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Stimulant action of pituitary adenylate cyclase-activating peptide on normal and drug-compromised peristalsis in the guinea-pig intestine

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1 Pituitary adenylate cyclase-activating peptide (PACAP) is known to influence the activity of intestinal smooth muscle. This study set out to examine the action of PACAP on normal and drug-inhibited peristalsis and to shed light on its site and mode of action.

2 Peristalsis in isolated segments of the guinea-pig small intestine was elicited by distension through a rise of the intraluminal pressure. Drug-induced motility changes were quantified by alterations of the peristaltic pressure threshold at which aborally moving peristaltic contractions were triggered.

3 PACAP (1–30 nM) stimulated normal peristalsis as deduced from a concentration-related decrease in the peristaltic pressure threshold (maximum decrease by 55%). The peptide's stimulant effect remained intact in segments pre-exposed to apamin (0.5 μM), N-nitro-L-arginine methyl ester (300 μM), naloxone (0.5 μM), atropine (1 μM) plus naloxone (0.5 μM) or hexamethonium (100 μM) plus naloxone (0.5 μM).

4 PACAP (10 nM) restored peristalsis blocked by morphine (10 μM), noradrenaline (1 μM) or N^6-cyclopentyladenosine (0.3 μM) and partially reinstated peristalsis blocked by Rp-adenosine-3′,5′-cyclic monophosphothioate triethylamine (100 μM) but failed to revive peristalsis blocked by hexamethonium (100 μM) or atropine (1 μM). The peptide's spectrum of properistaltic activity differed from that of naloxone (0.5 μM) and forskolin (0.3 μM).

5 The distension-induced ascending reflex contraction of the circular muscle was facilitated by PACAP (1–30 nM) which itself evoked transient nerve-mediated contractions of intestinal segment preparations.

6 These data show that PACAP stimulates normal peristalsis and counteracts drug-induced peristaltic arrest by a stimulant action on excitatory enteric motor pathways, presumably at the intrinsic sensory neurone level. The action of PACAP seems to involve multiple signalling mechanisms including stimulation of adenylate cyclase.

Keywords: Pituitary adenylate cyclase-activating peptide; naloxone; morphine; noradrenaline; adenylate cyclase; cholinergic neurones; enteric nervous system; sympathetic nervous system; intestinal peristalsis

Abbreviations: AER, ascending enteric reflex; CCK-8, cholecystokinin octapeptide; CPA, N^6-cyclopentyladenosine; L-NAME, N-nitro-L-arginine methyl ester; PACAP, pituitary adenylate cyclase-activating peptide; PPT, peristaltic pressure threshold; Rp-cyclic AMPS, Rp-adenosine-3′,5′-cyclic monophosphothioate triethylamine; VIP, vasoactive intestinal polypeptide

Introduction

Pituitary adenylate cyclase-activating peptide (PACAP) is structurally related to vasoactive intestinal polypeptide (VIP) and other members of the secretin/glucagon/VIP peptide family but encoded by a different gene. The PACAP precursor is processed to produce two molecular forms of the peptide, PACAP-38 and the C-terminally truncated PACAP-27 (Harmar *et al*., 1998). Within the digestive tract, PACAP is expressed in intrinsic neurones of the enteric nervous system and in extrinsic primary afferents of dorsal root ganglion origin (Sundler *et al*., 1992; Portbury *et al*., 1995; Hannibal *et al*., 1998). The finding that most PACAP in the guinea-pig intestine is contained in anally projecting interneurones of the myenteric plexus suggests that the peptide is involved in the neuronal regulation of gastrointestinal functions (Portbury *et al*., 1995). This conjecture is borne out by the ability of PACAP to influence neuronal and muscular activity in the gut.

In the guinea-pig small intestine, PACAP excites most AH/type 2 neurones in the myenteric plexus (Christofi & Wood, 1993) and elicits neurogenic contractions of the longitudinal muscle, which involve both acetylcholine and tachykinins as transmitters (Katsoulis *et al*., 1993). In addition, PACAP can relax intestinal smooth muscle by a direct action on myocytes (Jin *et al*., 1994; McConalogue *et al*., 1995; Katsoulis *et al*., 1996; Ekblad & Sundler, 1997; Parkman *et al*., 1997). The observation that, in the rat colon, PACAP is released by distension of the circular muscle and participates in the descending relaxation of the muscle elicited by stretch (Grider *et al*., 1994) points to a potential role of the peptide in intestinal motor coordination.

Although the actions of PACAP on intestinal effector systems have been extensively characterized, the overall impact of PACAP on propulsive motility in the intestine has not yet been explored. Since examination of drug effects on isolated motor pathways in the intestine does not necessarily allow to predict as to how propulsion will be influenced (Waterman *et al*., 1994) we set out to study the effect of PACAP-38, hereafter

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called PACAP, on peristaltic propulsion. Using an isolated preparation of the guinea-pig small intestine we found that PACAP potently and efficiently stimulated peristalsis and went on to characterize this effect with regard to sites of action, transduction mechanisms and neuronal systems involved. We also tested whether the facilitatory action of PACAP on propulsion could be exploited to overcome peristaltic blockade caused by adrenoceptor, opioid or adenosine receptor activation.

Methods

Propulsive peristalsis

Adult guinea-pigs (TRIK strain, IEP SAS Dobrá Voda, Bratislava, Slovakia) of either sex and 350–450 g body weight were stunned and bled. The distal small intestine (jejunum and ileum) was excised, 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. 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. For studying peristalsis, the distal small intestine was divided into eight segments, each being approximately 8 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. The system for eliciting and recording propulsive peristalsis has previously been described (Costall *et al*., 1993; Holzer *et al*., 1998). In brief, prewarmed Tyrode solution was continuously infused into the lumen of the segments at a rate of 0.5 ml min^{-1}. The intraluminal pressure at the aboral end of the segments was measured with a pressure transducer whose signal was, *via* an analogue/digital converter, fed into a personal computer and recorded and analysed with the software 'Peristal 1.0' (Heinemann Scientific Software, Graz, Austria).

The fluid passing through the gut lumen was directed into a vertical outlet tubing which ended 4.1 cm above the fluid level in the organ bath. When fluid was infused, the intraluminal pressure rose slowly until it reached a threshold at which peristalsis was triggered (Figure 1; Holzer *et al*., 1998). The aborally moving wave of peristaltic contraction resulted in a spike-like increase in the intraluminal pressure and caused emptying of the segment. The peristaltic pressure threshold (PPT) was used to quantify drug effects on peristalsis. Inhibition of peristalsis was reflected by an increase in PPT, and abolition of peristalsis manifested itself in a lack of propulsive motility in spite of an intraluminal pressure of 400 Pa as set by the position of the outlet tubing. Although in this case PPT exceeded 400 Pa, abolition of peristalsis was expressed quantitatively by assigning PPT a value of 400 Pa in order to obtain numerical results suitable for further statistical evaluation.

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. Thereafter the bath fluid was renewed and peristaltic motility initiated by intraluminal perfusion of the segments. After basal peristaltic activity had been recorded for a period of 30 min, the drugs to be tested were administered into the bath, i.e., to the serosal surface of the intestinal segments, at volumes not exceeding 1% of the bath volume. The corresponding vehicle solutions were devoid of any effect. Three experiments were carried out. Firstly, the concentration-related influence of PACAP (1–30 nM) on peristalsis was studied (Figure 2). Secondly, the susceptibility of the peristaltic motor effect of PACAP (10 nM) to a number of drugs was tested, these drugs being administered at appropriate time intervals before exposure to PACAP (Table 1). Thirdly, the ability of PACAP (10 nM) to revive blocked peristalsis was examined, in which case PACAP was added to the bath 20 min after the intestinal segments had been exposed to drugs that abolished peristalsis (Figure 3). In each case, the peristaltic motor activity of the segments was observed for 45 min after addition of PACAP to determine peak changes in peristalsis. Each drug was added only once to each segment and tested on at least five segments from five different guinea-pigs.

Ascending enteric reflex (AER) contraction

Ileal segments, about 6 cm in length, were secured horizontally in a 10-ml organ bath filled with oxygenated Tyrode's solution and maintained at 37°C (Holzer *et al*., 1993). The AER contraction of the circular muscle was triggered by radial distension of the intestinal wall, which was achieved by inflation of an intraluminal balloon made of latex rubber and inserted at the aboral end of the segment. The mechanical activity of the circular muscle was recorded at two sites orally from the site of distension. The 'close' recording site was about 1 cm, and the 'remote' recording site about 2 cm away from the site of balloon distension. Muscle activity was recorded under isotonic conditions *via* clips that gripped the mesentery close to the intestinal wall, the resting load being 10 mN (Holzer *et al*., 1993). Either end of the segments was secured so

Table 1 Action of PACAP (10 nM) to lower the peristaltic pressure threshold (PPT) under various drug treatments

Drug treatment	n	*Drug exposure time* (min)	*PPT (Pa) before exposure to PACAP*	*PPT (Pa) after exposure to PACAP*	*PPT (% of pre-PACAP)*
Control	35	5–40	71±5	42±2	63±3
PACAP-(6-38) (3 μM)	5	5	60±14	38±6	67±8
Apamin (0.5 μM)	7	30	48±7*	29±7	58±8
L-NAME (300 μM)	5	30	49±3*	30±4	60±6
Naloxone (0.5 μM)	6	30	62±8	39±7	61±4
Atropin (1 μM)+ naloxone (0.5 μM)	6	40 30	122±21	75±20	63±16
Hexamethonium (100 μM)+ naloxone (0.5 μM)	6	40 30	118±28	37±4	38±6*

The drug exposure time refers to the interval between administration of drug and that of PACAP. PPT (%) is expressed as a percentage of the PPT recorded before exposure to PACAP (10 nM). The values recorded after exposure to PACAP refer to the peak changes in PPT which occurred within 5 min post-administration. The values represent means±s.e.mean of *n* experiments as indicated. *$P<0.05$ versus control (two sample *t*-test).

as to prevent dislocation of the recording site by longitudinal contractions (Holzer, 1989) and to eliminate any contribution of longitudinal muscle activity to the circular muscle responses under study.

The experiments were begun after an equilibration period of 30 min, during which the bath fluid was repeatedly renewed. Thereafter the AER contraction was elicited by balloon inflation for periods of 5 s at 2-min intervals. Rest periods of 10 min were allowed between stimulation periods of 20 min (referred to as trains of 10 distension stimuli), and after each train of stimulation the bath medium was renewed by three successive washings. First, the preparations were standardized by the response to a train of maximal distension stimuli (balloon inflation to a diameter of 7 mm), which caused contractions that were not significantly different from those elicited by a maximally effective concentration of cholecystokinin octapeptide (CCK-8, 10 nM, $n=5$). The amplitude of all further reflex contractions was expressed as a percentage of the maximal AER contraction. The effect of PACAP was tested on trains of AER contractions elicited by balloon inflation to diameters of either 4, 5 or 6 mm. In each case, five AER contractions were elicited before, and five contractions after, PACAP (1–10 nM) had been added to the bath. The effect of PACAP was quantified by determining the peak changes in the AER contraction amplitude (Figure 4). At the end of each experiment, the neurogenic nature of the ascending reflex contractions was ascertained by addition of tetrodotoxin (0.5 μM) which invariably abolished the contractions (Holzer *et al.*, 1993).

Circular muscle activity

The effect of PACAP on circular muscle activity at baseline was studied with two different circular muscle preparations. The first preparation was identical with the segment preparation used for recording of the AER contraction, except that no inflatable balloon was inserted in the segment. After a 30-min equilibration period, during which the bath fluid was repeatedly renewed, the preparations were standardized by exposure to a maximally effective concentration of CCK-8 (10 nM). The responses to PACAP recorded subsequently were expressed relative to the contraction caused by CCK-8.

The second preparation was a whole-thickness circular strip which corresponded to a 2 mm wide ring of the ileum cut open at the mesenteric attachment (Barthó *et al.*, 1994). These strips were suspended vertically in a 7-ml organ bath filled with oxygenated Tyrode's solution and maintained at 37°C, contractions being recorded under isotonic conditions and a resting load of 2 mN. After an equilibration time of 120 min, during which the bath fluid was repeatedly renewed, the preparations were standardized by exposure to 10 nM CCK-8 (Barthó *et al.*, 1994).

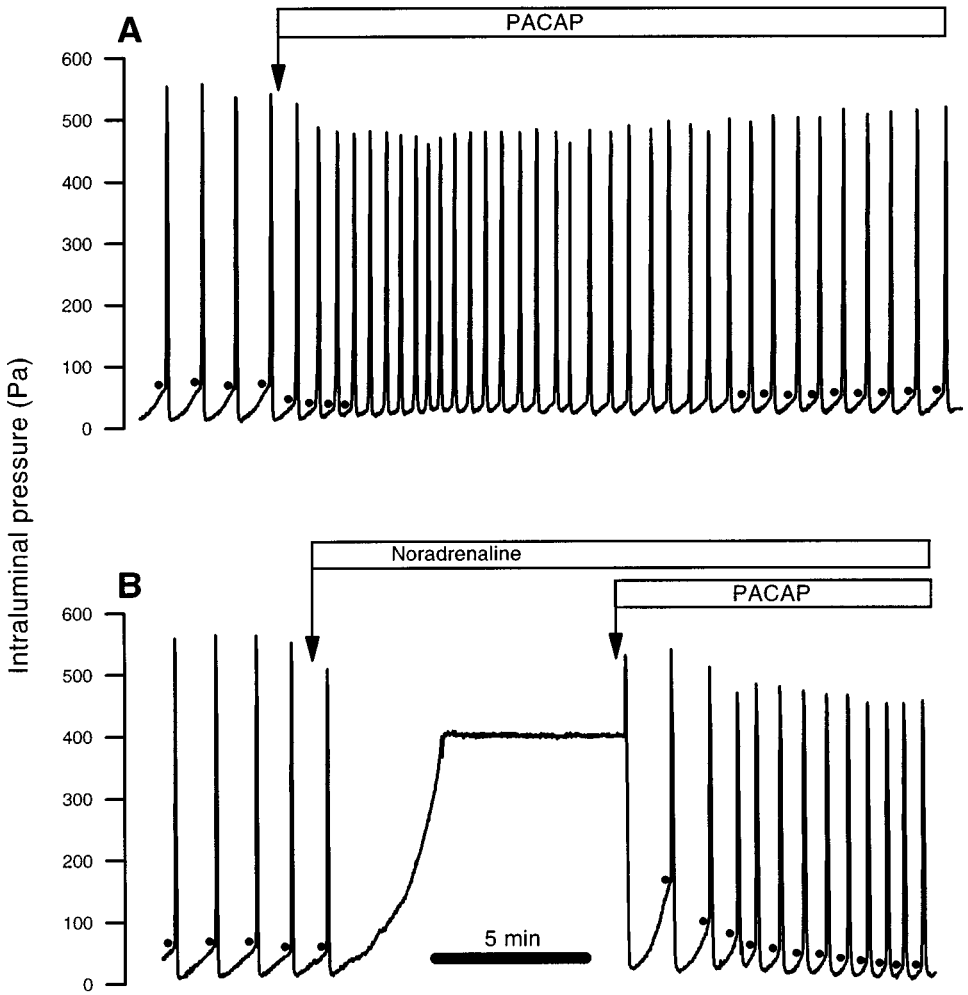

Figure 1 (A) Recording of the action of PACAP (10 nM) to stimulate peristalsis, which is reflected by a decrease in the peristaltic pressure threshold indicated by dots. (B) Effect of noradrenaline (1 μM) and PACAP (10 nM), added consecutively to the organ bath, on peristalsis. Noradrenaline abolishes peristalsis which is promptly restored by PACAP.

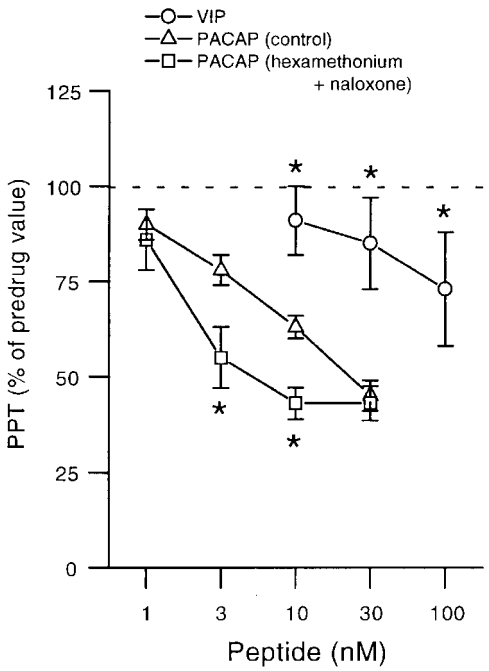

Figure 2 Concentration-dependent action of PACAP and VIP to lower the peristaltic pressure threshold (PPT). The action of PACAP to decrease PPT was recorded in the absence (control) and presence of hexamethonium (100 μM) plus naloxone (0.5 μM). Hexamethonium was administered 40 min, and naloxone 30 min, before addition of PACAP to the organ bath. The graphs show peak changes in PPT which occurred within 5 min after exposure to PACAP or VIP. PPT is expressed as a percentage of the threshold recorded before exposure to PACAP or VIP. The values represent means $\pm$ s.e.mean, $n \geqslant 5$. *$P < 0.05$ versus control (two sample t-test).

Subsequently to standardization, the circular muscle preparations were exposed to PACAP (1 – 30 nM) at 10 min intervals, the contact time for each peptide administration being 2 min. Additional experiments examined the susceptibility of the contractile effect of PACAP (30 nM) to certain drugs (Figure 5) which were administered to the bath 10 min before exposure to PACAP.

Drugs and solutions

Apamin (1 mM), atropine (1 mM), N^6-cyclopentyladenosine (CPA; 1 mM), hexamethonium (10 mM), morphine (1 mM), naloxone (1 mM), N-nitro-L-arginine methyl ester (L-NAME; 30 mM), noradrenaline (10 mM), tetrodotoxin (1 mM) and yohimbine (1 mM) were all obtained from Sigma (St. Louis, MO, U.S.A.) and dissolved in water. Human vasoactive intestinal polypeptide (VIP, 100 μM), PACAP-38 (1 mM), PACAP-(6 – 38) (100 μM) and CCK-8 (sulphated, 1 mM) were obtained from Peptide Institute (Osaka, Japan) and dissolved in water, except CCK-8 (100 μM) which was dissolved in 5% $NaHCO_3$. Forskolin (100 μM) and Rp-adenosine-3′,5′-cyclic monophosphothioate triethylamine (Rp-cyclic AMPS; 1 mM) were purchased from RBI (Natick, MA, U.S.A.) and dissolved in dimethyl sulphoxide. The concentrations referred to in brackets indicate the stock solutions which were diluted with Tyrode solution before use.

Data calculation and statistics

The PPT of three consecutive peristaltic contractions was averaged to determine the baseline values recorded immediately before administration of a drug. The same procedure was applied to calculate the peak values of drug-induced changes in PPT, unless peristalsis was abolished in which case PPT was assigned a value of 400 Pa. The effect of PACAP on the AER contractions was quantified by averaging the two contractions recorded immediately before exposure to PACAP and the two contractions which were most changed after addition of the peptide. Quantitative data are presented as means $\pm$ s.e.mean of n experiments, n referring to the number of guinea-pigs used in the test. The results were evaluated with the paired or two-sample Student's t-test. A probability value $P < 0.05$ was regarded as significant.

Results

Effect of PACAP to stimulate normal peristalsis

The PPT at baseline ranged from 60 – 100 Pa (Table 1, Figures 1 and 3). Addition of PACAP-38 (1 – 30 nM), hereafter called PACAP, to the organ bath lowered PPT in a concentration-related manner (Figure 2), which was associated with a rise of the frequency of peristaltic contractions (Figure 1A). The reduction of the contraction amplitude (Figure 1A) is a sequel of the frequency rise and not a specific response to PACAP, given that an increase in peristaltic frequency due to doubling of the luminal perfusion rate attenuated the contraction amplitude to a similar extent ($n = 5$; data not shown). PACAP's stimulant effect on peristalsis was quick in onset and reached a maximum within 1 – 5 min (Figure 1A), after which PPT returned slowly to the baseline value over the following 30 min. In some instances the initial decrease of PPT reversed after 15 – 20 min to a transient increase of PPT, but this late inhibition of peristalsis caused by PACAP was inconsistent and did not reach the level of statistical significance. Qualitatively similar effects were seen with VIP (10 – 100 nM) which also led to a concentration-dependent decrease of PPT. The potency of VIP to facilitate peristalsis, however, was lower than that of PACAP (Figure 2).

As shown in Table 1, the stimulant effect of PACAP (10 nM) on peristalsis remained unchanged by the antagonistic fragment PACAP-(6-38) (3 μM), the opioid receptor antagonist naloxone (0.5 μM), the nitric oxide synthase inhibitor L-NAME (300 μM) and the K^+ channel inhibitor apamin (0.5 μM). PACAP-(6-38) *per se* had no effect on peristalsis while, in confirmation of previous observations (Holzer *et al.*, 1997), apamin and L-NAME caused a persistent reduction of PPT on their own (Table 1). Naloxone also lowered PPT (Holzer *et al.*, 1998) but this effect was transient so that after an interval of 30 min, when PACAP was tested, PPT had returned to the baseline level (Table 1). In order to examine a contribution by cholinergic neurones, the facilitatory influence of PACAP on peristalsis was tested in the presence of atropine (1 μM) plus naloxone (0.5 μM) or hexamethonium (100 μM) plus naloxone. Atropine and hexamethonium alone suppressed peristaltic motor activity which, however, was restored when naloxone was added in the continued presence of atropine or hexamethonium (Table 1; Holzer *et al.*, 1998). While exposure to atropine plus naloxone failed to alter the ability of PACAP to facilitate peristalsis (Table 1), exposure to hexamethonium plus naloxone potentiated the facilitatory influence of PACAP on peristalsis (Table 1) as shown by a leftward shift of the concentration-response curve for PACAP (Figure 2).

Effect of PACAP to rescue blocked peristalsis

Peristalsis was blocked by atropine (1 μM), hexamethonium (100 μM), morphine (10 μM), noradrenaline (1 μM), CPA (0.3 μM) or Rp-cyclic AMPS (100 μM) at concentrations that in preliminary experiments ($n=6$) had been found to be minimal for abolishing peristalsis. Unlike naloxone (0.5 μM), PACAP (10 nM) and forskolin (0.3 μM), a direct adenylate

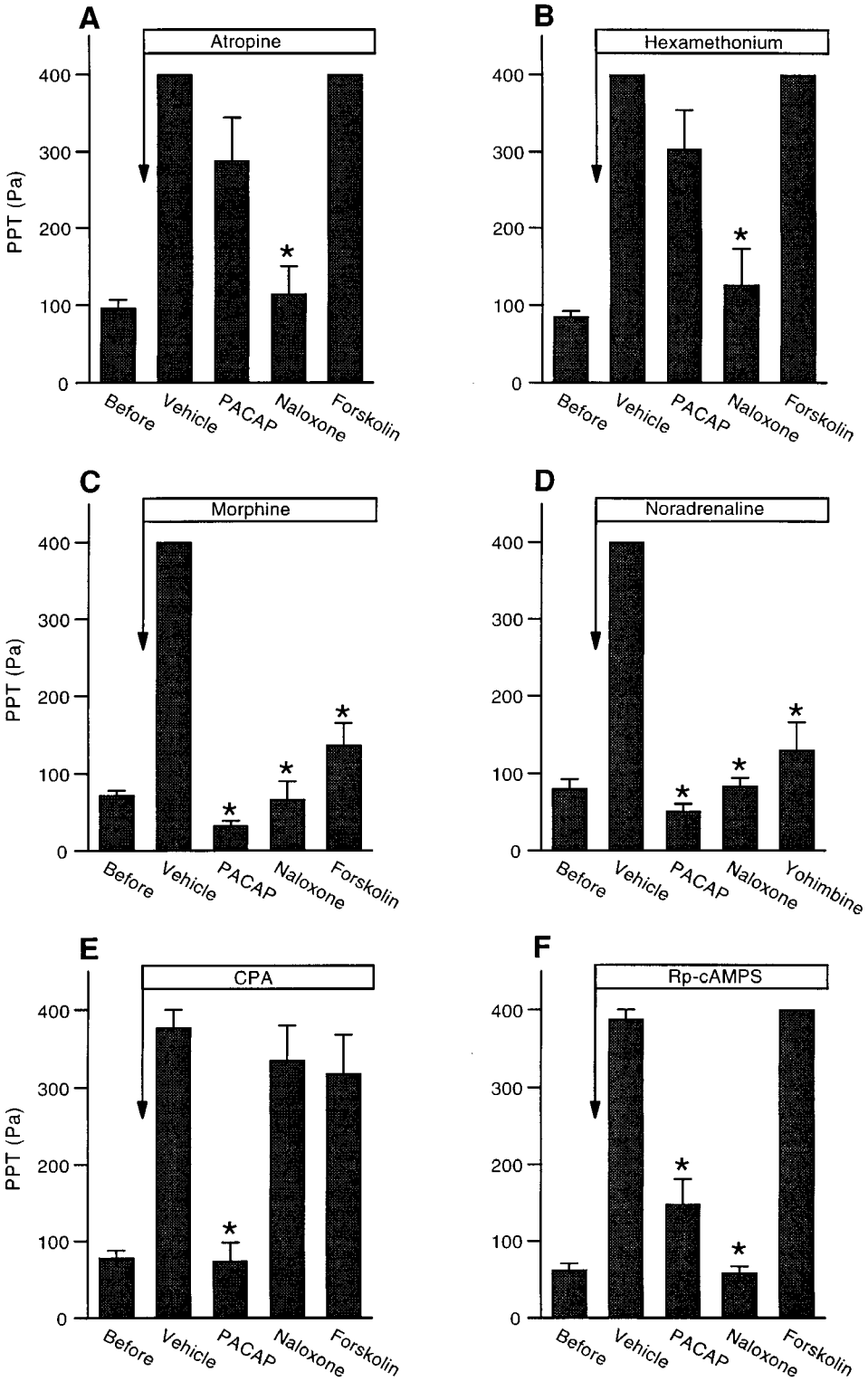

Figure 3 Action of PACAP (10 nM), naloxone (0.5 μM), forskolin (0.3 μM) and yohimbine (1 μM) to restore peristalsis which had been blocked for 20 min by administration of atropine (1 μM; A), hexamethonium (100 μM; B), morphine (10 μM; C), noradrenaline (1 μM; D), CPA (0.3 μM; E) or Rp-cyclic AMPS (100 μM; F). The graphs show the peristaltic pressure threshold (PPT) recorded immediately before exposure to any drug (before) and after administration of vehicle, PACAP, naloxone, forskolin or yohimbine to preparations in which atropine, hexamethonium, morphine, noradrenaline, CPA or Rp-cyclic AMPS had abolished peristalsis within 15 min (reflected by a PPT of 400 Pa). Thereafter the segments were observed for 45 min to record any recovery of peristalsis which, if it took place, was accomplished within 15 min and is illustrated by the peak changes in PPT. The values represent means$\pm$s.e.mean, $n\geqslant 5$. *$P<0.05$ versus vehicle (two sample t-test).

cyclase activator, failed to rescue peristalsis from blockade by atropine and hexamethonium to any significant extent (Figure 3A and B). PACAP, naloxone and forskolin, however, rescued peristalsis from suppression by morphine (Figure 3C). The effect of noradrenaline to block peristalsis was also cancelled out by PACAP and naloxone as well as by the α_2 adrenoceptor antagonist yohimbine (1 μM; Figures 1B and 3D). When peristalsis was suppressed by morphine or noradrenaline, PACAP not only restored peristalsis but even lowered PPT below the baseline level (Figure 3C and D) as was the case when PACAP was tested on normal peristalsis (Table 1, Figures 1 and 2). Forskolin and yohimbine, given alone, had no effect on normal peristalsis ($n=5$ for each drug; data not shown).

Further experiments compared PACAP, naloxone and forskolin in their ability to rescue peristalsis from blockade by the adenosine A_1 receptor agonist CPA and Rp-cyclic AMPS, an inhibitor of cyclic AMP-dependent protein kinases. As is shown in Figure 3E and F, only PACAP, but not naloxone and forskolin, restored peristaltic motility blocked by CPA. In contrast, peristalsis suppressed by Rp-cyclic AMPS was fully revived by naloxone, partially but significantly restored by PACAP but left blocked by forskolin (Figure 3F).

Effect of PACAP on the AER contraction

Intraluminal balloon inflation caused a distension-related AER contraction whose amplitude was larger at the close (Figure 4A) than at the remote (Figure 4B) recording site. Exposure of the segments to PACAP (1–10 nM) caused a slight increase in circular muscle tone (<10% of the maximal AER contraction), which sometimes was superimposed by

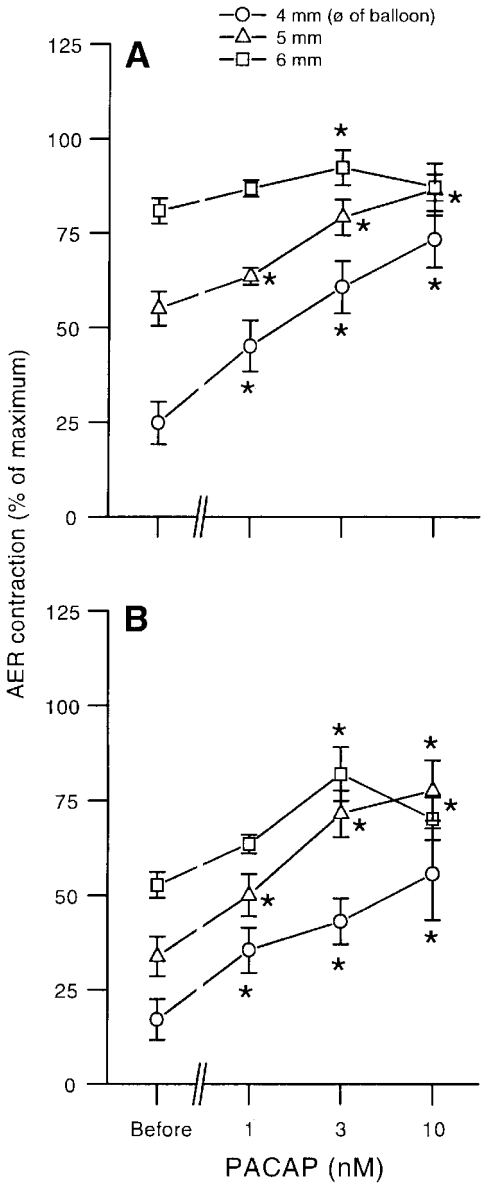

Figure 4 Concentration-related action of PACAP to augment ascending enteric reflex (AER) contractions elicited by inflation of an intraluminal balloon to diameters of 4, 5 or 6 mm. The contractions were recorded approximately 1 cm ('close'; A) and 2 cm ('remote'; B) orally from the distension site and are expressed as a percentage of the response to a maximal distension stimulus (balloon inflation to a diameter of 7 mm). The values, which are means ± s.e.mean, reflect the average amplitude of two AERs recorded immediately before (before) and after addition of PACAP when the peptide's effect was maximal. For the graphs all control values were pooled ($n=21-22$), although statistical comparisons were made between the individual treatment groups only ($n=7-8$). *$P<0.05$ versus 'before' (paired t-test).

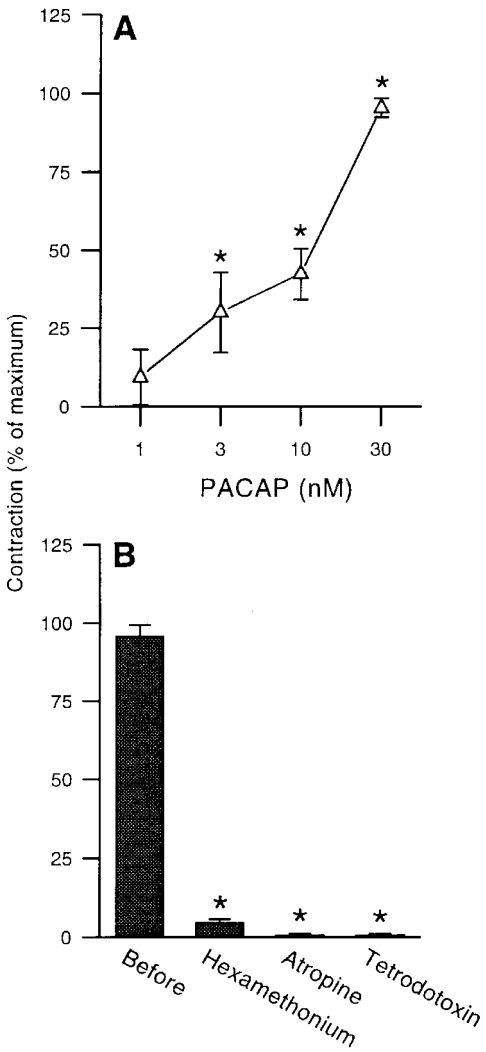

Figure 5 (A) Concentration-related action of PACAP to cause tonic-phasic contractions of the circular muscle in segment preparations. (B) Effect of PACAP (30 nM) to cause tonic-phasic contractions of the circular muscle under control conditions (before) and after a 10-min presence of hexamethonium (100 μM), atropine (1 μM) or tetrodotoxin (0.5 μM). The overall amplitude of the tonic-phasic contractions is expressed as a percentage of the response to 10 nM CCK-8. The values represent means ± s.e.mean, $n=8$ in A and 4–7 in B. *$P<0.01$ versus vehicle (A) or 'before' (B; paired t-test).

phasic contractions. This motor effect of PACAP was concentration-related (see below), transient and waned within 3 min. At the same time the amplitude of the AER contraction was augmented by PACAP (1–10 nM) in a concentration-dependent manner, this response peaking 3–5 min after exposure to the peptide. The effect of 3 and 10 nM PACAP to facilitate the AER contraction was significant at both recording sites and with all diameters of balloon inflation, except for the near-maximal contraction due to 6 mm balloon inflation at the close recording site (Figure 4A and B). The PACAP-induced enhancement of the AER contraction was more prominent with low than with large diameters of balloon inflation.

Effect of PACAP on circular muscle activity

As has been reported previously (Barthó *et al*., 1994), exposure to CCK-8 produces phasic contractions of the circular muscle superimposed on a sustained tonic response. PACAP (1–30 nM) caused a qualitatively similar reaction in the segment preparations of the circular muscle. The overall amplitude of the tonic-phasic contractions depended on the peptide concentration in the bath, 30 nM PACAP eliciting a contraction that equalled 95% of the response due to 10 nM CCK-8 (Figure 5A). Atropine (1 μM) and tetrodotoxin (0.5 μM) abolished the contraction due to 30 nM PACAP, and hexamethonium (100 μM) depressed it by 95% (Figure 5B). In contrast, the strip preparations of circular muscle failed to respond to PACAP (3–30 nM) with contraction or relaxation, although they contracted in response to 10 nM CCK-8 ($n=4$; data not shown).

Discussion

The present data show that PACAP potently stimulates propulsive motility in the guinea-pig isolated small intestine and rescues peristalsis from blockade caused by morphine, noradrenaline or adenosine. This spectrum of activity is consistent with the peptide's ability to excite most myenteric AH/type 2 neurones (Christofi & Wood, 1993), which are thought to be sensory neurones of the enteric nervous system (Furness *et al*., 1998). By stimulating enteric motor pathways PACAP not only elicits nerve-mediated contractions of the longitudinal muscle (Katsoulis *et al*., 1993) but, as demonstrated here, also evokes tonic-phasic contractions of the circular muscle, the major effector of intestinal peristalsis. As the contractile action was seen only in segment, but not strip, preparations it would appear that the peptide's action requires long neural pathways (see Waterman *et al*., 1994) that are interrupted in the strip preparations. This inference is borne out by the finding that PACAP-evoked contractions of the segment preparations were prevented by the nerve conduction blocker tetrodotoxin as well as by the muscarinic and nicotinic acetylcholine receptor antagonist atropine and hexamethonium, respectively. Since muscarinic receptors mediate both neuroneuronal and neuromuscular transmission in the gut (Tonini & Costa, 1990; Johnson *et al*., 1996) while nicotinic receptors participate in neuroneuronal transmission only (Kirchgessner & Liu, 1998) it follows that neuroneuronal transmission is obligatory for the contractile response to PACAP. It is very likely by this 'preganglionic' site of action that PACAP facilitates both peristalsis and the ascending enteric reflex (AER) contraction, a component of propulsive motility (Waterman *et al*., 1994). This conjecture is further supported by the similar potency with which PACAP stimulated peristalsis and the AER contraction.

Peristaltic facilitation in the guinea-pig small intestine is a novel action of PACAP which in the rat intestine has been found to participate in the descending muscle relaxation in response to stretch (Grider *et al*., 1994). The actions of PACAP and VIP are mediated by three specific receptors termed PAC_1, $VPAC_1$ and $VPAC_2$ receptors, with PACAP being more active than VIP at PAC_1 receptors but equipotent with VIP at $VPAC_1$ and $VPAC_2$ receptors (Harmar *et al*., 1998). Since PACAP was more potent in facilitating peristalsis than VIP, it is suggested that PACAP-induced stimulation of peristalsis is brought about by specific PAC_1 receptors. While the direct, relaxant effect of PACAP on intestinal smooth muscle is mediated by receptors that are antagonized by PACAP-(6-38) (Grider *et al*., 1994; Jin *et al*., 1994; Katsoulis *et al*., 1996; Parkman *et al*., 1997; Rattan & Chakder, 1997), the stimulant effect of PACAP on peristalsis remained unaltered by 3 μM PACAP-(6-38). It thus appears as if the PACAP receptors on excitatory pathways of the enteric nervous system are pharmacologically distinct from the inhibitory PACAP receptors on myocytes, which are fully blocked by 3 μM PACAP-(6-38) (Kishi *et al*., 1996). Higher concentrations of this expensive PACAP receptor antagonist were not affordable in our experiments. A further distinction between PACAP receptors on intestinal muscle and nerve is brought to light by apamin, a blocker of small conductance, calcium-dependent potassium channels which participate in inhibitory neuromuscular transmission brought about by purines (Waterman & Costa, 1994; McConalogue *et al*., 1995; Zagorodnyuk *et al*., 1996). The inhibitory action of PACAP on intestinal smooth muscle preparations is either inhibited (Schwörer *et al*., 1992; McConalogue *et al*., 1995; Katsoulis *et al*., 1996; Zagorodnyuk *et al*., 1996) or left unaltered (Grider *et al*., 1994; Ekblad & Sundler, 1997; Rattan & Chakder, 1997) by apamin. The current findings indicate that the excitatory action of PACAP on peristalsis is mediated by transduction processes that do not involve apamin-sensitive ion channels.

The excitatory neural pathways subserving peristalsis are essentially cholinergic, involve transmission *via* nicotinic and muscarinic receptors, and employ tachykinins as cholinergic cotransmitters (Tonini & Costa, 1990; Johnson *et al*., 1996; Holzer *et al*., 1998). A contribution of cholinergic mechanisms to the PACAP-induced facilitation of peristalsis was examined with the help of atropine and hexamethonium in combination with the opioid receptor antagonist naloxone which rescues peristalsis from blockade by muscarinic or nicotinic receptor antagonists (Holzer *et al*., 1998). The inability of atropine plus naloxone to prevent PACAP-induced stimulation of peristalsis shows that the peptide's properistaltic action does not specifically depend on intact transmission *via* muscarinic receptors and can be observed as long as peristalsis in the presence of atropine plus naloxone is maintained by endogenous tachykinins acting at NK_1 and NK_2 receptors (Holzer *et al*., 1998). It would hence appear that PACAP stimulates excitatory enteric pathways of peristalsis by acting on a unit that operates before neuroneuronal or neuromuscular transmission *via* muscarinic receptors comes into play. This target of action must also be situated ahead of neuroneuronal relays operated by nicotinic receptors, because hexamethonium plus naloxone likewise failed to prevent PACAP-evoked facilitation of peristalsis.

The properistaltic action of PACAP was in fact potentiated by hexamethonium plus naloxone, an observation that could reflect interruption of hexamethonium-sensitive inhibitory pathways of peristalsis that are stimulated by PACAP in

parallel with its action on excitatory pathways. However, since the peptide's facilitatory action on peristalsis remained unaltered by apamin, naloxone and L-NAME, an inhibitor of nitric oxide synthase, there is little room to hypothesize that PACAP stimulated peristalsis by interfering with endogenous purines, opioids or nitric oxide, three important inhibitory control systems of intestinal motility (Kromer, 1988; Makhlouf & Grider, 1993; Waterman & Costa, 1994; McConalogue *et al*., 1995; Zagorodnyuk *et al*., 1996; Holzer *et al*., 1997). These considerations corroborate the emerging hypothesis that PACAP promotes propulsive motility by stimulating excitatory pathways of peristalsis. The failure of PACAP to rescue peristalsis from blockade by atropine or hexamethonium suggests that PACAP facilitates peristaltic pathways by acting on intrinsic sensory neurones which lie ahead of cholinergic interneurones and motoneurones.

The ability of PACAP to restore peristalsis blocked by various drugs was tested in order to gain more insight into the site and mode of the peptide's action and to explore its potential in pathologically disturbed motility. Importantly, PACAP was able to revive peristalsis after blockade by morphine or noradrenaline whose action was mediated by μ opioid receptors and α_2 adrenoceptors, respectively, as shown with the help of naloxone and yohimbine. Although the major signalling pathway operated by α_2 adrenoceptors is inhibition of adenylate cyclase, μ opioid receptor agonists depress firing of myenteric neurones in the guinea-pig ileum *via* an action unrelated to adenylate cyclase (Karras & North, 1979). It is hence not possible to deduce from the current data that PACAP counteracts peristaltic inhibition by virtue of its adenylate cyclase-stimulating activity. This argument is corroborated by the observation that the spectrum of PACAP's properistaltic action differs from that of a supramaximally effective concentration of forskolin (0.3 μM; Zafirov *et al*., 1985) which is a direct activator of adenylate cyclase. Thus, peristalsis blocked by atropine or hexamethonium was revived by forskolin but not PACAP. In contrast, forskolin failed to restore peristalsis blocked by CPA, an agonist of adenosine A_1 receptors which are negatively coupled to adenylate cyclase, while PACAP reinstated CPA-suppressed peristalsis, which is consistent with the ability of CPA to oppose the PACAP-evoked depolarization of AH/type 2 neurones in the myenteric plexus (Christofi & Wood, 1993). The finding that PACAP, unlike forskolin, was able to partially counteract the peristaltic blockade caused by Rp-cyclic AMPS, an inhibitor of cyclic AMP-dependent protein kinases (Chik *et al*., 1996), indicates that the cellular action of PACAP involves additional signalling systems. This inference is in keeping with the reported coupling of PACAP receptors to adenylate cyclase, the phospolipase C/phosphoinositide pathway and various K^+ channels (Chik *et al*., 1996; Kishi *et al*., 1996; Van Rampelbergh *et al*., 1997; Harmar *et al*., 1998).

As is depicted in Figure 3, naloxone was able to terminate peristaltic arrest imposed not only by morphine, noradrenaline and Rp-cyclic AMPS but also by atropine and hexamethonium and thus exceeded PACAP in its spectrum of properistaltic activity. These differences in activity are likely to reflect different sites and mechanisms of action, and from the data obtained with naloxone it appears as if endogenous opioid peptides contribute to peristaltic shutdown caused by drug-induced interruption of various relays within the peristaltic motor pathways. The ability of naloxone to rescue peristalsis from blockade of divergent mechanisms is in keeping with the ability of opioid peptides to inhibit propulsive motility by interfering with multiple targets in the enteric nervous system (Kromer, 1988; Tonini *et al*., 1992). It is obvious that the effects of PACAP and naloxone to rescue peristalsis from inhibition caused by noradrenaline, morphine and CPA are of considerable therapeutic potential. This is particularly true if considered that noradrenaline released from sympathetic nerve terminals participates in adynamic ileus (Livingston & Passaro, 1990) and shutdown of propulsive motility is one of the major unwanted side effects of opiates.

In summary, the current data establish PACAP as a properistaltic peptide which seems to promote propulsive motility by increasing the excitability of sensory neurones within peristaltic motor pathways. This site of action of the adenylate cyclase activating peptide is consistent with the important role which adenylate cyclase plays in slow excitation of AH/type 2 neurones (Zafirov *et al*., 1985) now considered to be sensory neurones of the enteric nervous system (Furness *et al*., 1998). It would appear that the activity of these neurones is not only governed by the stimuli which they are supposed to perceive but also regulated by messengers derived from neighbouring neurones. PACAP may be one of the factors which when released from intrinsic enteric or extrinsic afferent neurones (Sundler *et al*., 1992; Portbury *et al*., 1995; Hannibal *et al*., 1998) enhances the excitability of intrinsic sensory neurones and in this way facilitates peristalsis. It remains to be elucidated whether certain states of disturbed intestinal motility are related to a disorder of the PACAP system. Should the current data obtained with PACAP have a bearing on human disease, pharmacokinetically improved analogues of the peptide acting selectively on neural PACAP receptors may be beneficial in overcoming peristaltic arrest caused by sympathetic activity, opiate treatment or intoxication and overproduction of adenosine.

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References

BARTHÓ, L., MAGGI, C.A., WILHELM, M. & PATACCHINI, R. (1994). Tachykinin NK_1 and NK_2 receptors mediate atropine-resistant ileal circular muscle contractions evoked by capsaicin. *Eur. J. Pharmacol.,* **259,** 187 – 193.

CHIK, C.L., BING, L.I., OGIWARA, T., HO, A.K. & KARPINSKI, E. (1996). PACAP modulates L-type Ca^{2+} channel currents in vascular smooth muscle: involvement of PKC and PKA. *FASEB J.,* **10,** 1310 – 1317.

CHRISTOFI, F.L. & WOOD, J.D. (1993). Effects of PACAP on morphologically identified myenteric neurons in guinea pig small bowel. *Am. J. Physiol.,* **264,** G414 – G421.

COSTALL, B., NAYLOR, R.J. & TULADHAR, B.R. (1993). 5-HT_4 receptor-mediated facilitation of the emptying phase of the peristaltic reflex in the guinea-pig isolated ileum. *Br. J. Pharmacol.,* **110,** 1572 – 1578.

EKBLAD, E. & SUNDLER, F. (1997). Distinct receptors mediate pituitary adenylate cyclase-activating peptide- and vasoactive intestinal peptide-induced relaxation of rat ileal longitudinal muscle. *Eur. J. Pharmacol.,* **324,** 61 – 66.

FURNESS, J.B., KUNZE, W.A.A., BERTRAND, P.P., CLERC, N. & BORNSTEIN, J.C. (1998). Intrinsic primary afferent neurons of the intestine. *Prog. Neurobiol.,* **54,** 1 – 18.

GRIDER, J.R., KATSOULIS, S., SCHMIDT, W.E. & JIN, J.G. (1994). Regulation of the descending relaxation phase of intestinal peristalsis by PACAP. *J. Auton. Nerv. Syst.*, **50,** 151 – 159.

HANNIBAL, J., EKBLAD, E., MULDER, H., SUNDLER, F. & FAHRENKRUG, J. (1998). Pituitary adenylate cyclase activating polypeptide (PACAP) in the gastrointestinal tract of the rat: distribution and effects of capsaicin or denervation. *Cell Tissue Res.*, **291,** 65 – 79.

HARMAR, A.J., ARIMURA, A., GOZES, I., JOURNOT, L., LABURTHE, M., PISEGNA, J.R., RAWLINGS, S., ROBBERECHT, P., SAID, S.I., SREEDHARAN, S.P., WANK, S.A. & WASCHEK, J.A. (1998). International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol. Rev.*, **50,** 265 – 270.

HOLZER, P. (1989). Ascending enteric reflex: multiple neurotransmitter systems and interactions. *Am. J. Physiol.*, **256,** G540 – G545.

HOLZER, P., LIPPE, I.T., HEINEMANN, Á. & BARTHÓ, L. (1998). Tachykinin NK_1 and NK_2 receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine *in vitro*. *Neuropharmacology,* **37,** 131 – 138.

HOLZER, P., LIPPE, I.T., LOTFI TABRIZI, A., LÉNÁRD, L. & BARTHÓ, L. (1997). Dual excitatory and inhibitory effect of nitric oxide on peristalsis in the guinea pig intestine. *J. Pharmacol. Exp. Ther.*, **280,** 154 – 161.

HOLZER, P., SCHLUET, W. & MAGGI, C.A. (1993). Ascending enteric reflex contraction: roles of acetylcholine and tachykinins in relation to distension and propagation of excitation. *J. Pharmacol. Exp. Ther.*, **264,** 391 – 396.

JIN, J.G., KATSOULIS, S., SCHMIDT, W.E. & GRIDER, J.R. (1994). Inhibitory transmission in tenia coli mediated by distinct vasoactive intestinal peptide and apamin-sensitive pituitary adenylate cyclase activating peptide receptors. *J. Pharmacol. Exp. Ther.*, **270,** 433 – 439.

JOHNSON, P.J., BORNSTEIN, J.C., YUAN, S.Y. & FURNESS, J.B. (1996). Analysis of contributions of acetylcholine and tachykinins to neuro-neuronal transmission in motility reflexes in the guinea-pig ileum. *Br. J. Pharmacol.*, **118,** 973 – 983.

KARRAS, P.J. & NORTH, R.A. (1979). Inhibition of neuronal firing by opiates: evidence against the involvement of cyclic nucleotides. *Br. J. Pharmacol.*, **65,** 647 – 652.

KATSOULIS, S., CLEMENS, A., SCHWÖRER, H., CREUTZFELDT, W. & SCHMIDT, W.E. (1993). PACAP is a stimulator of neurogenic contraction in guinea pig ileum. *Am. J. Physiol.*, **265,** G295 – G302.

KATSOULIS, S., SCHMIDT, W.E., SCHWARZHOFF, R., FÖLSCH, U.R., JIN, J.G., GRIDER, J.R. & MAKHLOUF, G.M. (1996). Inhibitory transmission in guinea pig stomach mediated by distinct receptors for pituitary adenylate cyclase-activating peptide. *J. Pharmacol. Exp. Ther.*, **278,** 199 – 204.

KIRCHGESSNER, A.L. & LIU, M.T. (1998). Immunohistochemical localization of nicotinic acetylcholine receptors in the guinea pig bowel and pancreas. *J. Comp. Neurol.*, **390,** 497 – 514.

KISHI, M., TAKEUCHI, T., SUTHAMNATPONG, N., ISHII, T., NISHIO, H., HATA, F. & TAKEWAKI, T. (1996). VIP- and PACAP-mediated nonadrenergic, noncholinergic inhibition in longitudinal muscle of rat distal colon: involvement of activation of charybdotoxin- and apamin-sensitive K^+ channels. *Br. J. Pharmacol.*, **119,** 623 – 630.

KROMER, W. (1998). Endogenous and exogenous opioids in the control of gastrointestinal motility and secretion. *Pharmacol. Rev.*, **40,** 121 – 150.

LIVINGSTON, E.H. & PASSARO, E.P. (1990). Postoperative ileus. *Digest. Dis. Sci.*, **35,** 121 – 132.

MAKHLOUF, G.M. & GRIDER, J.R. (1993). Nonadrenergic noncholinergic inhibitory transmitters of the gut. *News Physiol. Sci.*, **8,** 195 – 199.

MCCONALOGUE, K., LYSTER, D.J.K. & FURNESS, J.B. (1995). Electrophysiological analysis of the actions of pituitary adenylyl cyclase activating peptide in the taenia of the guinea-pig caecum. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **352,** 538 – 544.

PARKMAN, H.P., PAGANO, A.P. & RYAN, J.P. (1997). PACAP and VIP inhibit pyloric muscle through VIP/PACAP-preferring receptors. *Regul. Pept.*, **71,** 185 – 190.

PORTBURY, A.L., MCCONALOGUE, K., FURNESS, J.B. & YOUNG, H.M. (1995). Distribution of pituitary adenylyl cyclase activating peptide (PACAP) immunoreactivity in neurons of the guinea-pig digestive tract and their projections in the ileum and colon. *Cell Tissue Res.*, **279,** 383 – 392.

RATTAN, S. & CHAKDER, S. (1997). Excitatory and inhibitory actions of pituitary adenylate cyclase-activating peptide (PACAP) in the internal anal sphincter smooth muscle: sites of actions. *J. Pharmacol. Exp. Ther.*, **283,** 722 – 728.

SCHWÖRER, H., KATSOULIS, S., CREUTZFELDT, W. & SCHMIDT, W.E. (1992). Pituitary adenylate cyclase activating peptide, a novel VIP-like gut-brain peptide, relaxes the guinea-pig taenia caeci via apamin-sensitive potassium channels. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346,** 511 – 514.

SUNDLER, F., EKBLAD, E., ABSOOD, A., HÅKANSON, R., KÖVES, K. & ARIMURA, A. (1992). Pituitary adenylate cyclase activating peptide: a novel vasoactive intestinal peptide-like neuropeptide in the gut. *Neuroscience,* **46,** 439 – 454.

TONINI, M. & COSTA, M. (1990). A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex. *Neuroscience,* **38,** 787 – 795.

TONINI, M., WATERMAN, S.A., CANDURA, S.M., COCCINI, T. & COSTA, M. (1992). Sites of action of morphine on the ascending excitatory reflex in the guinea-pig small intestine. *Neurosci. Lett.*, **144,** 195 – 198.

VAN RAMPELBERGH, J., POLOCZEK, P., FRANCOYS, I., DELPORTE, C., WINAND, J., ROBBERECHT, P. & WAELBROECK, M. (1997). The pituitary adenylate cyclase activating polypeptide (PACAP I) and VIP (PACAP II/VIP1) receptors stimulate inositol phosphate synthesis in transfected CHO cells through interaction with different G proteins. *Biochim. Biophys. Acta,* **1357,** 249 – 255.

WATERMAN, S.A. & COSTA, M. (1994). The role of enteric inhibitory motoneurons in peristalsis in the isolated guinea-pig small intestine. *J. Physiol.*, **477,** 459 – 468.

WATERMAN, S.A., TONINI, M. & COSTA, M. (1994). The role of ascending excitatory and descending inhibitory pathways in peristalsis in the isolated guinea-pig small intestine. *J. Physiol.*, **481,** 223 – 232.

ZAFIROV, D.H., PALMER, J.M., NEMETH, P.R. & WOOD, J.D. (1985). Cyclic 3′,5′-adenosine-monophosphate mimics slow synaptic excitation in myenteric plexus neurons. *Brain Res.*, **347,** 368 – 371.

ZAGORODNYUK, V., SANTICIOLI, P., MAGGI, C.A. & GIACHETTI, A. (1996). The possible role of ATP and PACAP as mediators of apamin-sensitive NANC inhibitory junction potentials in circular muscle of guinea-pig colon. *Br. J. Pharmacol.*, **119,** 779 – 786.

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