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THE EFFECT OF APAMIN ON NON-ADRENERGIC, NON-CHOLINERGIC VASODILATOR MECHANISMS IN THE INTESTINES OF THE CAT

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SUMMARY

1. The effects of apamin, a polypeptide isolated from bee venom, on different vasodilator mechanisms in the small and large intestines were studied in atropinized cats.

2. In the large intestine vasodilatation in response to pelvic nerve stimulation was either abolished or markedly diminished by I.A. apamin. However, neither the contraction of colonic muscle which occurred under these conditions nor sympathetic vasoconstriction was significantly influenced by apamin, suggesting that the effect of the peptide was not a non-specific effect on nerves or vascular smooth muscle.

3. In the small intestine it was observed that the nervous vasodilatation induced by transmural electrical field stimulation or mechanical mucosal stimulation was either diminished or abolished by apamin.

4. Intestinal vasodilatation, caused by close I.A. infusions of 5-hydroxytryptamine (5-HT), was abolished by apamin. After giving apamin 5-HT infusions induced a vasoconstriction in five out of six experiments.

5. Vasodilatation induced by vasoactive intestinal polypeptide (VIP) was not significantly affected by apamin.

6. In a series of *in vitro* experiments on rat portal vein, dose–response curves of several putative intestinal neurotransmitters were determined in the presence and absence of apamin. The following substances were tested: VIP, substance P, bradykinin, 5-HT, ATP and adenosine. Apamin had no effect on the dose–response curves of any of these compounds.

7. The results are discussed in relation to the possibility that apamin may act by blocking the release of a putative peptidergic transmitter from nerve terminals.

INTRODUCTION

Historically autonomic nervous control of visceral function has been supposed to be mediated via adrenergic and cholinergic nerve fibres. However, experimental studies performed during the last two decades have demonstrated that autonomic control is also exerted via nerves which release other types of transmitter or

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transmitters. With regard to the enteric nervous system, an excellent recent review by Furness & Costa (1980) should be consulted for details of these developments.

In the gastrointestinal tract several functions have been ascribed to non-cholinergic, non-adrenergic nerves, such as the receptive relaxation of the stomach (Martinson, 1965; Jansson, 1969; Abrahamsson, 1973), intestinal relaxation accompanying the peristaltic reflex (Hirst, 1979), relaxation of the taenia coli evoked by transmural field stimulation (Bennett, Burnstock & Holman, 1966), intestinal vasodilatation produced by mechanical mucosal or electrical field stimulation (Biber, Jodal, Lundgren & Svanvik, 1970) and the colonic hyperaemia and muscular contraction seen during pelvic nerve stimulation (Hultén, Jodal & Lundgren, 1969). Several neurotransmitters have been proposed to mediate these mechanisms including adenosine triphosphate (ATP; Burnstock, 1972), vasoactive intestinal polypeptide (VIP; Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Schaffalitzky de Muckadell, 1978) and kinins (Fasth, 1973).

In the further analysis of such putative transmitters it would be of great advantage to be able to block the non-adrenergic, non-cholinergic responses described above. This report describes experiments on cats in which the vasodilator mechanisms in the small and large intestines have been abolished by the administration of apamin, a polypeptide originally isolated from bee venom (Haberman, 1972). These observations initiated a series of *in vitro* experiments on the isolated portal vein in an attempt to elucidate a possible receptor-blocking effect of apamin on the vascular smooth muscles. These experiments are also reported below. A preliminary report of some of the *in vivo* experiments has been published (Sjöqvist, Delbro, Jodal & Lundgren, 1980).

METHODS

Experiments on cats

Animals and general operative procedures. The experiments were performed on cats anaesthetized with α-chloralose I.V. (50 mg/kg body wt.) after induction with ether. The cats had been deprived of food for at least 24 hr with free access to water.

A slow I.V. infusion of a 10% glucose solution containing bicarbonate (100 mM-$NaHCO_3$) was started at the time of the induction of anaesthesia and continued thereafter throughout the experiment (0·1–0·2 ml./min). This infusion has previously been shown to maintain arterial pH at a normal level despite varying degrees of operative trauma (Haglund & Lundgren, 1972).

The femoral artery was cannulated, after heparinization (3–5 mg/kg body wt. I.V.) to record mean arterial blood pressure by means of a Statham pressure transducer (model P23AC) connected to a Grass polygraph.

The following operative procedures were common to each type of experiment performed in this study. After opening the abdomen by a mid-line incision, the great omentum and spleen were extirpated. The splanchnic nerves were cut bilaterally below the diaphragm. The right adrenal gland was denervated and the left gland excluded from the circulation by ligatures, to minimize changes in the adrenal output of catecholamines while maintaining release of adrenal steroids. A cannula was placed in a branch to the superior mesenteric artery for I.A. injections and infusions of drugs. Atropine (0·5–1·0 mg/kg) was given I.V.

Experiments on the colon. In the experiments performed on the colon ($n = 9$) the small intestine was extirpated. The inferior mesenteric artery was divided. This procedure preserves a normal blood flow to the feline large bowel (Hultén *et al.* 1969) as it is also supplied via the superior mesenteric artery. The vein running along the colonic border was ligated at the boundary between colon and sigmoid flexure. In this way it was ensured that only the venous effluent blood from the large intestine (except for the sigmoid flexure and rectum) was diverted through the colonic vein and

thence into the mesenteric vein. The mesenteric vein was cannulated and blood flow was recorded continuously by a drop recorder unit operating an ordinate writer and returned to the animal via the external jugular vein. The intestinal venous outflow pressure was kept constant at 8–10 mmHg throughout the experiments. In most experiments changes in the volume of the colonic lumen were also recorded by a volume transducer connected to the proximal end of the colon via plastic tubing.

The pelvic nerves were dissected free in most experiments and cut as they emerged from the sacral roots. Their distal ends were mounted on silver ring electrodes and subsequently stimulated at 5 V, 8 Hz and 5 msec by means of a Grass stimulator.

In each of these experiments the nerves surrounding the inferior mesenteric artery were cut and in most instances the distal ends were isolated and placed on ring electrodes for electrical stimulation (12 V, 8 Hz and 5 msec).

Experiments on the small intestine. In ten experiments a 15 cm length of intestinal segment (jejunum) was isolated with an intact vascular supply. The remainder of the small intestine and the colon were extirpated. The superior mesenteric vein, draining the small intestine and its lymph nodes, was cannulated. The intestinal venous blood was returned to the external jugular vein via a drop counter unit operating a pen recorder.

Mechanical stimulation of the intestinal mucosa was achieved by pulling a short fusiform plastic tube backwards and forwards through the jejunal lumen.

Electrical field stimulation across the intestinal wall was accomplished by introducing a cathode into the intestinal lumen and placing the intestine together with its intact vascular supply into a specially designed silver wire anode. The cathode consisted of a soft plastic tube (outer diameter 5 mm) with a flattened silver wire around its full length. The serosal side of the intestine was embedded in gauze soaked with saline in order to reduce the resistance between the electrodes. The outer and inner electrodes were connected to a Grass S 5 stimulator delivering pulses at a constant current via a specially designed constant-current generator. The stimulation characteristics were set at 40–80 Hz and 60–80 mA. For more technical details concerning the electrical field stimulation method, consult Biber, Fara & Lundgren (1973).

The distal ends of the severed splanchnic nerves were placed on silver ring electrodes in most experiments and stimulated at 8 Hz, 5 V and 5 msec.

Experiments on rat portal vein

Isolated portal veins from rats of the Sprague–Dawley strain of both sexes (Anticimex AB, Stockholm, Sweden) were used. The weights of the animals were 200–250 g. The rats were killed by a blow on the head under ether anaesthesia and the portal veins were carefully dissected free of surrounding tissue, cut open and tied at both ends with fine silk. Each preparation was mounted between a fixed hook and a force-displacement transducer (Grass FT03C) in an organ bath filled with modified Krebs–Henseleit solution kept at 38 °C. The solution contained (mM): NaCl, 122; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2 . 6\ H_2O$, 1·2; $NaHCO_3$, 15·5; KH_2PO_4, 1·2; glucose, 11·5 and was continuously perfused with 4 % CO_2 in O_2.

The vein was stretched to a resting passive force of 4 mN and left to equilibrate in the bath for at least 1 hr before each experiment. The active force of the longitudinal smooth muscle layer of the portal vein was continuously recorded and integrated *vs.* time during 1 min intervals using a Grass polygraph.

Drugs were introduced into the bath at 3 min intervals in a cumulative fashion (see Fig. 4). Only one drug was tested on each muscle.

Drugs

Solutions of the different drugs used in this study were prepared in the following way: VIP (purchased from Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden), substance P (Sigma Chemicals), bradykinin (Sigma Chemicals) and apamin (Serva) were dissolved in physiological saline containing 1 – 2 % bovine albumin (Serva). Albumin was added to the solution to reduce adhesion of the peptides to the glass and plastic walls of syringes and catheters.

5-HT (Sigma Chemicals), adenosine (Sigma Chemicals) and ATP (Sigma Chemicals) were dissolved in physiological saline.

Calculations

Neural effects on the intestinal vasculature were calculated from the relative change of regional flow resistance evoked by the stimulus. Colonic motility was estimated from the induced change in luminal volume.

In the *in vitro* experiments on the portal vein, drugs were introduced into the organ bath in a cumulative way at 3 min intervals. The integrated active force during the last 2 min of each test was averaged and taken to be the response except in the case of ATP where the integrated activity during the first minute had to be taken as the response (see below). The integrated activity was expressed as a percentage of the control, and dose–response relations were determined for each drug. The concentration that evoked 50% of the maximal response, ED_{50} and ID_{50} respectively, was calculated using a desk computer (Hewlett Packard) and a curve-fitting program. The parameter ED_{50} and E_{max} in the theoretical sigmoid dose–response curve, $E_{max} = x/(x+ED_{50})$, was adjusted to give the best least-square fit to the actual dose–response relationship. E_{max} is the maximal response and x is the concentration of the drug. For compounds which did not exhibit a sigmoid dose–response relation at the concentrations that were employed (ATP and adenosine) a linear interpolation formula was used to assess the concentration that evoked 50% of maximal response.

Statistics

Statistical significance was tested using non-parametrical tests such as the sign test and Wilcoxon's rank test. A probability of equality of 0·05 or less was considered to represent significant difference.

RESULTS

Experiments on the feline colon

Stimulation of the preganglionic fibres of the pelvic nerves to the colon evoked a transient, pronounced increase in colonic blood flow. This response was markedly diminished, and in three out of nine experiments was totally abolished, after administration of 1 or 2 mg apamin. In doses of 0·5 mg or less the vascular response was not significantly affected by the drug. The results from these nine experiments are summarized in Fig. 1.

Fig. 1 also shows the effects of apamin on the atropine-resistant contraction induced by pelvic nerve stimulation and on the effects of sympathetic stimulation. These two effects were very little influenced by apamin compared to the vasodilator effect, although in some experiments a transient reduction of the sympathetic vasoconstrictor response was seen during the first 10 min after administration of the peptide.

Intra-arterial infusions of VIP were performed in most experiments. The effects of apamin on the vascular response to VIP were more difficult to judge than the nervous effects reported in Fig. 1 since a strict comparison requires that the plasma concentrations of VIP be identical before and after apamin. To circumvent these difficulties VIP was infused at two rates and comparisons were made at comparable plasma concentrations as calculated from plasma flow and the infusion rate of VIP. Within 20 min after apamin, close I.A. infusion of VIP evoked a vascular response of $78 \pm 13\%$ (mean $\pm$ S.E. of mean, $n = 6$) of control.

Infusions of 5-HT were also made regularly in six experiments. It was found that the vasodilator action of the drug observed during control periods was reversed to a vasoconstriction after apamin ($P < 0{\cdot}01$). This effect is illustrated in Fig. 2 which shows the relative effects of 5-HT on blood flow before and at varying times after apamin. As with VIP, it was sometimes difficult to make the comparisons at the same

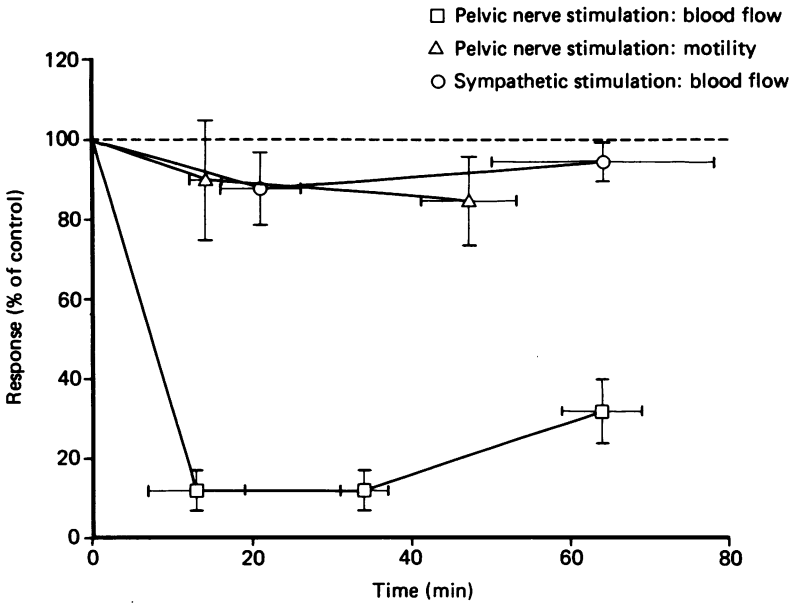

Fig. 1. The effect of giving apamin (1 or 2 mg) by close I.A. injection on the vasodilatation and muscular contraction in the colon evoked by pelvic nerve stimulation and on the colonic sympathetic vasoconstrictor response. Apamin was administered at time zero. The responses are expressed in per cent of the control responses. Note that only the vasodilator response on pelvic nerve stimulation was reduced by ampamin. Bars denote ±S.E. of means. Number of observations: colonic vasodilatation, nine; sympathetic vaso-constriction and colonic motility, five.

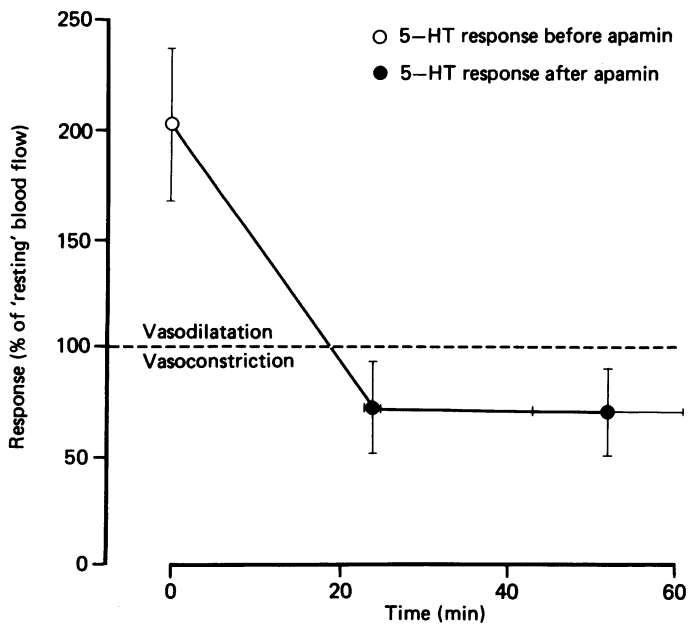

Fig. 2. The effect of apamin (1 or 2 mg) on the vascular response in the colon evoked by close I.A. infusion of 5-hydroxytryptamine. Apamin was given at time zero. Bars denote ±S.E. of means. $n = 6$.

plasma concentration of 5-HT. However, no such difference could explain the fact that the effect of 5-HT was reversed from vasodilatation to vasoconstriction following the administration of apamin.

Experiments on the feline small intestine

Transmural electrical field stimulation evoked an intestinal vasodilatation as has been reported earlier (Biber *et al.* 1973). This vasodilatation was markedly diminished in all experiments ($n = 6$) after I.A. apamin (1 mg or more, see above). In two of the experiments the response was totally abolished. The average response was $15 \pm 4\%$ of control 10–30 min after giving apamin. About 1 hr after apamin the transmural field response was $35 \pm 16\%$ of control.

In four experiments the effect of apamin on the intestinal vasodilatation observed in response to mechanical mucosal stimulation was studied. In all these experiments the drug markedly diminished this vascular response. No attempt was made to quantify the effect because it was difficult to stimulate the mucosa mechanically repeatedly in precisely the same way.

The effects of apamin on the VIP-induced vasodilatation were difficult to assess with certainty for reasons discussed above in relation to the colon. In five technically successful experiments the effect of VIP after apamin varied between 37 and 138% of control, the average value being 90%.

As in the colon, it was observed that in the small intestine the vasodilator effect of 5-HT infusion was reversed after giving apamin (Fig. 2). This response persisted for at least 2 hr after giving apamin.

In vitro experiments on the isolated rat portal vein

As pointed out above, it was difficult to ascertain the intestinal vascular effects of close I.A. infusions of VIP before and after apamin *in vivo*. In order to analyse the vascular response to VIP and other compounds more easily, series of *in vitro* experiments on the isolated rat portal vein were performed. The experiments were carried out in two series, one with and one without apamin, as it was found that a second application of an excitatory compound evoked a less pronounced quantitative response at each concentration.

Fig. 3*A* illustrates the effects of VIP on the spontaneous myogenic activity of the rat portal vein. It is evident that apamin did not influence the dose–response curve of VIP. Log ID_{50} measured with and without apamin ($-8{\cdot}00 \pm 0{\cdot}37$; $n = 7$ and $-7{\cdot}81 \pm 13$; $n = 9$ respectively; mean ± S.E. of mean) did not differ significantly.

Three other peptides which have been suggested to be putative neurotransmitters in the gastrointestinal tract were also tested on the rat portal vein. Fig. 3*B* and *C* illustrates the results of experiments with substance P and bradykinin, respectively. Both these compounds evoked a contraction of the vascular smooth muscle *in vitro*, although they are known to cause vasodilatation *in vivo*. It is clear from these results that apamin did not markedly influence the dose–response relation of the two peptides although there was a small but significant reduction of the maximal active contraction elicited by substance P in the presence of apamin. The log ED_{50} values of substance P ($-5{\cdot}27 \pm 0{\cdot}10$; $n = 8$) and bradykinin ($-5{\cdot}40 \pm 0{\cdot}07$; $n = 6$) were not changed by apamin ($-5{\cdot}44 \pm 18$; $n = 8$ and $-5{\cdot}14 \pm 0{\cdot}12$, $n = 6$, respectively).

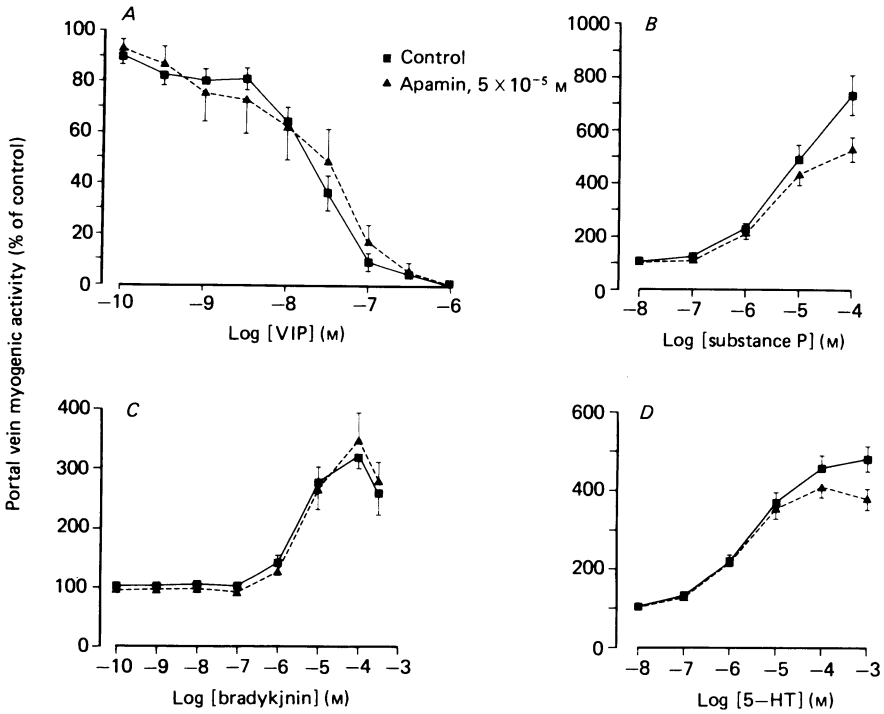

Fig. 3. The effect of apamin on the myogenic activity of the isolated rat portal vein induced by vasoactive intestinal polypeptide (VIP; *A*), substance P (*B*), bradykinin (*C*) and 5-hydroxytryptamine (5-HT; *D*). Bars indicate ± S.E. of means. For number of observations see Table 1.

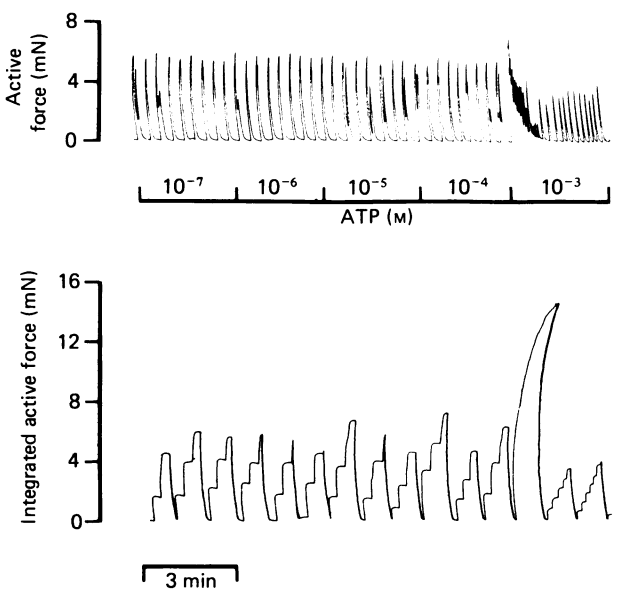

Fig. 4. The effect of adenosine triphosphate (ATP) on the myogenic activity of a portal vein. The upper tracing shows the active force exhibited by the vascular smooth muscle cells. In the lower panel the integrated active force is given. Note that the effect of ATP is seen first at a concentration of 10^{-3} M.

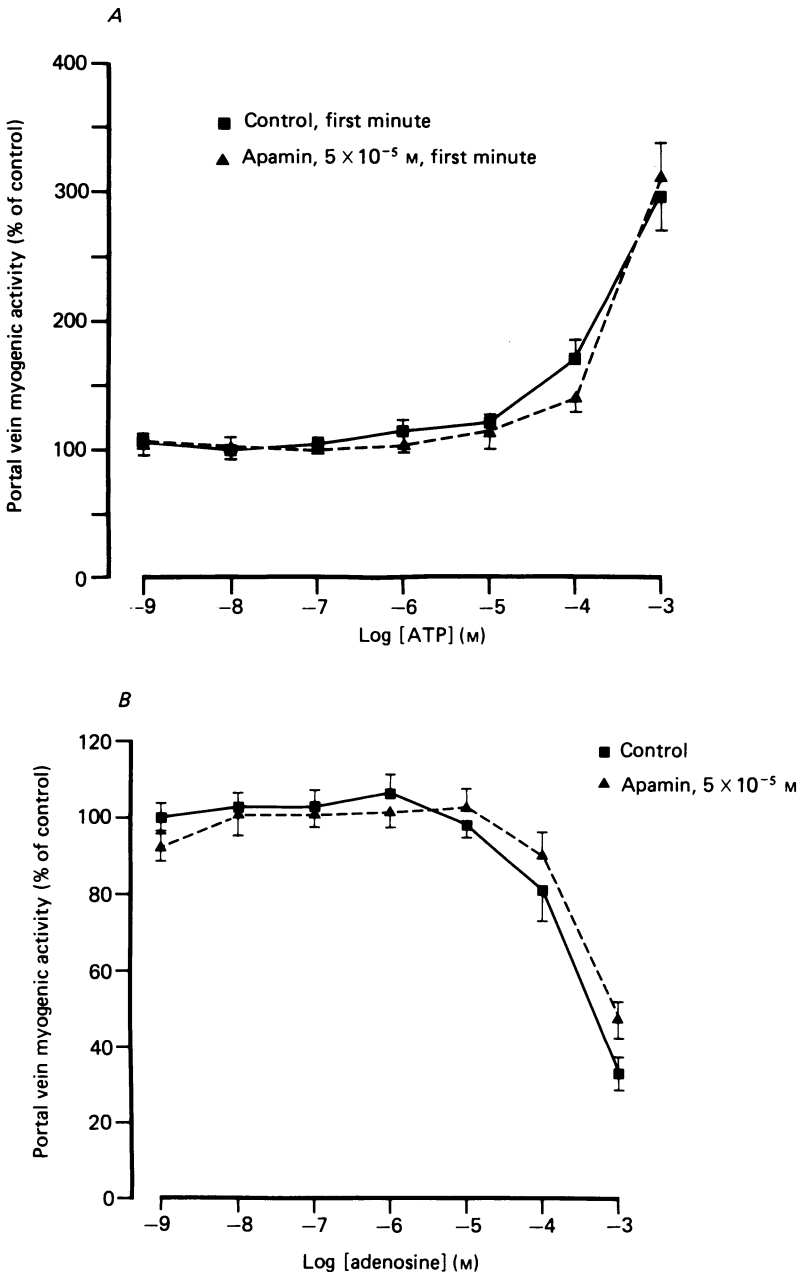

Fig. 5. *A*, the effect of ATP on the rat portal vein with and without apamin. The vascular smooth muscle response was calculated from the integrated response observed during the first minute after application of ATP (see Fig. 4). *B*, the effect of apamin on the adenosine-evoked changes in portal vein myogenic activity. Bars indicate ± S.E. of means.

Leu–enkephalin was investigated in the same way as substance P and bradykinin. However, even large (3×10^{-4} M) concentrations of enkephalin had no effect on the vascular smooth muscle of the rat portal vein.

5-HT, studied in the way described above, before and after apamin showed that apamin did not change the dose–response relation (Fig. 3*D*) or the log ED_{50} ($-5{\cdot}58 \pm 0{\cdot}06$; $n = 12$ and $5{\cdot}79 \pm 0{\cdot}09$; $n = 8$, respectively). A series of experiments was also performed showing that the vascular smooth muscle response to 5-HT was not mediated via nerves since tetrodotoxin did not abolish the response.

The effects of ATP on vascular smooth muscle of the rat portal vein was also studied. The tracing from one of these experiments is shown in Fig. 4. It is evident that, at a concentration of 10^{-4}-M or less, ATP had no effect on the portal vein. A concentration of 10^{-3}-M evoked a complex response involving an initial transient, strong contraction followed by weaker contractions at a higher frequency compared with the control. A similar effect of ATP on the rat portal veins has been reported by Sjöberg & Wahlström (1975). Apamin neither influenced the pattern of response nor changed the dose–response relation of the contractile response during the first minute (Fig. 5*A*). In two experiments indomethacin (10 μg/ml.) was added to the organ bath without influencing the response to ATP.

A breakdown product of ATP, adenosine, was also investigated in the same way (Fig. 5*B*). In the upper concentration range this compound reduced the active contractions of the vascular smooth muscle of the rat portal vein. Apamin did not influence this response. Neither the log ED_{50} for ATP ($-3{\cdot}5 \pm 0{\cdot}11$; $n = 10$) nor the log ID_{50} for adenosine ($-3{\cdot}52 \pm 0{\cdot}93$; $n = 6$) was changed by apamin ($-3{\cdot}35 \pm 0{\cdot}03$; $n = 6$ and $-3{\cdot}39 \pm 0{\cdot}38$; $n = 6$, respectively).

DISCUSSION

The results of this study indicate that apamin abolishes or markedly diminishes the non-cholinergic, non-adrenergic vascular effects induced by the pelvic nerves to the colon and via the intramural nerve fibres in the small intestine. This effect cannot be ascribed to any non-specific effect of apamin on autonomic nerves or smooth muscles since both the sympathetic vasoconstriction and the atropine-resistant colonic contraction were largely unaffected by apamin (Fig. 1). These observations strongly suggest that the vascular and smooth muscle contractile responses to pelvic nerve stimulation are mediated via two different non-cholinergic, non-adrenergic neutrotransmitters. This conclusion is supported by the observation that it is possible to induce the vasodilatation selectively by stimulating the pelvic nerves electrically in 'bursts' rather than at a steady, continuous frequency (Andersson & Järhult, 1981), and that aprotinin, a protease inhibitor, blocks the vascular but not the motor response to pelvic nerve stimulation (Fasth, Hultén, Nordgren & Zeitlin, 1981).

In an attempt to ascertain whether apamin blocks receptors of any of the proposed neurotransmitters in the intestine (see Furness & Costa, 1980), dose–response curves for such putative neurotransmitters were determined using the isolated rat portal vein in the presence and absence of apamin; this vascular smooth muscle preparation is considered to be a good model for the study of precapillary resistance vessels (Ljung, 1970). None of the vascular effects of the substances tested (ATP, adenosine, 5-HT,

VIP, substance P and bradykinin) seemed to be influenced by apamin. We therefore conclude that, if this peptide exerts its effect at the level of the vascular smooth muscle cell, it does not do so by blocking receptors of any of the substances tested in this study. This conclusion is substantiated as regards VIP by observations made on uterine muscle tissue (Ottesen, 1981) and taenia coli (Mackenzie & Burnstock, 1980). However, a presynaptic action is not ruled out by these observations.

Apamin has been used in several studies of the neurally evoked hyperpolarization of taenia coli. This response is mediated via a non-cholinergic, non-adrenergic mechanism as first reported by Bennett *et al.* (1966). Burnstock, Campbell, Satchell & Smythe (1970) proposed that ATP is the neurotransmitter mediating this response, based on the observation that ATP mimicks the response to transmural electrical field stimulation. The hyperpolarization of taenia coli evoked by this procedure is blocked by apamin (Shuba & Vladimirova, 1980; Maas & den Hertog, 1979; Mackenzie & Burnstock 1980; Maas, 1981). It has also been shown that the ATP-induced hyperpolarization is abolished by apamin (Shuba & Vladimirova, 1980; Maas & den Hertog, 1979; Mackenzie & Burnstock, 1980; Maas, den Hertog, Ras & den Akker, 1980; Brown & Burnstock, 1981). The latter observation is at variance with the findings of this study (Figs. 4 and 5) for reasons which are at present obscure.

Apamin has also been shown to block the α-adrenergic relaxation of taenia coli (Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979; Maas & den Hertog, 1979; Muller & Baer, 1980) although not in a competive manner (Jenkinson, 1981). An α-blocking effect was also noted in this study during the first 10 min after giving apamin by close I.A. injection when the sympathetic vasoconstriction was diminished in some experiments. This property of the peptide has been ascribed to blockage of the noradrenaline-induced increase in potassium permeability (Banks *et al.* 1979; Maas *et al.* 1980; Burgess, Claret & Jenkinson, 1981). The present results suggest that this effect is transient *in vivo*, in the face of a rather sustained inhibition of the non-adrenergic, non-cholinergic vasodilatations in the small and large intestines. This observation suggests that apamin may exert more than one effect on autonomic nerves and their effector cells.

As pointed out by Maas (1981) in his analyses of apamin on the neurally evoked hyperpolarization of the taenia coli, such experiments do not exclude a presynaptic action. A presynaptic site of action is not uncommon for toxins, as exemplified by the botulinum toxin (Gundersen, 1980).

A presynaptic action of apamin is suggested by the experiments with 5-HT which demonstrated that the vasodilatation evoked in the small intestine by comparatively low concentrations of 5-HT was in most experiments reversed after apamin (Fig. 2), an effect that could not be ascribed to any interaction between 5-HT and apamin at the level of the effector cell (Fig. 3). We have earlier shown that tetrodotoxin (TTX), which blocks nerve conduction, given by close I.A. injection, produces the same reversal of the vascular action of 5-HT (Biber, Fara & Lundgren, 1973). Based on studies of VIP release we have proposed that the vasodilator effect of 5-HT is mediated via its release of VIP (Eklund, Fahrenkrug, Jodal, Lundgren, Schaffalitzky de Muckadell & Sjöqvist, 1980) which is then presumably inhibited by TTX. A similar action of apamin could account for the observations in the present study.

Two other observations which have been made recently in our laboratory also

suggest that apamin may influence the release of VIP from the autonomic nerve fibres by a presynaptic action. First, apamin displaces VIP from receptors on isolated plasma membranes from the rat brain in a competitive fashion (J. Fahrenkrug, S. Gammeltoft, P. Staun-Olsen, B. Ottesen & A. Sjöqvist, unpublished observations). Secondly, release of VIP during pelvic stimulation (Fahrenkrug *et al.* 1978), transmural electrical field stimulation and during I.A. infusion of 5-HT (Eklund *et al.* 1980) is inhibited after apamin (A. Sjöqvist, J. Fahrenkrug, M. Jodal & O. Lundgren, unpublished observations). Further experiments to explore this possibility are in progress in our laboratory.

Two polypeptides, aprotinin (fifty-eight amino acid residues) and apamin (eighteen amino acid residues), have been shown either to inhibit or totally block the vasodilatory effect in the colon in response to pelvic nerve stimulation (Fasth *et al.* 1981). A comparison of the amino acid composition of the two compounds suggest that they may exert their influence via a similar mode of action. Both peptides are positively charged at pH 6–7 ('basic'). In the case of aprotinin the positive charge is located on one part of the molecule. Furthermore, in the aprotinin molecule the lysine residue in position 15 is considered to be of great importance for binding to enzymes (Werle, 1972). The following sequence (14–16) is found around this residue: cys–lys–ala. The cysteine in position 14 is coupled via a disulphide bridge to a cysteine residue in position 38. An identical sequence is found in apamin in positions 3–5, the cysteine in position 3 making contact with cysteine in position 11. Similarly, the sequence 12–14 in the apamin molecule (ala–arg–arg) is considered to be of importance for the function of apamin (Jenkinsson, 1981). A similar sequence is found in the aprotinin molecule in position 40–42 (ala–lys–arg). It should be pointed out that arginine and lysine are considered to be so-called conservative exchanges, i.e. they are considered to be mutually interchangeable with similar functional and/or steric roles. Based on such considerations we suggest that the inhibitory action of aprotinin and apamin on the non-adrenergic, non-chlorinergic vasodilatation in the colon may well be mediated via the same action of the compounds.

Fasth *et al.* (1981) used the protease inhibitor aprotinin in an attempt to substantiate the kinin hypothesis of Hilton & Lewis (1955*a*, *b*). It is of interest to note that apamin seems not to have any protease inhibitory activity since it can be broken down by both trypsin and chymotrypsin (Haux, Sawerthal & Haberman, 1967). If the action of aprotinin and apamin on the pelvic nerve response is mediated via the same mechanism, it cannot be the enzyme inhibitory action of aprotinin that was studied by Fasth *et al.* (1981).

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REFERENCES

ABRAHAMSSON, H. (1973). Studies on the inhibitory nervous control of gastric motility. *Acta physiol. scand.* (suppl.) **390**, 1–38.

ANDERSSON, P.-O. & JÄRHULT, J. (1981). Separation of colonic motor and blood flow responses to pelvic nerve stimulation in the cat. *Acta physiol. scand.* **113**, 263–265.

BANKS, B. E. C., BROWN, C., BURGESS, G. M., BURNSTOCK, G., CLARET, M., COCKS, T. M. & JENKINSON, D. H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature, Lond.* **282**, 415–417.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **182**, 541–558.

BIBER, B., FARA, J. & LUNDGREN, O. (1973). Intestinal vasodilatation in response to transmural electrical field stimulation. *Acta physiol. scand.* **87**, 277–282.

BIBER, B., FARA, J. & LUNDGREN, O. (1973). Intestinal vascular responses to 5-hydroxytryptamine. *Acta physiol. scand.* **87**, 526–534.

BIBER, B., JODAL, M., LUNDGREN, O. & SVANVIK, J. (1970). Intestinal vasodilatation after mechanical stimulation of the jejunal mucosa. *Experientia* **26**, 263–264.

BROWN, C. M. & BURNSTOCK, G. (1981). Evidence in support of the P_1/P_2 purinoceptor hypothesis in the guinea-pig taenia coli. *Br. J. Pharmac.* **73**, 617–624.

BURGESS, G. M., CLARET, M. & JENKINSON, D. H. (1981). Effects of quinine and apamin on the calcium-dependent potassium permeability of mammalian hepatocytes and red cells. *J. Physiol.* **317**, 67–90.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.* **24**, 509–581.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *J. Pharmacol.* **40**, 668–688.

EKLUND, S., FAHRENKRUG, J., JODAL, M., LUNDGREN, O., SCHAFFALITZKY DE MUCKADELL, O. B. & SJÖQVIST, A. (1980). Vasoactive intestinal polypeptide, 5-hydroxytryptamine and reflex hyperaemia in the small intestine of the cat. *J. Physiol.* **302**, 549–557.

FAHRENKRUG, J., HAGLUND, U., JODAL, M., LUNDGREN, O., OLBE, L. & SCHAFFALITZKY DE MUCKADELL, O. B. (1978). Nervous release of vasoactive intestinal polypeptide in the gastrointestinal tract of cats: possible physiological implications. *J. Physiol.* **284**, 291–305.

FASTH, S. (1973). The effect of bradykinin on gastrointestinal motility and circulation. Thesis. A.B. Götatryckeriet, Göteborg, Sweden.

FASTH, S., HULTÉN, L., NORDGREN, S. & ZEITLIN, I. J. (1981). Studies on the atropine resistant sacral parasympathetic vascular and motility responses in the cat colon. *J. Physiol.* **311**, 421–429.

FURNESS, J. B. & COSTA, M. (1980). Types of nerves in the enteric nervous system. *Neuroscience* **5**, 1–20.

GUNDERSEN, C. B. (1979). The effects of botulinum toxin on the synthesis, storage and release of acetylcholine. *Prog. Neurobiol.* **14**, 99–119.

HABERMANN, E. (1972). Bee and wasp venoms. *Science, N.Y.* **177**, 314–322.

HAGLUND, U. & LUNDGREN, O. (1972). Reactions within consecutive vascular sections of the small intestine of the cat during prolonged hypotension. *Acta physiol. scand.* **84**, 151–163.

HAUX, P., SAWERTHAL, H. & HABERMANN, E. (1967). Sequenzanalyse des Bienengift-Neurotoxins (Apamin) aus seinen tryptischen und chymotryptischen Spaltstüken. *Hoppe-Seyler's Z. physiol. Chem.* **348**, 737–738.

HILTON, S. M. & LEWIS, G. P. (1955*a*). The cause of the vasodilatation accompanying activity in the submandibular salivary gland. *J. Physiol.* **128**, 235–248.

HILTON, S. M. & LEWIS, G. P. (1955*b*). The mechanism of the functional hyperaemia in the submandibular salivary gland. *J. Physiol.* **129**, 253–271.

HIRST, G. D. S. (1979). Mechanisms of peristalsis. *Br. med. Bull.* **35**, 263–268.

HULTÉN, L. M., JODAL, M. & LUNDGREN, O. (1969). Extrinsic nervous control of colonic blood flow. *Acta physiol. scand.* (suppl.) **335**, 39–49.

JANSSON, G. (1969). Extrinsic nervous control of gastric motility. An experimental study in the cat. *Acta physiol. scand.* (suppl.) **326**, 1–72.

JENKINSON, D. H. (1981). Peripheral actions of apamin. *Trends in pharmacol. Sci.* **2**, 318–320.

LJUNG, B. (1970). Nervous and myogenic mechanisms in the control of a vascular neuroeffector system. *Acta physiol. scand.* (suppl.) **349**, 33–68.

MAAS, A. J. J. (1981). The effect of apamin on responses evoked by field stimulation in guinea-pig taenia caeci. *Eur. J. Pharmacol.* **73**, 1–9.

MAAS, A. J. J. & DEN HERTOG, A. (1979). The effect of apamin on the smooth muscle cells of the guinea-pig. *Eur. J. Pharmacol.* **58**, 151–156.

MAAS, A. J. J., DEN HERTOG, A., RAS, R. & VAN DEN AKKER, J. (1980). The action of apamin on guinea-pig taenia caeci. *Eur. J. Pharmacol.* **67**, 265–274.

MACKENZIE, I. & BURNSTOCK, G. (1980). Evidence against vasocactive intestinal polypeptide being the non-adrenergic, non-cholinergic inhibitory transmitter released from nerves supplying the smooth muscle of the guinea-pig taenia coli. *Eur. J. Pharmacol.* **67**, 255–264.

MARTINSON, J. (1965). Studies on the efferent vagal control of the stomach. *Acta physiol. scand.* **65** (suppl.) 255, 1–27.

MULLER, M. J. & BAER, H. P. (1980). Apamin, a nonspecific antagonist of smooth muscle relaxants. *Naunyn-Schmiedebergs Arch. Pharmacol.* **311**, 105–107.

OTTESEN, B. (1981). Vasoactive intestinal polypeptide (VIP): effect on rabbit uterine smooth muscle *in vivo* and *in vitro*. *Acta physiol. scand.* **113**, 193–199.

SHUBA, M. F. & VLADIMIROVA, I. A. (1980). Effect of apamin on the electrical responses of smooth muscle to adenosine 5′-triphosphate and to non-adrenergic non-cholinergic nerve stimulation. *Neuroscience* **5**, 853–859.

SJÖBERG, G. & WAHLSTRÖM, B. A. (1975). The effect of ATP and related compounds on spontaneous mechanical activity in the rat portal vein. *Acta physiol. scand.* **94**, 46–53.

SJÖQVIST, A., DELBRO, D., JODAL, M. & LUNDGREN, O. (1980). The effect of Apamin^{R} on nonadrenergic, noncholinergic nervous vasodilatations in the cat small intestine. *Experientia* **36**, 1202.

WERLE, E. (1972). Trasylol: a short survey on its history, biochemistry and activities. in *New Aspects of Trasylol Therapy*, vol. 5, ed. BRENDEL, W. & HABERLAND, G. L., pp. 9–19. Stuttgart: F. K. Schattauer Verlag.