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MECHANISM OF ACTION OF VASOACTIVE INTESTINAL POLYPEPTIDE ON MYOMETRIAL SMOOTH MUSCLE OF RABBIT AND GUINEA-PIG

BY T. B. BOLTON, R. J. LANG AND B. OTTESEN*
From the Department of Pharmacology, St. George's Hospital Medical School, London SW17 0RE

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SUMMARY

1. The action of vasoactive intestinal peptide (VIP) on the electrical and mechanical activity of strips of longitudinal myometrial smooth muscle from rabbits and guinea-pigs treated with oestradiol was studied in the sucrose-gap apparatus.

2. In myometrial strips which spontaneously exhibited regular contractions, or which were induced to contract rhythmically by the application of oxytocin, VIP reduced both the frequency and the force of contraction.

3. Contractions were associated with bursts of action potential discharge. In guinea-pig, the membrane potential reached its most negative value shortly following a burst and a slow decay of negativity followed ('generator potential'). VIP inhibited the decay of this negativity and increased the duration of the period between bursts. In rabbit myometrical strips, electrical discharges occurred less regularly but VIP also had an inhibitory action. The inhibitory action of VIP was not affected by the β-adrenoreceptor blocker propranolol, by tetrodotoxin, or by apamin.

4. Using the double sucrose-gap apparatus, bursts of action potentials and contractions were elicited with depolarizing electrical pulses in the absence of oxytocin. Changes in membrane resistance were also estimated by eliciting hyperpolarizing electrotronic potentials. VIP hyperpolarized the membrane and inhibited contractions as depolarizing pulses now failed to reach threshold for action potential discharge or fewer action potentials were discharged. A small (about 10%) reduction in membrane resistance was frequently observed during the hyperpolarization.

5. If a single action potential was elicited in the presence of VIP, the tension generated by the muscle was less than in its absence.

6. In a calcium-free high-potassium (126 mM) solution, readmitting calcium produced contraction; VIP inhibited this contraction. Activation of β-receptors by means of isoprenaline had a similar effect but unlike isoprenaline the action of VIP was not blocked by propranolol.

7. It is suggested that the primary action of VIP is on the calcium economy of the myometrial smooth muscle cell, possibly to accelerate sequestration and/or extrusion of calcium from the cell. In some way this is associated with inhibition of

* Present address: Institute of Medical Physiology B, Panum Institute, University of Copenhagen, Denmark.

the generator potential, hyperpolarization, and with a small increase in permeability of the membrane to potassium.

INTRODUCTION

Vasoactive intestinal peptide (VIP) was first isolated from porcine intestine by Said & Mutt (1970). It is a peptide of twenty-eight amino acid residues structurally related to glucagon and secretin (Mutt & Said, 1974). VIP is known to relax smooth muscle in a variety of organs and tissues e.g. blood vessels (Said & Mutt, 1970; Piper, Said & Vane, 1970), gastro-intestinal tract (Morgan, Schmalz & Szurszewski, 1978), gall-bladder (Piper *et al.* 1970), trachea (Piper *et al.* 1970) and uterus (Ottesen, Ulrichsen, Wagner & Fahrenkrug, 1979).

A number of groups have produced evidence which could support a transmitter role for VIP. By means of immunochemistry or radioimmunoassay it has been shown that appreciable VIP-like immunoreactivity is present in brain, gastrointestinal tract, salivary glands, pancreas and other tissues (Fahrenkrug, 1979). Immunohistochemistry has revealed VIP-like immunoreactive substance associated with the nerves in these tissues and with nerves to the smooth muscle of blood vessels in several vascular beds (e.g. Larsson, Fahrenkrug, Schaffalitzky de Muckadell, Sundler, Håkanson & Rehfeld, 1976; Alm, Alumets, Håkanson, Owman, Sjöberg, Sundler & Walles, 1980; Edvinsson, Fahrenkrug, Hanko, Owman, Sundler & Uddman, 1980; see Fahrenkrug, 1979 for a review). Such nerves, containing VIP or VIP-like immunoreactive substance apparently arise at least in some situations from cell bodies outside the central nervous system and close to the organ innervated (Alm *et al.* 1980; Jessen, Polak, van Noorden, Bloom & Burnstock, 1980).

Stimulation of autonomic nerves supplying a number of organs results in an increase in the amount of VIP-like immunoreactive substance in the venous effluent. In the cat colon, vasodilation resulting from nerve stimulation, or mechanical stimulation of the mucosa, was associated with increased release of VIP-like immunoreactive material (Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Schaffalitzky de Muckadell, 1978). Essentially similar results have been obtained in response to stimulation of vagal fibres (Edwards, Bircham, Mitchell & Bloom, 1978) and in opossum, relaxation of the lower oesophageal sphincter upon vagal nerve stimulation was reduced by VIP antiserum (Goyal, Rattan & Said, 1980). Thus, it could be that VIP is present in nerves from where it is released during nerve discharge; upon release it may cause relaxation of the smooth muscle of blood vessels (i.e. vasodilation) or inhibition of smooth muscle activity in the organ innervated. VIP is considered by some to be a candidate for the non-adrenergic inhibitory transmitter in the gut (Fahrenkrug *et al.* 1978).

VIP has been found to inhibit contractions of myometrial smooth muscle from a number of mammalian species (Ottesen *et al.* 1979; Ottesen, Wagner & Fahrenkrug, 1980; Ottesen, Larsen, Fahrenkrug, Stjernqvist & Sundler, 1981). It occurs in appreciable amounts in the corpus uteri of rabbits but less is found in the corpus uteri of guinea-pigs (Alm *et al.* 1980; Ottesen *et al.* 1981). VIP-like immunoreactivity was associated with nerves supplying myometrial smooth muscle, particularly towards the cervix; more was found in the rabbit than in the guinea-pig (Ottesen *et al.* 1981).

Little is known about the mechanism of action of VIP on smooth muscle. It would appear to exert a direct action which is generally inhibitory (cf. Cohen & Landry, 1980). In canine antrum, Morgan *et al.* (1978) found that VIP uncoupled electromechanical coupling during spontaneous and acetylcholine-induced electrical and mechanical activity. A brief report of the present investigations has been made to The Physiological Society (Bolton, Lang & Ottesen, 1981).

METHODS

Rabbits and guinea-pigs were pretreated with 50–100 μg oestradiol benzoate (Intervet Laboratories Ltd) daily or on alternate days for 3–7 days. Animals were killed, bled and the cervical ends of the uterine horns excised. Strips of longitudinal muscle (1–2 mm wide, 1–2 cm long) were dissected and the mucosal layer was removed.

Tension recordings

Single strips were suspended either in a 5 ml. organ bath and attached to an isometric tension transducer (FT03C Grass Instruments) or suspended in a small vertical tube and connected to an isotonic tension transducer (George Washington Ltd.) (Brading & Sneddon, 1980; Bolton & Clark, 1981). During isotonic tension recordings the strips were constantly perfused (at 2 ml./min) with Krebs solution warmed to 37 °C and previously bubbled with 95 % O_2 and 5 % CO_2. Rapid solution changes could be made by transfering the inflow tube from one solution to another. During isometric tension recordings in the organ bath the bathing solution was stationary, the solution being warmed to 37 °C and oxygenated constantly. Drugs were added in a cumulative manner.

Electrical Recordings

Intracellular recordings were made at 35 °C from uterine strips with glass micro-electrodes, filled with 3 M-potassium chloride and having resistances of 30–60 MΩ, in a small bath as described previously (Bolton, 1972).

Extracellular recordings of electrical activity at 35 °C were made in the single or double sucrose gap in a manner similar to that described by Bülbring & Tomita (1969). In the sucrose gap, tension was recorded by connecting one end of the myometrial strips to an isometric force transducer (AE 803, Aksjeselskapet Mikro-electronikk, Horten, Norway, in a circuit described by Eisner & Lederer, 1979). The rate of flow of the Krebs solution was kept constant at about 2 ml./min. Drugs were applied in a small volume by slow injection by means of a motor driver syringe into the flowing solution or by adding them to the solutions in the reservoirs.

Solutions

A modified Krebs solution was used of the following composition (mM): Na^+ 137, K^+ 5·9, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 133·6, HCO_3^- 15·4, $H_2PO_4^-$ 1·2, glucose 11·4. This solution was usually warmed to 37 °C and bubbled with a 95 % O_2 and 5 % CO_2 gas mixture creating a pH of about 7·2. In experiments where calcium contractures were studied a high-potassium depolarizing solution containing 126 mM-potassium and 17 mM-sodium was used. Where calcium-free solutions were used, calcium (and a corresponding amount of chloride) was omitted. When studying the release of calcium by stimulants such as carbachol or oxytocin, the calcium stores were filled by incubating for a short time in a solution in which potassium concentration was raised to 47·2 mM so reducing the sodium concentration to 79 mM. Calcium contractures were studied in magnesium-free solution. In experiments the results of which are summarized in Fig. 1 a modified Krebs solution of the following composition (mM) Na^+ 143, K^+ 5·5, Ca^{2+} 2·5, Mg^{2+} 1·0, Cl^- 127·5, SO_4^{2-} 1·0, HCO_3^- 25, $H_2PO_4^-$ 1·0, glucose 6·0, with a pH of 7·4 was used.

Drugs

Apamin (Serva), carbachol chloride (Sigma) DL isoprenaline sulphate (Burroughs Wellcome & Co.) oxytocin (Sandoz) propranolol hydrochloride (I.C.I.), tetrodotoxin (Sigma) and vasoactive intestinal (poly) peptide (purified porcine VIP, from V. Mutt, Karolinska Institutet, Stockholm, Sweden (Mutt & Said, 1974)).

RESULTS

Organ bath studies

Strips of longitudinal myometrial smooth muscle from rabbit or guinea-pig showed spontaneous contractions within 1 hr when suspended in oxygenated physiological salt solution at 37 °C. If VIP was applied to a quiescent muscle it produced no change in tension or length. Regular contractions could be elicited by introducing oxytocin (2×10^{-8} M) to the bathing solution. The frequency and force of these was reduced

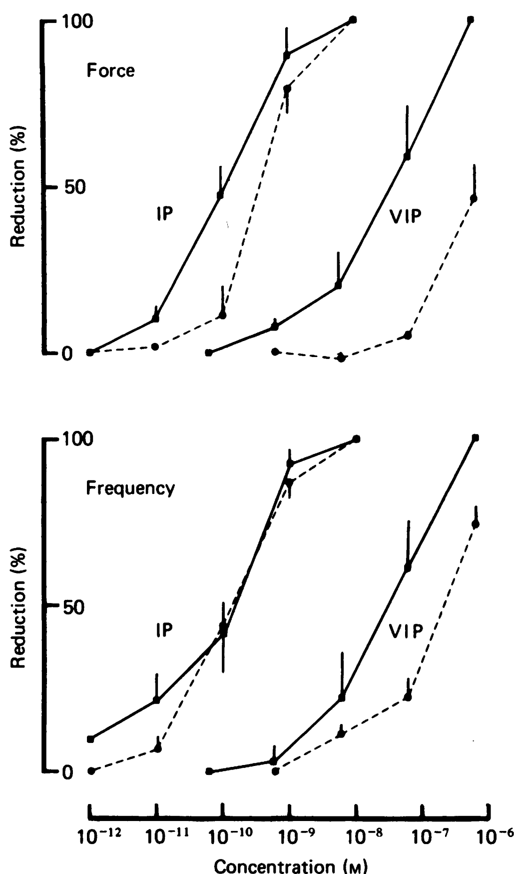

Fig. 1. Effects of VIP and isoprenaline (IP) on the force and frequency of isometric contractions of myometrial strips from (■——■) rabbit and (●---●) guinea-pig. Regular contractions were first elicited by the addition of 2×10^{-8} M-oxytocin to the bath and VIP or IP were added cumulatively. The values plotted are the means and 1 S.E. of results on six to nine strips from at least three animals.

by the addition of VIP ($6 \times 10^{-9} - 6 \times 10^{-7}$ M) to the bathing solution. If contractions were abolished this was taken as 100% reduction in frequency and force. The frequency and force of contractions of rabbit myometrial strips were about equally sensitive to the inhibitory action of VIP and more sensitive than guinea-pig myometrial strips. In the latter frequency was slightly more sensitive than tension. Strips from both species were, however, at least a 100 times more sensitive to the inhibitory action of isoprenaline, a β-receptor stimulant (Fig. 1).

The inhibitory action of VIP was not due to a specific antagonism at the oxytocin receptor as similar inhibitions were produced of regular contractions elicited by the addition of carbachol, by raising the external potassium concentration, or by prostaglandin (see Ottesen *et al.* 1980). The inhibitory action of VIP in the presence of these stimulating agents was studied in less detail.

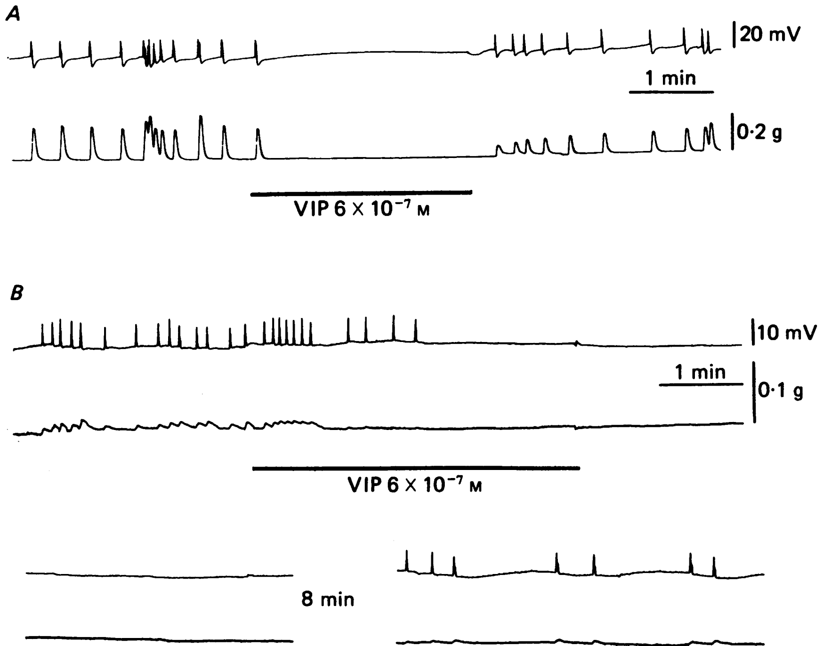

Fig. 2. Effect of VIP on electrical and mechanical activities of rabbit myometrial strips recorded with the single sucrose gap. In *A* oxytocin (4×10^{-9} M) was infused throughout. In *B* activity was spontaneous. Upper and lower records in *B* are continuous but an 8 min section of the lower record has been omitted.

Electrophysiological studies

Strips of rabbit or guinea-pig longitudinal myometrial muscle about 1 mm wide were introduced into a form of single sucrose-gap apparatus. Sometimes they exhibited spontaneous contractions (Fig. 2*B*) but generally they were quiescent. Contractions were induced in quiescent preparations by the addition of 2×10^{-9}–10^{-8} M-oxytocin to the solution. In rabbit, contractions occurred less regularly than in guinea-pig. Each contraction was associated with one or more action potentials of up to 20 mV in size when recorded extracellularly in the sucrose gap, often followed by an apparent hyperpolarization. This was probably caused by contraction altering the size of the junction potentials between solutions which are a feature of this type of recording (Fig. 2*A*). A few experiments were done in which the membrane potential was recorded in rabbit myometrium with intracellular micro-electrodes. No action potentials were observed if the muscle was not stimulated with oxytocin. If oxytocin was applied (4×10^{-9} M) then action potentials were discharged, generally in bursts

of two or three, without conspicuous slow waves of depolarization as seen in guinea-pig (see Fig. 4). The membrane potential was about 55 mV and the action potentials could show 5–10 mV overshoot (Fig. 3). They were often up to several hundred milliseconds in duration measured at their half maximum size.

In the guinea-pig, contractions were generally of greater duration than in the rabbit and the electrical changes associated with these complex. Generally they consisted

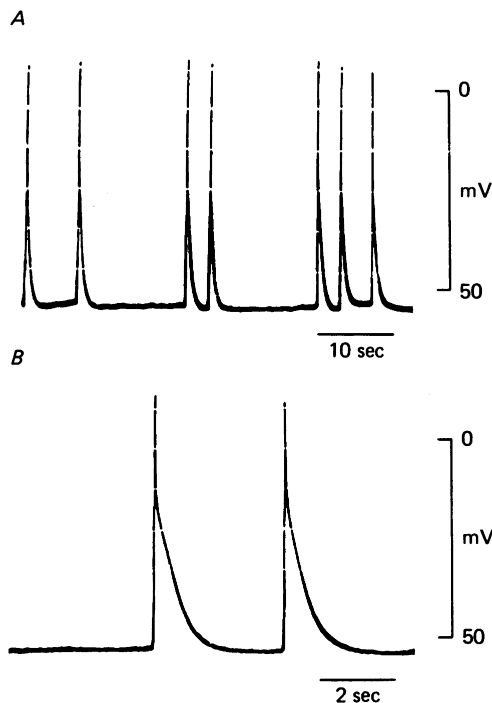

Fig. 3. Intracellular micro-electrode recordings of oxytocin (4×10^{-9} M) induced electrical activity of rabbit myometrial smooth muscle. *A*, activity seen at slower sweep speed consists of sporadic bursts of two or three action potentials. *B* shows single action potentials at faster sweep speed.

of an abrupt depolarization or spike followed by a plateau which gave way to oscillations of potential that developed into small action potentials. After a burst of these the membrane repolarized abruptly and then more slowly to reach a peak of negativity. A slow decline of this negativity then occurred ('generator potential') preceding the next action potential burst (Fig. 4*A*, *B*).

VIP (6×10^{-7} M) inhibited contractions and the associated discharge of action potentials. At lower concentrations (6×10^{-9}–6×10^{-8} M) in the rabbit the frequency of contractions and their associated bursts of action potentials were reduced. The force of contractions occurring during the action of VIP was also reduced and this was often associated with a reduction in the number of action potentials in a burst. A concentration of 6×10^{-7} M-VIP generally arrested contractions in rabbit (Fig. 2) and, during recovery, contraction force was reduced. Concentrations of VIP much below 6×10^{-7} M had little effect in the guinea-pig but this concentration increased

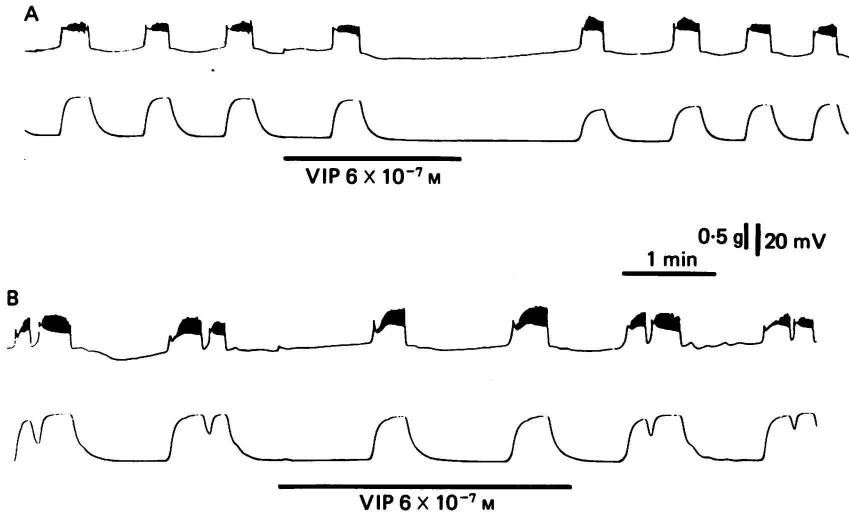

Fig. 4. Effect of VIP on the electrical and mechanical activities of guinea-pig myometrial strips recorded with the single sucrose gap. VIP inhibits contractions (*A*) or reduces their duration (*B*). In both records VIP reduces the rate of rise of the 'generator potential'.

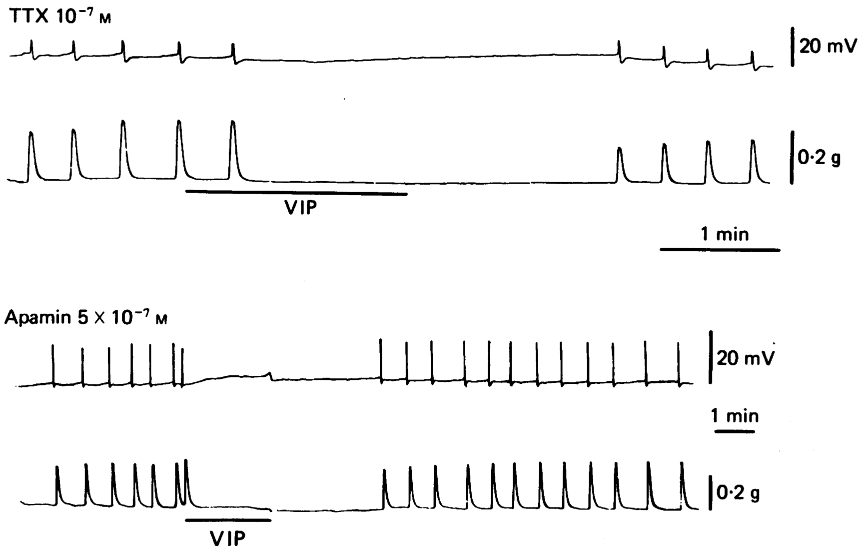

Fig. 5. The action of VIP (6×10^{-7} M) is unchanged in the presence of tetrodotoxin (TTX, 10^{-7} M) or apamin (5×10^{-7} M). Single sucrose gap records.

the period between contractions (Fig. 4*A*) or reduced their duration (Fig. 4*B*). The main effect of VIP in guinea-pig was to reduce the rate of rise of the generator potential or inhibit it for a period.

The inhibitory actions of VIP (6×10^{-7} M) were not apparently affected if the solution contained TTX (10^{-7} M) apamin (5×10^{-7} M) or propranolol (10^{-6} M) (Fig. 5).

These results indicate that VIP is unlikely to exert its effects indirectly via nerves and has its effects via a receptor distinct from the β-adrenoreceptor. As apamin blocks the actions of several substances which apparently increase the potassium permeability of smooth muscle (non-adrenergic inhibitory transmitter, α-receptor stimulants and ATP; Vladimirova & Shuba, 1978; Banks, Brown, Burgess, Burnstock, Claret, Cocks

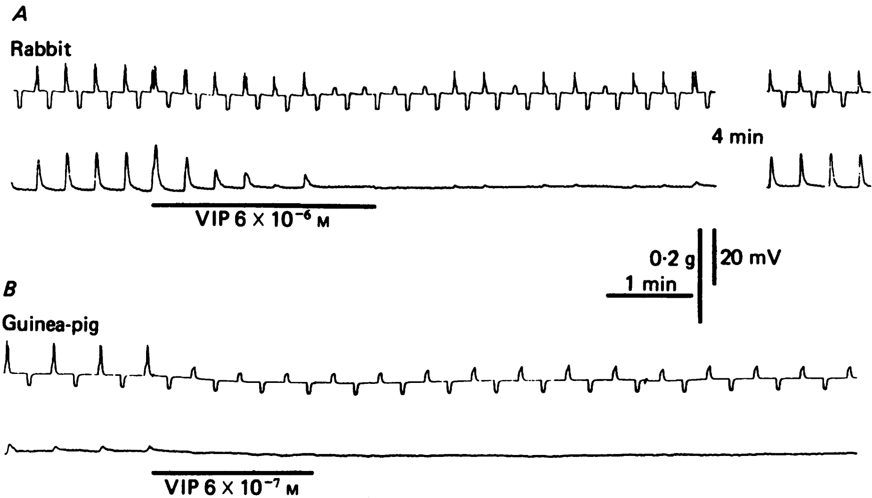

Fig. 6. Effect of VIP on the membrane potential and conductance of myometrial strips in the double sucrose gap. *A*, rabbit; depolarizing pulses were 5×10^{-6} A, hyperpolarizing pulses were 5×10^{-6} A. *B*, guinea-pig; depolarizing pulses were 9×10^{-8} A, hyperpolarizing pulses were 5×10^{-7} A.

& Jenkinson, 1979; Maas & den Hertog, 1979; Shuba & Vladimirova, 1980) this result indicates that if VIP increases potassium permeability then this is not essential to its inhibitory action or alternatively, the potassium channels opened by VIP are not sensitive to blockade by apamin.

Effects on membrane conductance. Experiments were done in the double sucrose gap apparatus in which rectangular depolarizing electrical pulses (2–3 sec duration) were applied every 30 sec or so. These elicited action potential discharge and contraction. Alternating with these, similar hyperpolarizing pulses were applied which gave rise to electrotonic potentials. In the guinea-pig, VIP (6×10^{-7} M) produced a small hyperpolarization of the membrane (2–5 mV) during which in several preparations a distinct but small (10–15 %) reduction in the hyperpolarizing electrotonic potential was observed (Fig. 6*B*). The reduction in the size of the electrotonic potential indicates an increase in the membrane conductance brought about by VIP and the hyperpolarization of the resting membrane suggest that this involves an increase in the potassium permeability. The effect of this hyperpolarization was to shift the membrane potential during depolarizing current pulses below threshold so that action potentials were not generated and contractions did not occur. VIP also produced hyperpolarization in solutions of raised (20 mM) or lowered (1·2 or 0·6 mM) potassium concentration. However, hyperpolarizations were too small and not sufficiently consistent for any systematic effect of varying the potassium concentration to be

detected. In unpublished experiments, C. Benham has observed in this laboratory that 10^{-6} M-VIP produced a small increase in the efflux of ^{86}Rb from quiesient strips of guinea-pig myometrium, suggesting an increase in potassium permeability.

In the rabbit, 6×10^{-6} M-VIP was needed to produce similar effects to those described above in the guinea-pig on membrane potential and conductance even

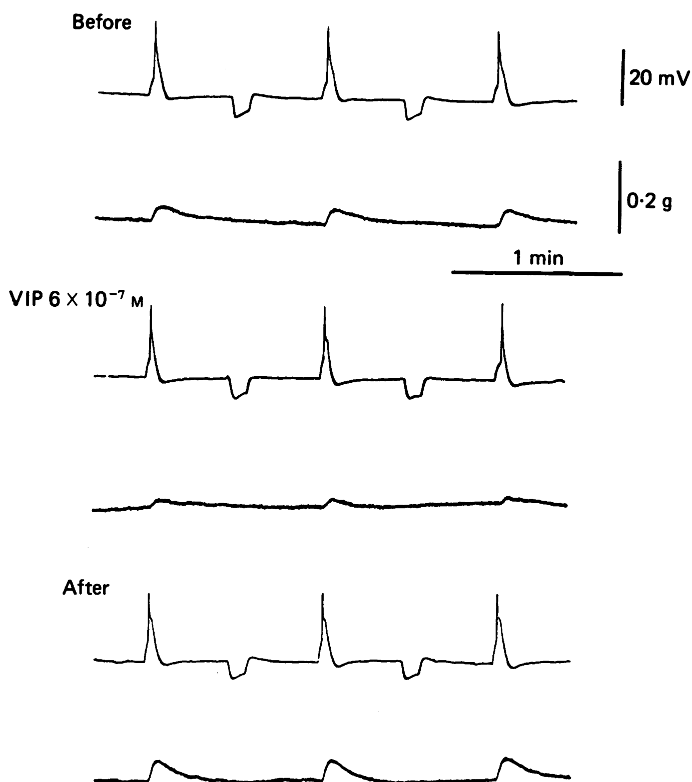

Fig. 7. VIP reduces the force of contraction elicited by a single action potential in the double sucrose gap. In this experiment the hyperpolarizing electronic potential was not detectably affected.

though rabbit strips were generally more sensitive to the inhibitory actions of VIP on spontaneous and oxytocin-stimulated contractions. Frequently during the action of VIP, the depolarizing pulse elicited fewer action potentials and smaller contractions. However, it was noticed that if a single action potential was elicited its associated tension response was less in the presence of VIP (Fig. 6*A*).

Tension response to a single action potential. The effect of VIP (6×10^{-7} M) on the tension response to a single action potential evoked by a depolarizing pulse was examined in more detail in guinea-pig strips. Depolarizing pulse strength and duration were selected so that a single action potential was evoked in the absence of VIP. This was associated with a small phasic contraction (Fig. 7). VIP was then introduced. Generally it was necessary to increase pulse strength to evoke a single similar, matching, action potential in the presence of VIP. The tension response

associated with this was reduced. After returning to VIP-free solution the tension response recovered.

These experiments suggested that the rise in intracellular calcium associated with the discharge of an action potential may be attenuated during the action of VIP. Similar results to those described here for VIP, have been reported for β-adrenoreceptor activation by isoprenaline in taenia smooth muscle by Bülbring & Kuriyama (1973). It seems less likely, although possible, that VIP somehow reduces the inward flux of calcium across the cell membrane associated with an action potential, because the

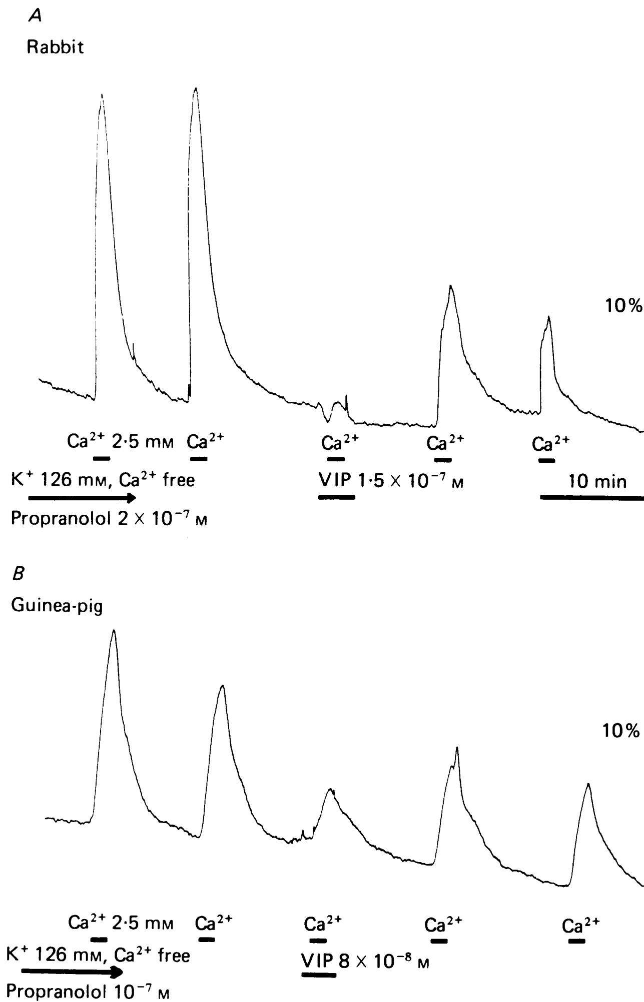

Fig. 8. Effect of VIP on calcium contractures in rabbit (*A*) and guinea-pig (*B*) myometrial strips. In a high-potassium (126 mM) calcium-free solution, calcium (2·5 mM) was readmitted for 90 sec (as indicated by the short bars) producing shortening which was initially between 10 and 20 % of that obtained with oxytocin (10^{-8} M) (Vertical bar indicates 10 % of this maximal shortening). VIP at the indicated concentration was introduced shortly before readmitting calcium and reduced or abolished the contracture. Partial recovery of the calcium contracture only was achieved after returning to VIP-free solution. Both experiments were done in the presence of propranolol.

electrical record (albeit extracellular and so subject to alterations in spatial and temporal dispersion within the node) was essentially unchanged in VIP.

Effects on calcium contractures and oxytocin contractions

Readmitting calcium to a calcium-free high-potassium solution produced a contracture. This presumably results primarily from the influx of calcium into the cell

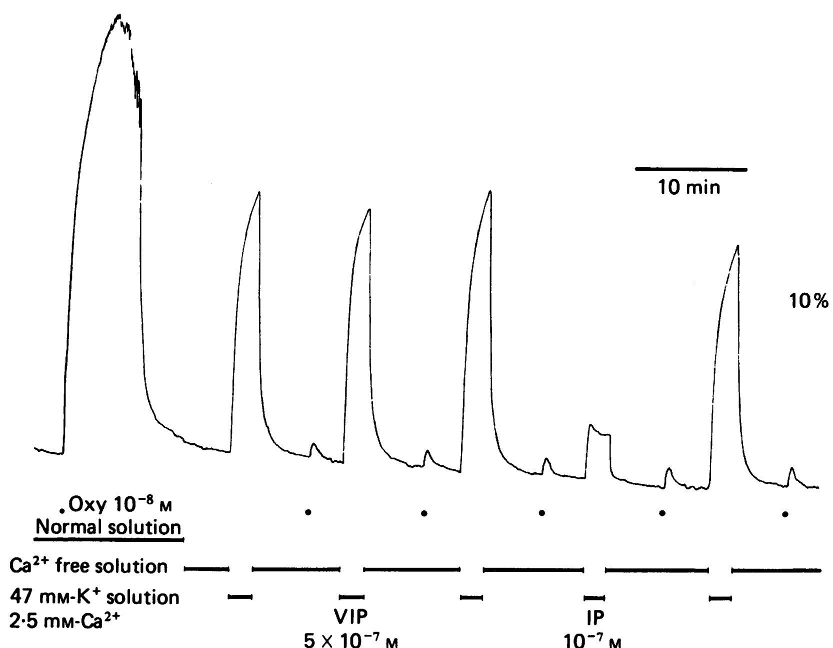

Fig. 9. Effect of VIP or isoprenaline (IP) on refilling of drug-releasable calcium stores in guinea-pig myometrial strips. Responses to oxytocin (Oxy, 10^{-8} M, applied for 10 sec at ●) were very small 5 min after a 2 min application of a high-potassium (47·2 mM) calcium-containing (2·5 mM) solution indicating that drug-induced release of stored calcium in myometrium was small. The presence of VIP (5×10^{-7} M) or isoprenaline (IP, 10^{-7} M) during the application of high-potassium solution reduced its contractile effect (very slightly in the case of VIP) and in contrast to taenia (not shown) the subsequent response to oxytocin was little, if at all, affected. In this record the vertical calibration is 10% of resting length.

under these conditions (Jenkinson & Morton, 1967; Ohashi, Ohga & Saito, 1973). VIP ($6 \times 10^{-8} - 6 \times 10^{-7}$ M) reduced or blocked the contracture which occurred upon readmitting calcium in both guinea-pig and rabbit strips (Fig. 8). Isoprenaline ($1\text{–}2 \times 10^{-7}$ M) produced similar effects but its action, unlike that of VIP, was abolished by propranolol ($1{\cdot}2 \times 10^{-7}$ M) a β-receptor blocking agent. In calcium-free high-potassium solution the muscle often continued to lengthen for 30 min or more, so that calcium contractures were observed on a falling base line. Also, the size of the calcium contracture declined with repetition or time so that complete recovery of response size after inhibition by VIP was not achieved.

In taenia it has been described how responses to high concentrations of carbachol persist for a period in calcium-free solutions (Ohashi, Takewaki & Okada, 1974;

Casteels & Raeymaekers, 1979; Brading & Sneddon, 1980). This suggests that under these conditions carbachol, acting via muscarinic receptors, can release calcium from storage sites within the cell. Such releasable stores can be filled by prior incubation in a high-potassium calcium containing solution. We have been able to confirm that β-receptor activation with isoprenaline, while reducing the contractile response to high-potassium solution in taenia (cf. Jenkinson & Morton, 1967) potentiated the contraction to a subsequent addition of carbachol in calcium-free solution (composition otherwise normal). However, we have been unable to demonstrate appreciable potentiation in myometrial strips although isoprenaline produced a large reduction of the potassium contraction (Fig. 9). Similar experiments were done with VIP in guinea-pig strips. VIP produced at most only a small reduction in the potassium contraction in concentrations up to 5×10^{-7} M and no noticeable potentiation of the response to a subsequent test with oxytocin given in calcium-free solution (Fig. 9). Carbachol was less potent than oxytocin in producing contractions in calcium-free solution, or normal solution. These results may indicate that if myometrial smooth muscle has significant calcium stores they are not readily released by activation of oxytocin or muscarinic receptors.

DISCUSSION

These experiments suggest that VIP interferes with the calcium economy of the smooth muscle cell. It cannot be definitely excluded that VIP reduces the influx of calcium through ion channels which open during the discharge of an action potential, or which are permanently open in high-potassium solution, since these may be the same population of ion channels (Bolton, 1979, p. 628; but see Shuba, 1980). However, as the electrical discharge, a single evoked action potential, was ostensibly unchanged in the presence of VIP, such an explanation would imply that VIP shifted the balance of ions carrying inward current so that less calcium and more of another cation (such as sodium) was responsible for the action potential in the presence of VIP. This seems less likely than that VIP attenuates the rise in intracellular calcium which occurs upon action potential discharge, or upon restoring calcium to a calcium-free high-potassium solution; it may do this by accelerating calcium sequestration within the cell, by increasing its extrusion, or both. Such a mechanism as envisaged would require sufficiently vigorous calcium pumping mechanisms so that a substantial proportion of calcium entering the cell, or released from stores within it, fails to reach the contractile proteins under these conditions. It is also feasible that VIP may act intracellularly to reduce the sensitivity of the contractile proteins to intracellular calcium, but at present there is no evidence for this. A similar explanation to the one advanced above has been invoked to account for the inhibitory effects of β-receptor activation. Both the contractile responses to a single action potential (Bülbring & Kuriyama, 1973; Bülbring & den Hertog, 1980) and to readmitting calcium to a calcium-free high-potassium solution (Jenkinson & Morton, 1967; Ohashi *et al.* 1973) are reduced by β-receptor activation. However, we have been unable to show that VIP at 5×10^{-7} M enhances the storage of calcium *releasable* by a subsequent dose of stimulant in uterus, although we could confirm that β-receptor activation produces such an effect in taenia (Casteels & Raeymaekers, 1979). β-receptor activation in

myometrium also apparently failed to significantly affect responses to strong stimulants in calcium-free solution in our hands, so it may be that stimulants (we used oxytocin and carbachol) are less able to release bound calcium in uterus than in taenia.

Morgan *et al.* (1978) observed effects of VIP on canine gastric antral smooth muscle. They found no effect of VIP on resting membrane potential or action potential shape (except a small curtailment of the plateau when its duration was increased by pentagastrin) but an appreciable reduction in contractile force, however engendered. These observations of Morgan *et al.* (1978) using lower concentrations of VIP fit well with ours; it was with higher concentrations that we detected effects on membrane potential and conductance. Interestingly, however, Morgan *et al.* observed that VIP *increased* the frequency of contraction by a maximum of about twofold. Morgan *et al.* also postulated that VIP interfered with calcium movements, possibly accelerating calcium storage within the cell.

It is, nevertheless, obscure how the postulated action of VIP on calcium sequestration/binding is related to the suppression of action potential discharge which was seen. It is noteworthy that a similar type of effect is seen upon β-receptor activation in uterus (Bülbring & Tomita, 1969) and both β-receptor activation and VIP increase cyclic AMP levels in smooth muscle (Frandsen, Krishna & Said, 1978). VIP apparently reduced the rate of rise of the 'generator potential' in guinea-pig strips and may well have exerted a similar action in rabbit myometrium but the electrophysiological records using external recording were less satisfactory and the electrical activity less regular, so that such an effect may have been obscured. The frequency of electrical discharges was clearly reduced, and this could have resulted from an action on some pacemaker region similar to that described for guinea-pig.

In both species an effect on membrane conductance was only demonstrable at higher concentrations and even then the effect was small. Again, parallel observations have been made with β-receptor activation (myometrium, Diamond & Marshall, 1969; Kroeger & Marshall, 1973; Marshall, 1977; taenia, Bülbring & den Hertog, 1980). It may be that a small and essentially undetectable effect of VIP on membrane conductance at lower concentrations is the basis of its inhibitory effect on the 'generator potential' although this does not seem very likely. We are inclined to think that the effect on the membrane conductance probably represents a small increase in the potassium permeability of the membrane, an epiphenomenon not directly related to the inhibitory action on action potential discharge, but possibly a reflection of increased calcium binding to it. At present such ideas must be regarded as highly speculative. It is quite striking, nevertheless, that the effects of VIP and β-receptor activation are at the same time similar, and yet diverse, implying a common and as yet unknown causation.

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