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PACAP-(6–38) inhibits the effects of vasoactive intestinal polypeptide, but not PACAP, on the small intestinal circular muscle

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Abstract

Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide-(1–38) (PACAP) have been found to stimulate distension-induced peristaltic motility in the guinea-pig isolated small intestine. In this study, we tested whether the putative VIP/PACAP receptor antagonist PACAP-(6–38) counteracts the properistaltic effect of VIP and PACAP in isolated segments of the guinea-pig small intestine. VIP (100 nM) and PACAP (30 nM) had a stimulatory effect, i.e., lowered the peristaltic pressure threshold at which peristaltic waves were triggered and enhanced the frequency of peristaltic waves. PACAP-(6–38) (3 μM) was per se without effect on peristalsis but prevented or reversed the peristaltic motor stimulation caused by VIP, when it was given before or after the agonist, respectively. PACAP-(6–38), however, failed to antagonize the properistaltic effect of PACAP. In ileal circular strips treated with tetrodotoxin (1 μM) and indomethacin (3 μM), spontaneous myogenic activity was inhibited by VIP (5–30 nM). This effect was significantly reduced by a pretreatment with PACAP-(6–38) (3 μM). A similar inhibition by PACAP-(1–38) (10–500 nM) was not influenced by the antagonist. It is concluded that PACAP-(6–38) is a VIP receptor antagonist, both in the peristaltic motor pathways and at the level of the circular muscle of the guinea-pig small intestine. The lack of a motor effect of PACAP-(6–38) on its own indicates that VIP acting on PACAP-(6–38)-sensitive receptors (located on neurons and/or the smooth muscle) is unlikely to participate in peristaltic motor regulation.

Keywords: Peristaltic reflex; Circular muscle contraction; Small intestine; (Guinea-pig); VIP receptor antagonist; PACAP (pituitary adenylate cyclase-activating peptide)

1. Introduction

Vasoactive intestinal polypeptide (VIP), a 28 amino acid peptide, is an important messenger of the enteric nervous system. Immunohistochemistry has revealed that VIP-positive perikarya and nerve fibres are present in the myenteric and submucosal plexus of several animal species (Dockray, 1994). In the guinea-pig small intestine, myenteric VIP neurons project in an anal direction to innervate other myenteric ganglia as well as the circular muscle layer (Costa and Furness, 1983). Functional studies indicate that VIP is a nonadrenergic noncholinergic (NANC) transmitter that contributes to the regulation of gastrointestinal motility, secretion and blood flow (Dockray, 1994).

VIP causes relaxation of most gastrointestinal smooth muscle preparations. In the guinea-pig ileum, however, VIP has an additional action inasmuch as it excites myenteric neurons and, by releasing acetylcholine and substance P from these neurons, leads to contraction of the ileal musculature (Williams and North, 1979; Cohen and Landry, 1980; Katsoulis et al., 1992). While VIP is thought to be an inhibitory transmitter of enteric nerve pathways regulating propulsion in the rat colon (Grider, 1993), it is not yet known whether the excitatory action of VIP on myenteric neurons has a bearing on peristaltic motor regulation in the guinea-pig small intestine. This possibility was tested here by use of the pituitary adenylate cyclase activating polypeptide (PACAP) fragment PACAP-(6–38), which is a putative VIP receptor antagonist.

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Although in certain tissues this compound behaves as a PACAP receptor antagonist (Robberecht et al., 1992), in the guinea-pig gut PACAP-(6–38) has been found to inhibit the effects of exogenous VIP on the myenteric plexus of the ileum (Barthó et al., 2000) and on the smooth muscle of the taenia caeci and ascending colon (Lénárd et al., 2000; Rózsai et al., 2001). When examined on peristaltic motor activity in the guinea-pig small intestine, PACAP-(6–38) at a concentration of 3 μM does not per se modify distension-induced propulsive motility and fails to prevent the properistaltic action of exogenous PACAP (Heinemann and Holzer, 1999). In the current study we set out to test whether PACAP-(6–38) inhibits the stimulant and inhibitory effects of exogenous VIP on intestinal peristalsis and myogenic circular muscle movements, respectively, thereby addressing a possible role of endogenous VIP in the regulation of circular muscle motility during peristalsis.

2. Materials and methods

2.1. Recording of peristalsis

The small intestine of adult guinea-pigs (TRIK strain, either sex, 350–450 g body weight) was isolated, flushed of luminal contents and placed, for up to 4 h, in Tyrode solution kept at room temperature and oxygenated with a mixture of 95% O_2 and 5% CO_2 (Heinemann and Holzer, 1999). The composition of the Tyrode solution was (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6. The jejunum and ileum were divided into 8 segments, each being approximately 10 cm long. Four intestinal segments were set up in parallel and secured horizontally in organ baths containing 30 ml of Tyrode solution at 37 °C. In order to elicit propulsive peristalsis, pre-warmed Tyrode solution was continuously perfused through the lumen of the segments at a rate of 0.5 ml/min (Shahbazian et al., 2001). The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, via an analog/digital converter, fed into a personal computer and recorded and analyzed with the software "Peristal 1.0" (Heinemann and Holzer, 1999).

The fluid passing through the gut lumen was directed into a U-shaped vertical outlet tubing which ended 4.1 cm (equivalent to a hydrostatic pressure of 400 Pa) above the fluid level in the organ bath. When fluid was perfused, the intraluminal pressure rose slowly until it reached a threshold at which peristalsis was triggered. The aborally moving wave of peristaltic contraction resulted in a spike-like increase in the intraluminal pressure, the peristaltic wave, which caused emptying of the segment if the maximal pressure of the peristaltic wave exceeded the level of 400 Pa as set by the position of the outlet tubing.

2.2. Evaluation of peristalsis

The recordings of peristalsis were subjected to computerized analysis in order to determine the peristaltic pressure threshold which is the intraluminal pressure at which a peristaltic wave is triggered. Stimulation of peristalsis was reflected by a decrease in peristaltic pressure threshold, which was often associated with an increase in the frequency of peristaltic waves, whereas inhibition of peristalsis manifested itself in a rise of peristaltic pressure threshold.

2.3. Experimental protocol

The preparations were allowed to equilibrate in the organ bath for a period of 30 min during which they were kept in a quiescent state, i.e., perfused with the outlet tubing set at 0 Pa hydrostatic pressure. Thereafter, the bath fluid was renewed and peristaltic motility initiated by elevating the position of the outlet tubing to the equivalent of 400 Pa hydrostatic pressure. After basal peristaltic activity had been recorded for a 30-min period, the drugs under study were added to the bath, i.e., to the serosal surface of the intestinal segments, at volumes not exceed-

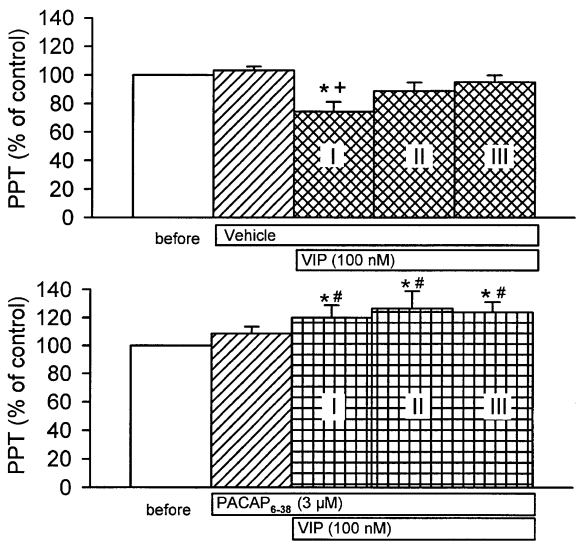

Fig. 1. Effect of PACAP-(6–38) to prevent the action of VIP to lower the peristaltic pressure threshold (PPT). Before: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period before administration of vehicle (upper panel) or PACAP-(6–38) (lower panel) were evaluated and averaged to obtain a 100% control value. Vehicle and PACAP-(6–38) alone: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period after administration of vehicle and PACAP-(6–38) were averaged and expressed as a percentage of the control value. I, II, III: The first three peristaltic waves recorded after administration of VIP were evaluated individually and expressed as a percentage of the control value. The values shown are means ± S.E.M. of six experiments. $^{*}P < 0.05$ vs. before, $+P < 0.05$ vs. vehicle or PACAP-(6–38), $\#P < 0.05$ vs. respective value in upper panel.

ing 1% of the bath volume. The corresponding vehicle solutions were devoid of any effect.

The effects of PACAP-(6–38) on the properistaltic motor actions of VIP and PACAP were examined in two experimental protocols involving separate preparations. In the first protocol termed "method of prevention", PACAP-(6–38) was added to the organ bath 5 min before the segments were exposed to VIP or PACAP. The peristaltic pressure thresholds of the peristaltic waves recorded during the 5 min period before administration of PACAP-(6–38) were evaluated and averaged to obtain a 100% control value. The peristaltic pressure thresholds of the peristaltic waves recorded during the 5 min period after administration of PACAP-(6–38) were likewise averaged and expressed as a percentage of the control value. Finally, the first three peristaltic waves recorded after administration of VIP or PACAP were evaluated individually and expressed as a percentage of the control value (Figs. 1 and 2).

In the second protocol termed "method of reversal", the agonists VIP and PACAP were first added to the organ bath. After their peristaltic motor effect had been fully developed, usually within 3 min, PACAP-(6–38) was administered. In this arrangement, the peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period before administration of VIP or PACAP were evaluated and averaged to obtain a 100% control value. Then, the peristaltic pressure threshold of the peristaltic wave recorded after the motor effect of VIP and PACAP had been fully established was evaluated and expressed as a percentage of the control value. Finally, the first three peristaltic waves recorded after administration of PACAP-(6–38) were evaluated individually and expressed as a percentage of the control value (Figs. 3 and 4).

The vehicle of PACAP-(6–38) was tested in separate experiments involving both experimental protocols.

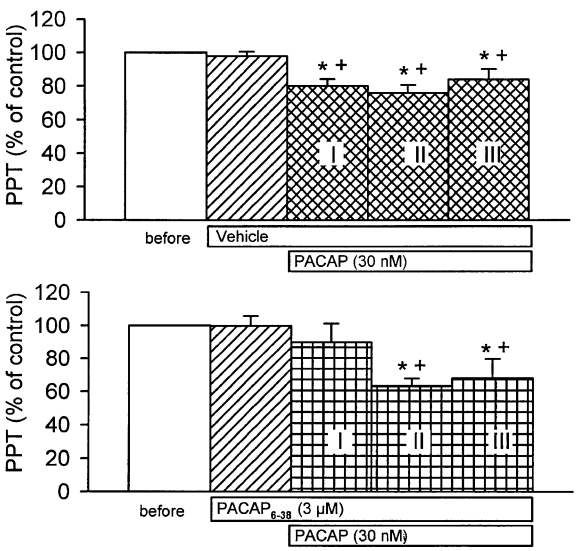

Fig. 2. Effect of PACAP-(6–38) on the action of PACAP to lower the peristaltic pressure threshold (PPT). Before: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period before administration of vehicle (upper panel) or PACAP-(6–38) (lower panel) were evaluated and averaged to obtain a 100% control value. Vehicle and PACAP-(6–38) alone: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period after administration of vehicle and PACAP-(6–38) were averaged and expressed as a percentage of the control value. I, II, III: The first three peristaltic waves recorded after administration of PACAP were evaluated individually and expressed as a percentage of the control value. The values shown are means ± S.E.M. of six experiments. * $P < 0.05$ vs. before, + $P < 0.05$ vs. vehicle or PACAP-(6–38). There was no statistically significant difference between the respective values in the upper and lower panel.

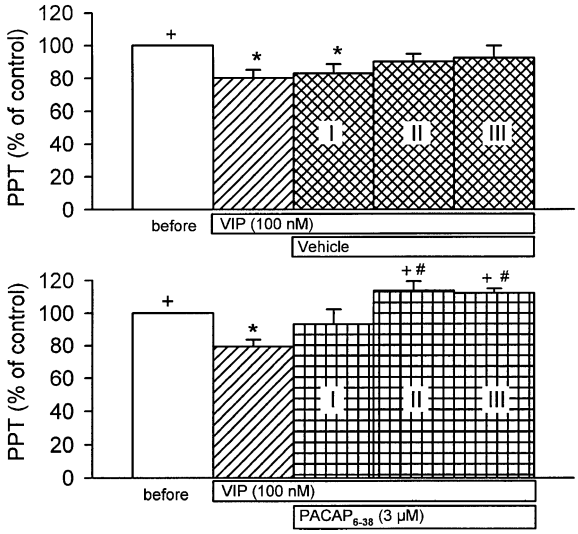

Fig. 3. Effect of PACAP-(6–38) to reverse the action of VIP to lower the peristaltic pressure threshold (PPT). Before: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period before administration of VIP were evaluated and averaged to obtain a 100% control value. VIP alone: The peristaltic pressure threshold of the peristaltic wave recorded after the motor effect of VIP had been fully established was evaluated and expressed as a percentage of the control value. I, II, III: The first three peristaltic waves recorded after administration of vehicle (upper panel) and PACAP-(6–38) (lower panel) were evaluated individually and expressed as a percentage of the control value. The values shown are means ± S.E.M. of six experiments. * $P < 0.05$ vs. before, + $P < 0.05$ vs. VIP alone, # $P < 0.05$ vs. respective value in upper panel.

2.4. Recording circular muscle activity

Whole-thickness circular strips of the ileum were prepared from rings of 1.5–2 mm width as described earlier (Barthó et al., 1992). Regular spontaneous activity was induced with indomethacin (3 μM) (Maggi et al., 1994). Quantitative evaluation of the phasic motor activity was performed as described by Barthó et al. (1991). Briefly, amplitudes of phasic contractions were summed up for each min of observation and expressed as fraction of the maximal spasm evoked by KCl (80 mM) at the end of the experiment. All experiments were performed with tetrodotoxin (1 μM) present in the organ bath. We examined the effects of VIP and PACAP on the spontaneous contractile activity of circular muscle strips, as well as their modification by PACAP-(6–38).

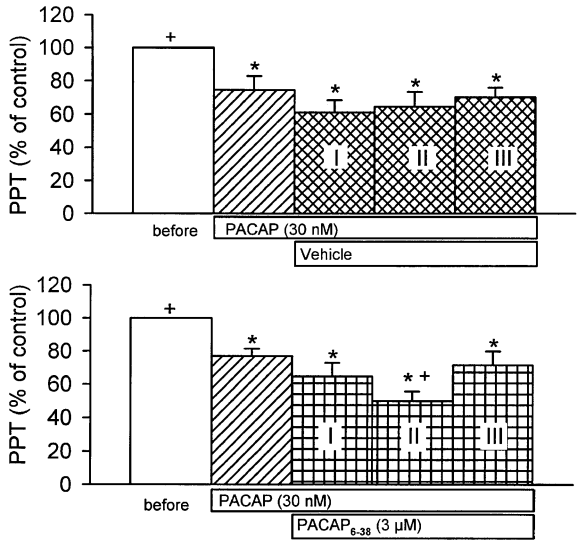

Fig. 4. Effect of PACAP-(6–38) to reverse the action of PACAP to lower the peristaltic pressure threshold (PPT). Before: The peristaltic pressure thresholds of the peristaltic waves recorded during the 5-min period before administration of PACAP were evaluated and averaged to obtain a 100% control value. PACAP alone: The peristaltic pressure threshold of the peristaltic wave recorded after the motor effect of PACAP had been fully established was evaluated and expressed as a percentage of the control value. I, II, III: The first three peristaltic waves recorded after administration of vehicle (upper panel) and PACAP-(6–38) (lower panel) were evaluated individually and expressed as a percentage of the control value. The values shown are means ± S.E.M. of 5–6 experiments. * $P <$ 0.05 vs. before, + $P < 0.05$ vs. PACAP alone. There was no statistically significant difference between the respective values in the upper and lower panel.

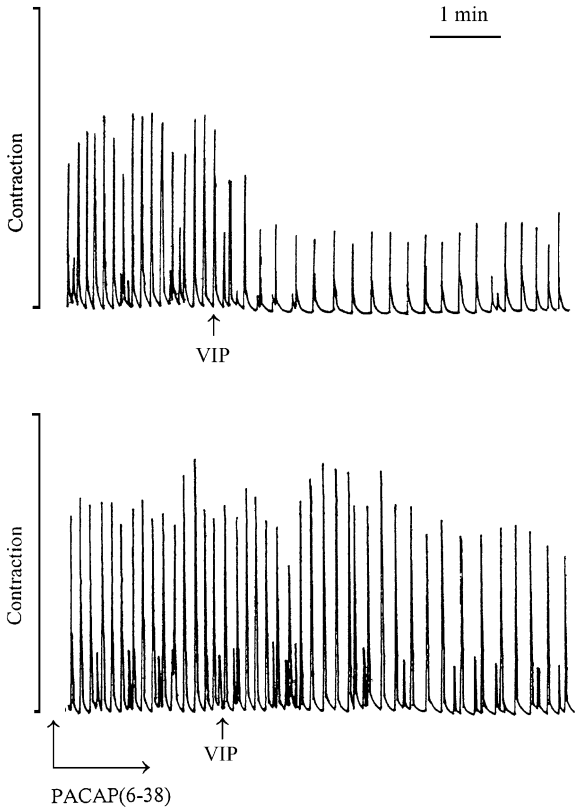

Fig. 5. Effect of PACAP-(6–38) (3 μM) on the inhibitory action of VIP (3 nM) on the spontaneous contractions of ileal circular strips in the presence of tetrodotoxin (1 μM) and indomethacin (3 μM). Vertical calibration bars show 50% of the KCl-provoked maximal spasm.

2.5. Drugs and solutions

The sources of drugs and their stock solutions (given in brackets) were as follows. VIP (300 μM) and PACAP (PACAP-(1–38); 1 mM) were obtained from Peptide Institute (Osaka, Japan). PACAP-(6–38) (1 mM) was either purchased from Bachem (Bubendorf, Switzerland) or synthesized at the Department of Medicinal Chemistry of the University of Szeged Medical School (Lénárd et al., 2000). tetrodotoxin (1 mM), and indomethacin (10 mM) were from Sigma (Budapest, Hungary). Stock solutions were prepared in 96% ethanol (indomethacin) or in distilled water (all other drugs).

2.6. Statistics

The peristaltic pressure threshold data are expressed as a percentage of the control peristaltic pressure threshold measured during the 5-min period before any drug administration and presented as means ± S.E.M. Statistical comparisons of consecutive peristaltic pressure threshold recordings were made with one-way analysis of variance for repeated measures followed by Dunnett's test. Independent samples were compared with each other by Student's *t*-test. For comparing two consecutive samples of circular muscle movements a *t*-test for related samples was used. A probability value of $P < 0.05$ was taken as significant.

3. Results

When all recordings were pooled, peristaltic pressure threshold at baseline was found to be 63.7 ± 2.7 Pa ($n =$ 47). Addition of VIP (100 nM) or PACAP (30 nM) to the organ bath stimulated peristalsis, i.e., decreased peristaltic

Table 1
The inhibitory action of VIP (5–30 nM) on the contractile activity of circular muscle strips in the presence of tetrodotoxin (1 μM) and indomethacin (3 μM), before and after the administration of PACAP-(6–38) (3 μM; contact time, 15 min)

	Control	PACAP-(6–38)	$P <$
Before VIP	5.41 ± 0.66	5.61 ± 0.69	n.s.
Min 1	4.84 ± 0.77	5.15 ± 0.68	n.s.
Min 2	3.06 ± 0.58	3.87 ± 0.43	0.05
Min 3	2.02 ± 0.46	2.80 ± 0.35	0.05
Min 4	1.31 ± 0.47	2.32 ± 0.41	0.05
Min 5	1.14 ± 0.41	2.19 ± 0.44	0.01

Cumulative contractile activity was recorded for each minute at minutes 1 to 5 of administration. Each pair of samples was compared by means of *t*-test. Number of preparations, $n = 7$.

Table 2
The inhibitory action of PACAP-(1–38) (PACAP; 10–500 nM) on the contractile activity of circular muscle strips in the presence of tetrodotoxin (1 μM) and indomethacin (3 μM), before and after the administration of PACAP-(6–38) (3 μM; contact time, 15 min)

	Control	PACAP-(6–38)	$P <$
Before PACAP	3.07 ± 0.47	2.82 ± 0.48	n.s.
Min 1	2.76 ± 0.36	2.56 ± 0.53	n.s.
Min 2	1.40 ± 0.27	1.35 ± 0.33	n.s.
Min 3	0.87 ± 0.29	0.61 ± 0.30	n.s.
Min 4	0.47 ± 0.29	0.43 ± 0.30	n.s.
Min 5	0.46 ± 0.46	0.31 ± 0.31	n.s.

Cumulative contractile activity was recorded for each minute at minutes 1 to 5 of administration. Each pair of samples was compared by means of *t*-test. Number of preparations, $n = 6$, except for min 5 where it was $n = 5$.

pressure threshold and enhanced the frequency of peristaltic waves. The stimulant effect of PACAP was more sustained than that of VIP (Figs. 1–4).

Administration of PACAP-(6–38) (3 μM) or its vehicle had no effect on peristaltic pressure threshold (Figs. 1 and 2). The effect of PACAP-(6–38) on the peristaltic motor effects of VIP and PACAP was examined in vehicle-controlled experiments (Figs. 1–4). When given before exposure to the agonist peptides, PACAP-(6–38) (3 μM) prevented the properistaltic action of VIP but not that of PACAP (Figs. 1 and 2). When added to the bath after the agonist effect had been fully developed, PACAP-(6–38) rapidly reversed the stimulant effect of VIP on peristalsis but failed to stop that of PACAP (Figs. 3 and 4).

In circular strips treated with indomethacin rhythmic spontaneous activity developed 1–3 h after the preparations had been set up. These movements were not inhibited by tetrodotoxin (1 μM). The effect of VIP or PACAP-(1–38) was tested in the presence of tetrodotoxin. VIP caused an inhibition of the circular muscle contractions, with a threshold concentration of 3 nM in most preparations. A single VIP concentration (between 5 and 30 nM) that caused a sub-total inhibition in a reproducible manner was chosen for each preparation. PACAP-(6–38) (3 μM, contact time, 15 min) significantly reduced but did not abolish the inhibitory action of VIP (5–30 nM) (Fig. 5, Table 1). A similar inhibitory action of PACAP-(1–38) (10–500 nM) was not influenced by PACAP-(6–38) (3 μM; Table 2). PACAP-(6–38) on its own failed to influence circular muscle movements (data not shown).

4. Discussion

The present study has shown that the antagonistic peptide fragment PACAP-(6–38) inhibits the stimulant action of VIP on intestinal peristalsis, whereas the properistaltic action of PACAP is spared. This finding is in line with our previous observations that neurogenic contractions of the guinea-pig small intestinal longitudinal muscle evoked by VIP are more readily inhibited by PACAP-(6–38) than contractions evoked by PACAP (Barthó et al., 2000). Of the rat receptors for VIP and/or PACAP, PACAP-(6–38) acts at the $VPAC_2$ and PAC_1 type (Harmar et al., 1998). Provided that this classification is valid for the guinea-pig as well, it would seem that the properistaltic effect of VIP is mediated by $VPAC_2$ receptors, whereas that of PACAP is at least in part brought about by other receptors.

At the level of the smooth muscle, both VIP and PACAP have been found to attenuate the motility of gastrointestinal preparations (Dockray, 1994). However, while VIP relaxes circular muscle strips taken from the guinea-pig ileum (Katsoulis et al., 1992), it is at the same time able to excite neurons in the myenteric plexus (Williams and North, 1979; Cohen and Landry, 1980). This activity and the ability of VIP to facilitate the propagation of action potentials in cholinergic motor neurons (Kadlec et al., 1990) is in keeping with the current observation that VIP stimulates distension-induced peristalsis in the guinea-pig small intestine. Since VIP-containing enteric nerve fibres are present in the myenteric plexus and smooth muscle layers of the gut, it is tempting to assume that VIP plays a double role in peristaltic motor regulation, being a neuro-neuronal excitatory transmitter and/or modulator as well as an inhibitory neuro-muscular messenger of descending motor pathways.

In the tetrodotoxin-treated circular muscle strip VIP inhibited spontaneous contractions, an effect also reduced by PACAP-(6–38). This, along with the inability of the antagonist to inhibit PACAP-induced depression of smooth muscle contractility, might indicate that VIP and PACAP act, at least partly, through separate receptors located on the smooth muscle.

In confirmation of a previous report (Heinemann and Holzer, 1999), we have found that the antagonistic peptide fragment PACAP-(6–38) does not per se modify propulsive motility. Since our data indicate that at the neuronal level (and, at least partly, at the smooth muscle as well) PACAP-(6–38) can be regarded as a VIP receptor antagonist, we infer that a neuronal stimulant action of endogenous VIP does not play a major role in the enteric control of peristaltic motor activity. Likewise, the direct inhibitory effect of endogenous VIP on the circular muscle seems not to be a major determinant of peristalsis in our experimental models.

Acknowledgements

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