, hexamethonium (2×10^{-4} M) and atropine (2×10^{-6} M) were present throughout. *A*, stimulation of the lumbar colonic nerves (20 Hz, 4 s) evoked a slow, non-cholinergic e.p.s.p. which was 5 mV in amplitude. *B*, during application of non-immune rabbit serum by pressure ejection (900 ms; 20 lbf/in^2; 0·15 Hz) for 2 min, the slow non-cholinergic e.p.s.p. attained an amplitude of 4 mV. *C*, during application of rabbit VIP antiserum by pressure ejection for 2 min the amplitude of the slow non-cholinergic e.p.s.p. was 2 mV. All recordings made from the same neurone.

that VIP may participate in mediating reflexes between the colon and i.m.g. Therefore, the colon–i.m.g. preparation was studied to determine if radial distension of the colon evoked a slow depolarization which was VIP dependent.

Effect of VIP antiserum on depolarization due to colonic distension. In these experiments, VIP antiserum was applied by pressure ejection in an attempt to block slow depolarizations evoked by colon distension. The colon was distended to raise the intraluminal pressure to an initial value of 15 cmH_2O. This distension resulted in subsequent rhythmic contractions of the colon which caused intraluminal pressure periodically to exceed 20 cmH_2O followed by a relaxation and fall in pressure to slightly below 15 cmH_2O. Such distensions proved sufficient to cause the slow e.p.s.p. to be maximal. Colonic distension was maintained until a steady maximum depolarization was achieved. Two minutes was allowed between the end of one

distension and the start of another. When VIP antiserum was tested, it was applied by a series of 900 ms pressure pulses at 0·15 Hz. The antiserum was applied for a minimum of 2 min before distension of the colon was initiated and application was continued during the duration of the distension. Colonic distension and antiserum application were ceased simultaneously. Frequently, colonic distension failed to

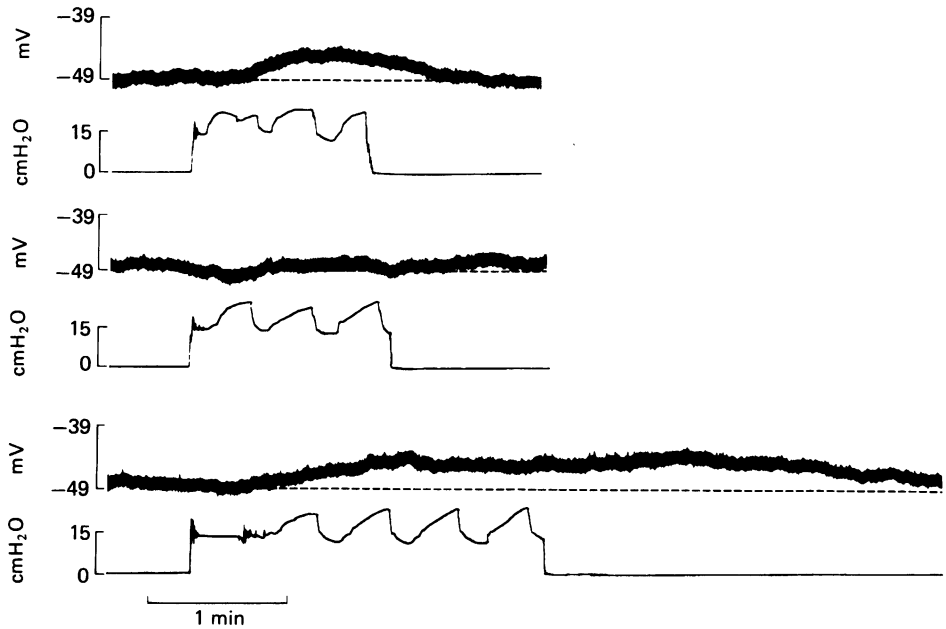

Fig. 10. Effect of VIP antiserum on the slow e.p.s.p. evoked by distension of the distal colon. In each panel: top trace intracellular recording from a neurone in the i.m.g., bottom trace intraluminal pressure in the colonic segment. Throughout this experiment, hexamethonium (2×10^{-4} M) and atropine (2×10^{-6} M) were present in the Krebs solution bathing only the i.m.g. Upper panel: pressure in the segment of colon was increased to 15 cmH_2O by injection of normal Krebs solution. This distension caused a 4 mV slow, non-cholinergic depolarization of the membrane potential of a neurone in the i.m.g. Middle panel: during application of rabbit VIP antiserum by pressure ejection (20 lbf/in^2; 900 ms; 0·15 Hz), distension of the colon caused a slow, non-cholinergic depolarization which was 1 mV in amplitude. Lower panel: 2 min after ending application of VIP distension of the colon evoked a slow, non-cholinergic depolarization which was 5 mV in amplitude. All recordings were made from the same neurone.

produce a slow e.p.s.p. or evoked one of only small amplitude (2 mV or less). For these experiments we studied only cells which exhibited slow depolarizations of 3 mV or more which could be exactly reproduced. Even a 1 mV difference in slow e.p.s.p. amplitude from distension to distension was grounds for rejection. The time course of the distension-induced depolarizations was much more variable than the amplitude. This probably reflects the fact that intraluminal pressure in a 6–8 cm segment of colon does not accurately indicate the extent of distension of that small portion of the segment which sends afferent input to the single neurone being

recorded from. For this reason, we based our quantification of the slow e.p.s.p. only on its maximum amplitude and adjusted distension times to allow the maximum to be reached from distension to distension in a given cell. In two of three cells which met the above criteria, VIP antiserum applied by pressure ejection reduced the mean amplitude of the slow e.p.s.p. by 50% from $5{\cdot}0 \pm 1{\cdot}4$ mV to $2{\cdot}5 \pm 0{\cdot}7$ mV. One such response is illustrated in Fig. 10. The third cell was unaffected by VIP antiserum.

DISCUSSION

The VIP-dependent depolarization and increase in excitability observed in the present study were similar to the effects of VIP observed in rat cortex and toad spinal cord (Phillis, Kirkpatrick & Said, 1978; Phillis & Kirkpatrick, 1980), guinea-pig myenteric plexus (Zafirov, Palmer, Nemeth & Wood, 1985), rat hippocampus (Dodd, Kelly & Said, 1979), carp retina horizontal cells (Lasater, Watling & Dowling, 1983), and rat medial preoptic-septal neurones (Haskins, Samson & Moss, 1982). The slow onset and long duration of VIP-induced depolarization were similar to those seen in the superior cervical ganglion of the cat (Kawatani, Rutigliano & DeGroat, 1985*b*; Rutigliano, Kawatani & DeGroat, 1985).

The present results indicate that the depolarizing action of VIP was due to a direct action on the postsynaptic membrane because the depolarizing effect of VIP was resistant to cholinergic blocking agents and was still observed in a low-Ca^{2+} high-Mg^{2+} solution.

The ionic mechanism of the VIP-induced depolarization was not studied in detail but the effects on slope resistance suggest that a change in the conductance to a single ionic species may not be sufficient to explain the two types of resistance changes observed. A simultaneous inactivation of K^+ conductance and activation of Na^+ conductance, with both resulting in membrane depolarization, could result in different net resistance changes depending upon the relative contributions of the two currents in a given cell. The difference in resting input resistance between the two cell populations provided some support for this hypothesis since the cells with a lower resting input resistance, and thus presumably having higher resting K^+ conductance, responded to VIP with an increase in slope resistance while the neurones with a higher resting input resistance responded with a decrease in slope resistance. Thus, the effect of VIP on membrane input resistance in i.m.g. neurones differs from the effects of VIP seen in the carp retina horizontal cell (Lasater *et al.* 1983) and guinea-pig myenteric neurones (Zafirov, Palmer, Nemeth & Wood, 1985) where only a decrease and increase, respectively, were observed. There is precedence for multiple conductance changes occurring during slow muscarinic depolarization and during slow depolarization due to nerve stimulation in sympathetic ganglia (Kuba & Koketsu, 1978) including the guinea-pig i.m.g. In the guinea-pig i.m.g., both increases and decreases in input resistance during non-cholinergic slow e.p.s.p.s have been reported (Neild, 1978; Konishi *et al.* 1979*b*). It has been suggested that these conflicting results can be explained by the occurrence of multiple conductance changes including Na^+ conductance (G_{Na}) activation and K^+ conductance (G_K) inactivation (Jiang & Dun, 1981). The slow depolarization evoked by substance P also appears to be due to an interaction of G_{Na} activation and G_K inactivation

(Minota, Dun & Karczmar, 1981; Dun & Minota, 1981). More recent voltage-clamp data support the theory that substance P does not evoke slow depolarization in the guinea-pig i.m.g. by only inhibiting the M-current (Brown & Griffith, 1984).

VIP has been reported to enhance acetylcholine release from intramural nerves in the small intestine (Cohen & Landry, 1980), at the frog neuromuscular junction (Gold, 1982) and at cholinergic synapses in the myenteric plexus (Yau, Youther & Verdun, 1985). We observed no alteration in the effect of exogenous VIP during synaptic blockade. In addition, we saw no effect of VIP on the amplitude of evoked fast e.p.s.p.s or the amplitude or frequency of spontaneous e.p.s.p.s in the absence of the postsynaptic depolarization and its concomitant resistance changes. The data therefore suggested that VIP has no obvious presynaptic effect in the i.m.g. In this respect, our data supported previous observations made by Mo & Dun (1984) in the guinea-pig i.m.g. and by Kawatani, Rutigliano & DeGroat (1985*a*) in the superior cervical ganglion of the cat.

VIP has been shown to modulate cholinergic muscarinic transmission either by altering the affinity of muscarinic receptors for acetylcholine (Lundberg, 1981; Mo & Dun, 1984; Kawatani *et al.* 1985*a*), by enhancing the transduction mechanisms leading to muscarinic depolarization, or by acting in synergism with acetylcholine on the ion channels involved in the slow muscarinic depolarization (Kawatani *et al.* 1985*b*). It is unlikely that the effects of VIP seen in the present study were due to muscarinic receptor modification because the effects of VIP were observed during blockade of acetylcholine release. However, the present experiments do not rule out the possibility that VIP can facilitate other types of muscarinic receptor-mediated responses.

Although VIP did not affect nicotinic transmission directly, VIP did increase ganglion cell excitability when the ganglion received on-going mechanosensory input from the colon. Since no presynaptic effect on the amplitude or frequency of fast nicotinic e.p.s.p.s was observed, and since VIP altered the passive membrane properties of post-ganglionic neurones, we concluded that the facilitation observed in the presence of VIP was due to movement of the membrane potential closer to the threshold for firing of individual fast nicotinic synaptic potentials. In this sense, the facilitatory action of VIP in the guinea-pig i.m.g. was non-selective. In those cells in which the VIP-induced depolarization was due to an increase in membrane input resistance, the facilitatory effect may also have been due to an increase in the amplitude of the individual e.p.s.p.s, as would be expected from the change in resistance. The present experiments do not provide evidence either in favour or against this notion. Conversely, in those cells in which there was a decrease in membrane input resistance, the amplitude of the individual fast e.p.s.p.s would be expected to be reduced. However, the data indicated that the shift of the membrane depolarization nearer to threshold for firing action potentials was a more important factor in determining whether an action potential would occur than the decrease in resistance responsible for depolarization.

The physiological significance of the present study with regard to synaptic transmission in the guinea-pig i.m.g. was that non-cholinergic slow depolarizations may be due to release of VIP. Previously, non-cholinergic slow depolarizations observed in the guinea-pig i.m.g. during electrical stimulation of preganglionic

nerves have been attributed solely to release of substance P from the peripheral processes of primary afferent neurones located in dorsal root ganglia (Konishi *et al.* 1979*b*; Matthews & Cuello, 1984). However, substance P or a related peptide may not be the only endogenous transmitter which causes the non-cholinergic e.p.s.p. Pretreatment with capsaicin, which almost completely depletes the guinea-pig i.m.g. of substance P-like immunoreactivity (Dalsgaard, Vincent, Schultzberg, Hökfelt, Elfvin, Terenius & Dockray, 1983*b*), and desensitization to exogenously applied substance P depress non-cholinergic slow e.p.s.p.s by only 50% (Peters & Kreulen, 1984), suggesting the involvement of other peptides. In the present study, complete suppression of the slow non-cholinergic depolarization induced by electrical nerve stimulation also was not observed either during desensitization to exogenously added VIP or during the presence of VIP antiserum. Furthermore, VIP antiserum decreased the amplitude of the slow, non-cholinergic e.p.s.p. in only 40% of the cells studied. Thus, based on the evidence obtained in this and previous studies (Peters & Kreulen, 1984), it appears that both substance P and VIP may be transmitters for electrically evoked, slow non-cholinergic e.p.s.p.s in the guinea-pig i.m.g. In addition to these two peptides, bombesin, cholecystokinin (Dalsgaard *et al.* 1983*b*), neurotensin (Reinecke, Forssmann, Thiekötter & Triepel, 1983) and dynorphin (Vincent, Dalsgaard, Schultzberg, Hökfelt, Christensson & Terenius, 1984) are contained in nerve fibres in the i.m.g. of the guinea-pig. The role of these peptides in electrically evoked, slow non-cholinergic e.p.s.p.s awaits future investigation.

The physiological significance and relevance of the present study with regard to colonic motility lies in the observation that distension of the colon, a 'physiologic' stimulus, resulted in a non-cholinergic depolarization that was partially antagonized by VIP-specific antiserum. These results suggested the occurrence of a mechanosensory afferent pathway which utilized VIP or a VIP-like substance as a transmitter. Thus, in addition to acetylcholine and substance P (Peters & Kreulen, 1986), VIP should also be considered a candidate transmitter mediating colonic reflexes in the i.m.g.

When the data from the present study are considered together with other studies on the physiological role of the i.m.g., the following picture emerges. In addition to receiving cholinergic mechanosensory input, neurones in the i.m.g. also receive mechanosensory inputs which appear to utilize substance P and VIP. Like the cholinergic pathway, the mechanosensory transducer lies in the wall of the colon and functions as tension mechanoreceptors (Kreulen & Peters, 1986). Since the slow non-cholinergic depolarization induced by radial distension of the colon recorded in any one neurone is not completely blocked after interruption of either substance P or VIP transmission, it appears that individual neurones receive more than one type of non-cholinergic afferent input. The depolarization and increase in excitability exerted by substance P and VIP would be expected to amplify central and peripheral synaptic inputs arriving in the i.m.g., thereby enhancing sympathetic inhibitory drive to the colon. In contrast, the central enkephalinergic pathway represents a second-order modulating system by virtue of its presynaptic inhibitory action on substance P and cholinergic nerve fibres. The inhibitory effect of the enkephalinergic pathway leads to a decrease in sympathetic inhibitory drive to the colon and thereby an increase in colonic motility (Shu *et al.* 1987). Thus, central and peripheral peptidergic and

cholinergic nerve fibres play an integral role in regulating colonic activity. The physiological role of the other peptidergic pathways identified as containing dynorphin-, neurotensin-, cholecystokinin- and bombesin-like immunoreactivity remains to be determined.

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