ELSEVIER

European Journal of Pharmacology 389 (2000) 217–224

ejp

www.elsevier.nl/locate/ejphar

Electrical and mechanical effects of vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide in the rat colon involve different mechanisms

Lídia Plujà, Ester Fernández, Marcel Jiménez *

Department of Cell Biology, Physiology and Immunology, Veterinary Faculty, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Catalunya, Spain

Received 6 October 1999; received in revised form 25 October 1999; accepted 29 October 1999

Abstract

This work aimed to study the effects of pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) on the mechanical and electrical activity of the circular muscle of the rat colon and the mechanisms involved in such effects. Spontaneous mechanical activity was studied in vitro in an organ bath and the membrane potential was recorded using the microelectrode technique. Both VIP and PACAP (0.1 μM) caused an immediate, sustained and tetrodotoxin (1 μM)-resistant inhibition of the cyclic spontaneous mechanical activity and hyperpolarization. The small-conductance Ca^{2+}-activated K^+ channel blocker, apamin (1 μM), did not change the VIP- and PACAP-induced relaxation but reduced the hyperpolarization induced by PACAP whereas it did not change that induced by VIP. In contrast, the purinoceptor antagonist, suramin (100 μM), blocked the hyperpolarization caused by PACAP and VIP but failed to change their mechanical inhibitory effects. Moreover, the putative PACAP and VIP receptor antagonists, PACAP-(6–38) and VIP-(10–28), respectively, both 3 μM, failed to change the effects of either peptide and modified neither the inhibitory junction potential nor the relaxation induced by electrical-field stimulation. Thus, these results suggest that the mechanisms mediating relaxation are not strictly coupled to the mechanisms mediating hyperpolarization. This could be due to activation of two distinct mechanisms of action after agonist receptor interaction.

Keywords: PACAP (pituitary adenylate cyclase-activating peptide); VIP (vasoactive intestinal peptide); Apamin; Suramin; PACAP-(6–38); VIP-(10–28); (Rat); Colon

1. Introduction

Pituitary adenylate cyclase activating peptide (PACAP) is a vasoactive intestinal peptide (VIP)-like peptide that was first isolated from ovine hypothalamus (Miyata et al., 1989). PACAP has been found, with immunohistochemical techniques, in nerve cell bodies and fibers of the gastrointestinal tract of many species, including the rat (Sundler et al., 1992; McConalogue et al., 1995a; Portbury et al., 1995). More recently, the presence of PACAP in the gastrointestinal tract of the rat was confirmed with radioimmunoassay and chromatography techniques (Hannibal et al., 1998). The synthesis of PACAP in enteric neurons was confirmed with in situ hibridization methods (Hannibal et al., 1998).

PACAP is a neurotransmitter that might be involved in inhibitory actions in the gastrointestinal tract (see for review Shuttleworth and Keef, 1995). The inhibitory effect of PACAP has been described for many species and for several areas of the gastrointestinal tract (Mungan et al., 1991; Schwörer et al., 1992; McConalogue et al., 1995a; Parkman et al., 1997) including human colon longitudinal muscle (Schwörer et al., 1993). In vitro studies show that PACAP relaxes the rat colon (Mungan et al., 1991; Kishi et al., 1996) and ileum (Katsoulis et al., 1993; Ekblad and Sundler, 1997). PACAP might participate in the descending relaxation phase of the peristaltic reflex (Grider et al., 1994). Electrical-field stimulation and stretching of colon segments induce PACAP release and descending relaxation of the rat colon smooth muscle (Grider et al., 1994).

Three types of VIP/PACAP receptors have been described. PACAP-1 receptors exhibit high affinity for PACAP. The VIP-1/PACAP-2 and the VIP-2/PACAP-3

* Corresponding author. Tel.: +34-93-5811898; fax: +34-93-5812006.
E-mail address: marcel.jimenez@uab.es (M. Jiménez).

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00773-6

receptors show equally high affinity for VIP and PACAP. Smooth muscle cells of rabbit and guinea-pig stomach and taenia coli only express VIP-2/PACAP-3 receptors (Teng et al., 1998). However, results of pharmacological and functional studies suggest that VIP and PACAP use different receptors and pathways. PACAP is more potent than VIP to relax precontrated smooth muscle from different areas of the gastrointestinal tract, including the rat colon (Mungan et al., 1991) and ileum (Katsoulis et al., 1993; Ekblad and Sundler, 1997). Apamin, a small-conductance Ca^{2+}-activated K^+ channel blocker, inhibits the PACAP- but not the VIP-induced relaxation in the human colon and the guinea-pig taenia caeci (Schwörer et al., 1992, 1993). Three receptors have recently been suggested to exist in the rat ileal longitudinal muscle (Ekblad and Sundler, 1997): (1) a PACAP receptor (sensitive to apamin), (2) a PACAP receptor that might be activated by PACAP-27 and PACAP-38 and (3) a VIP receptor.

The pathway involved in PACAP-induced relaxation is controversial and might be different, depending on the species and area of the gastrointestinal tract considered. These differences are mainly related to the sensitivity to apamin, suramin and nitric oxide (NO) synthase inhibitors.

(1) In the guinea-pig taenia caeci and colon, apamin reduces the relaxation and hyperpolarization induced by PACAP (Schwörer et al., 1992; McConalogue et al., 1995b; Zagorodnyuk et al., 1996). However, the PACAP-induced relaxation is not modified by apamin in the rat ileum (Katsoulis et al., 1993) and apamin does not block the increase in cAMP induced by PACAP in the guinea-pig caecum (McConalogue et al., 1995a).

(2) Suramin, a purinoceptor antagonist, might also block the effects of PACAP. In the guinea-pig taenia caecum, suramin reduces the hyperpolarization induced by PACAP but does not affect the inhibitory junction potential (McConalogue et al., 1995b). In contrast, in the circular muscle of the guinea-pig colon, suramin blocks the hyperpolarization induced by PACAP and the fast component of the inhibitory junction potential (IJP) (Zagorodnyuk et al., 1996).

(3) NO synthase inhibitors have been reported to abolish the inhibitory effect of VIP and PACAP in the rat colon, suggesting that NO pathways might be activated by these neurotransmitters (Grider et al., 1994). However, NO is not involved in PACAP-induced relaxation in the longitudinal muscle of the human colon (Schwörer et al., 1993).

We have recently reported that the cyclic mechanical activity in the rat proximal colon is due to myogenic cyclic depolarizations. Electrical-field stimulation induces a biphasic IJP followed by a relaxation. The fast component of the IJP is sensitive to apamin and to suramin whereas the second component is sensitive to NO synthase inhibitors (Plujà et al., 1999). Our results suggest that ATP and NO might be two inhibitory neurotransmitters involved in nonadrenergic, noncholinergic inhibitory pathways. A similar conclusion was reached for the rat distal colon, using a nicotinic agonist (Börjesson et al., 1997). However, according to the results described above, the use of apamin, suramin and NO synthase inhibitors (Plujà et al., 1999) does not allow one to rule out the possibility that PACAP might be a neurotransmitter involved in nonadrenergic noncholinergic (NANC) relaxations. Accordingly, the aim of this study was to study PACAP and VIP pathways inducing hyperpolarization and relaxation in the circular muscle of the rat colon.

2. Material and methods

2.1. Tissue preparation

Male Sprague–Dawley rats (8–10 weeks old), weighing 300–350 g, were kept at a constant temperature (21–23°C), with a lighting cycle of 12 h light/12 h dark, and fasted overnight (18 h) but allowed ad libitum access to water. The rats were killed by decapitation and bled (this procedure was approved by the Ethical Committee of the Universitat Autònoma de Barcelona). The colon was then removed and placed in Krebs solution consisting of (in mM): glucose 10.10; NaCl 115.48; $NaHCO_3$ 21.90; KCl, 4.61; NaH_2PO_4 1.14; $CaCl_2$ 2.50 and $MgSO_4$ 1.16, bubbled with a mixture of 5% CO_2–95% O_2 (pH 7.4). All studies (microelectrode and organ bath) were carried out under NANC conditions (atropine, propranolol and phentolamine, 1 μM). The mucosal and submucosal layers were carefully removed and circular muscle strips were cut 1 cm long and 0.3 cm wide.

2.2. Recordings of spontaneous mechanical activity

Muscle strips were attached with silk threads to a stable mount in the bottom of a 10-ml organ bath filled with carbogenated Krebs solution at 37 ± 1°C. The upper end was tied to an isometric force transducer (Harvard VF-1) connected to an amplifier and then to a computer. Data were digitized (25 Hz) and simultaneously displayed and collected using Datawin2 software (Panlab-Barcelona) coupled to an ISC-16 A/D card installed in a PC Pentium computer. A tension of 1 g was applied and the tissue was allowed to equilibrate for 1 h. After this period, the strips displayed spontaneous phasic activity. The amplitude, duration and frequency of the contractions in response to drugs were measured before and after drug addition.

2.3. Microelectrode recordings

Muscle strips were placed (circular muscle side up) in a Sylgard-coated chamber and continuously perfused with carbogenated Krebs solution at 37 ± 1°C. Preparations were allowed to equilibrate for approximately 1 h before experiments started. Circular muscle cells were impaled with glass microelectrodes ($R = 40$–60 MΩ) filled with 3 M

KCl. The membrane potential was measured using a standard electrometer Duo773 (WPI, FL, USA). The data were displayed on a digital storage oscilloscope 4026 (Racal-Dana, England), and simultaneously digitized (100 Hz) and collected using EGAA software coupled to an ISC-16 A/D card (RC Electronics, CA, USA) installed in a 486 PC computer. As we previously reported, the presence of nifedipine (1 μM) did not modify either the resting membrane potential or the IJPs induced by electrical-field stimulation (Plujà et al., 1999). Thus, all intracellular recordings were made in the presence of nifedipine, since the absence of spontaneous contractions significantly increased the duration of impalements.

2.4. Inhibitory junction potential and mechanical inhibition induced by electrical-field stimulation

Electrical-field stimulation was used in order to induce an IJP. Train stimulation had the following characteristics: total duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms and increasing amplitude strengths (5, 10, 12, 15, 17, 20 and 25 V). In order to characterise the fast and the slow component of the IJP, the amplitude and duration of the hyperpolarization were measured in the control and in the presence of the antagonist (Plujà et al., 1999). The same equipment was used to evaluate the effect of electrical-field stimulation on mechanical activity. Repeated pulses (one each 0.5 s) of 10, 15 and 25 V (pulse duration 0.3 ms, frequency 20 Hz, train duration 100 ms) were used to inhibit the spontaneous activity (Plujà et al., 1999).

2.5. Solutions and drugs

The following drugs were used: nifedipine, *N*-nitro-L-arginine (L-NOARG), phentolamine (Sigma Chemicals, St. Louis, USA); tetrodotoxin, atropine sulphate, propranolol, suramin, apamin (Research Biochemicals International, Natick, USA); vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP 27) (Peptide Institute, Osaka, Japan); VIP-(10–28) (Peninsula Laboratories, Belmont, USA); PACAP-(6–38) (Bachem, UK). Stock solutions were made by dissolving drugs in distilled water except for nifedipine which was dissolved in ethanol.

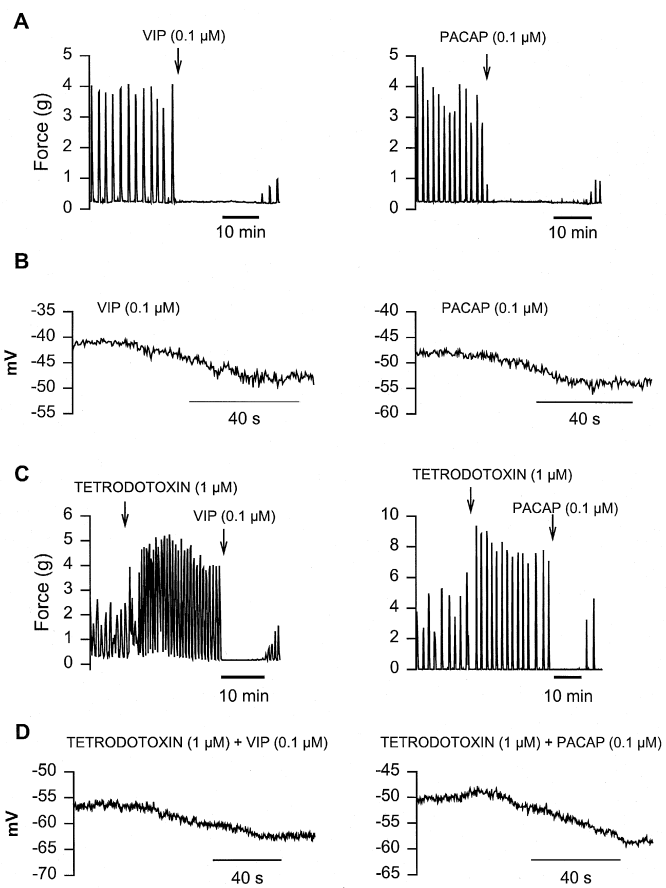

Fig. 1. (A) Recordings showing the effects of VIP (0.1 μM) and PACAP (0.1 μM) on spontaneous mechanical activity. (B) Intracellular microelectrode recordings showing the effects of VIP (0.1 μM) and PACAP (0.1 μM) on the resting membrane potential under control conditions. (C) Mechanical recordings showing the effect of VIP (0.1 μM) and PACAP (0.1 μM) in the presence of tetrodotoxin (1 μM) on spontaneous cyclic contractions. (D) Intracellular recordings that show the responses to VIP (0.1 μM) and PACAP (0.1 μM) of the resting membrane potential in the presence of tetrodotoxin (1 μM).

2.6. Statistical analysis

The data are expressed as means ± S.E.M. A paired Student's *t*-test was used to compare mechanical activity and membrane potential measurements in the absence and in the presence of drugs. A two-factor (volts and treatment) paired analysis of variance (ANOVA) was used to compare the amplitude and duration of the IJP. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Effects of VIP and PACAP on the spontaneous mechanical activity and on the resting membrane potential

The resting membrane potential of circular muscle cells from the rat colon was -45.0 ± 2.4 mV ($n = 26$). The circular muscle of the rat colon showed spontaneous mechanical activity consisting of cyclic contractions which occurred at a frequency of 0.64 ± 0.05 contractions/min and averaged 3.3 ± 0.1 g amplitude and 22.6 ± 1.4 s duration ($n = 29$).

VIP (0.1 μM, $n = 6$) and PACAP (0.1 μM, $n = 5$) abolished the spontaneous contractions (Fig. 1A). The neural blocker, tetrodotoxin (1 μM, $n = 6$), increased the amplitude (2.7 ± 0.6 vs. 4.8 ± 0.7 g; $P < 0.01$) and frequency (0.80 ± 0.25 vs. 1.14 ± 0.33 contractions/min; $P < 0.05$) and did not modify the duration of cyclic spontaneous contractions. In the presence of tetrodotoxin (1 μM), both VIP and PACAP (0.1 μM, $n = 4$) abolished the spontaneous mechanical activity also (Fig. 1C).

Perfusion of VIP (0.1 μM, $n = 5$; $P < 0.01$) caused a hyperpolarization of 6.8 ± 1.4 mV of the membrane potential (Fig. 1B). This hyperpolarization was resistant to tetrodotoxin (1 μM) (9.3 ± 1.6 mV, $n = 4$) (Fig. 1D). PACAP (0.1 μM, $n = 4$) hyperpolarized the muscle cells by 8.0 ± 1.8 mV ($P < 0.05$, Fig. 1B). In the presence of tetrodotoxin (1 μM, $n = 5$) a PACAP-induced hyperpolarization was also observed (10.0 ± 2.5 mV) (Fig. 1D).

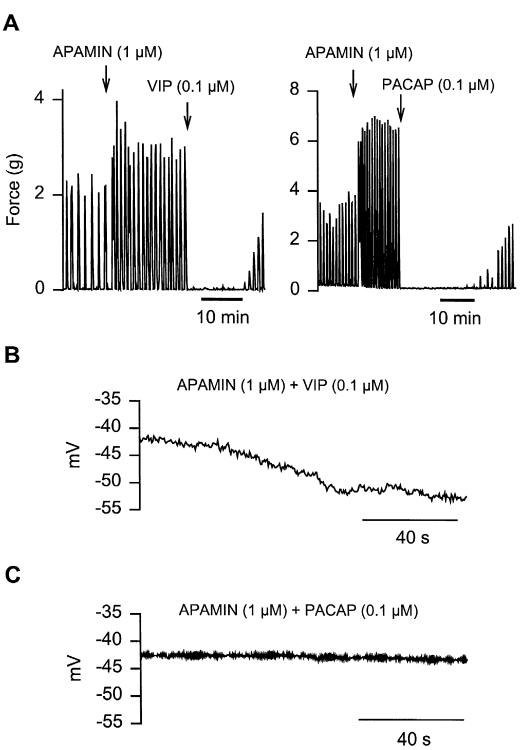

Fig. 2. (A) Mechanical recordings showing the effects of VIP (0.1 μM) and PACAP (0.1 μM) on the spontaneous contractile activity in the presence of apamin (1 μM). Intracellular microelectrode recordings showing the effect of VIP (0.1 μM) and PACAP (0.1 μM) on the resting membrane potential in the presence of apamin (1 μM) (B and C, respectively).

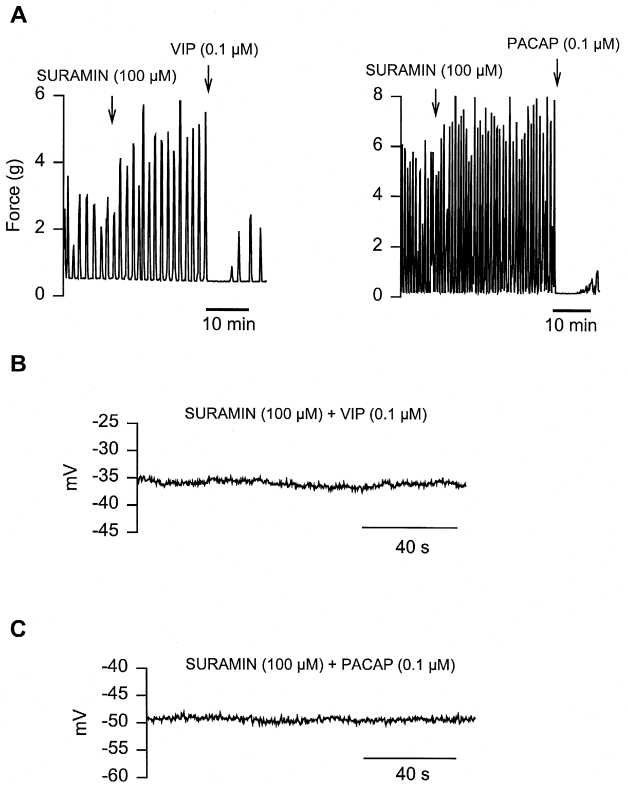

Fig. 3. (A) Mechanical recordings showing the effects of VIP (0.1 μM) and PACAP (0.1 μM) on the spontaneous cyclic activity in the presence of suramin (100 μM). Intracellular microelectrode recordings showing the effect of VIP (0.1 μM) and PACAP (0.1 μM) on the resting membrane potential in the presence of suramin (100 μM) (B and C, respectively).

3.2. Effect of apamin on the VIP and PACAP response

The small-conductance Ca^{2+}-activated K^+ channel blocker, apamin (1 μM, $n = 6$), increased the amplitude (3.5 ± 0.5 vs. 4.9 ± 0.6 g; $P < 0.01$) and frequency (0.65 ± 0.17 vs 0.94 ± 0.13 contractions/min; $P < 0.05$) and did not modify the duration of phasic contractions. Apamin (1 μM) failed to block either VIP or PACAP (0.1 μM,

$n = 4$)-induced relaxations of the circular colon muscle (Fig. 2A).

The VIP (0.1 μM)-induced hyperpolarization was resistant to apamin (1 μM) (6.0 ± 1.5 mV, $n = 5$) (Fig. 2B). In the presence of apamin (1 μM, $n = 6$), PACAP (0.1 μM) did not hyperpolarize the smooth muscle. Though a residual effect (3.3 ± 1.4 mV) was observed, it did not reach statistical significance (Fig. 2C).

3.3. Effect of suramin on the VIP and PACAP response

In the presence of suramin (100 μM, $n = 8$), a purinoceptor blocker, the amplitude and frequency of contractions increased (3.2 ± 0.4 vs. 4.6 ± 0.5 g and 0.52 ± 0.08 vs. 0.76 ± 0.12 contractions/min, respectively; both $P < 0.05$) whereas their duration was not affected. VIP and PACAP (0.1 μM, $n = 4$) abolished the spontaneous mechanical activity in the presence of suramin (100 μM) (Fig. 3A). In the presence of suramin (100 μM), neither VIP (0.1 μM, $n = 6$) nor PACAP (0.1 μM, $n = 6$) hyperpolarized circular muscle cells (Fig. 3B and C).

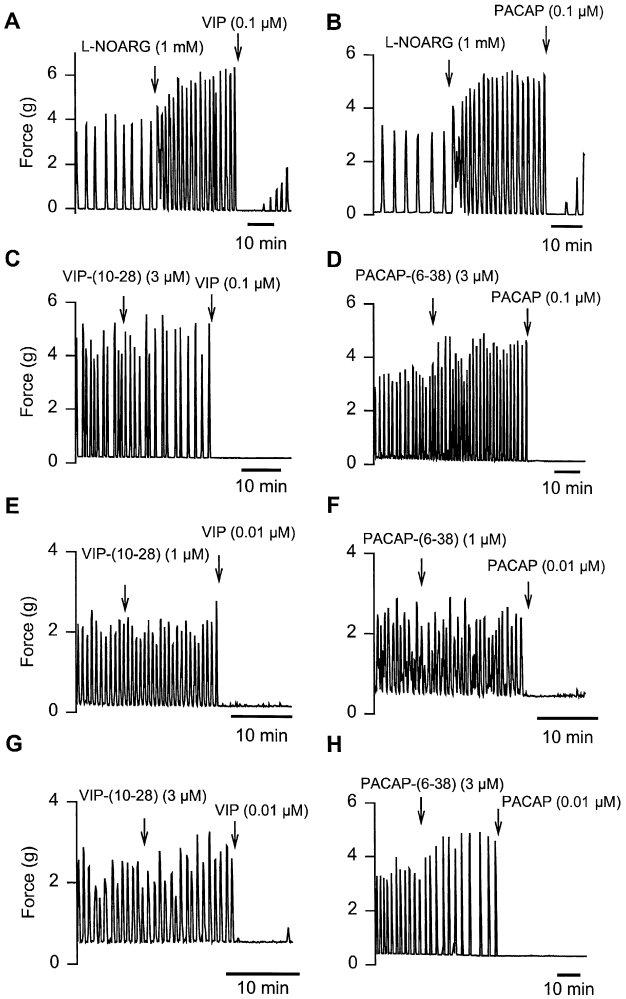

Fig. 4. Mechanical recordings showing the effects of VIP (A) and PACAP (B) (both 0.1 μM) on the spontaneous contractile activity in the presence of L-NOARG (1 mM). Panels C, E and G show the effects of VIP (0.01 μM and 0.1 μM) in the presence of VIP-(10–28) (1–3 μM). Panels D, F and H show the effects of PACAP (0.01 μM and 0.1 μM) in the presence of PACAP-(6–38) (1–3 μM).

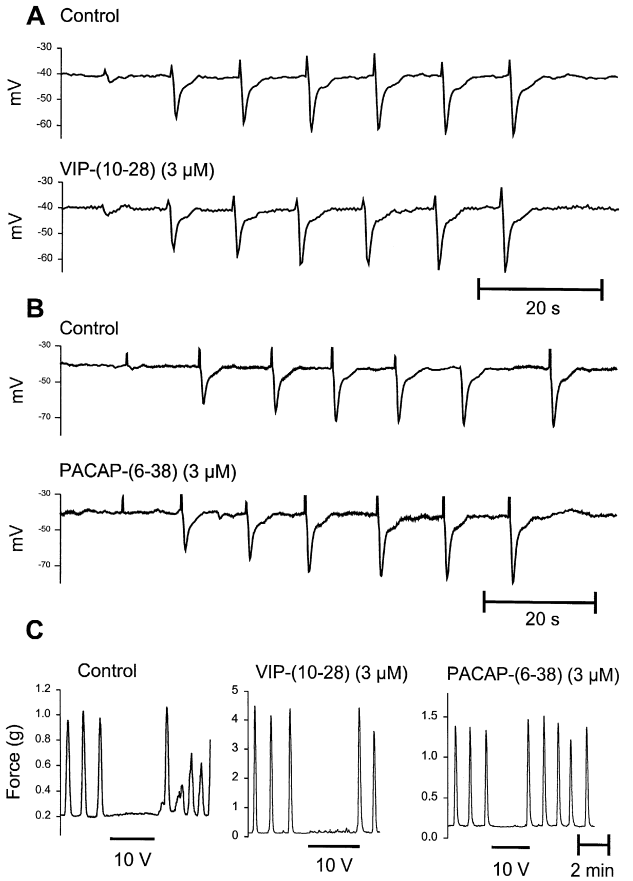

Fig. 5. Effect of electrical-field stimulation on the inhibitory junction potential (IJP) and mechanical activity in presence of VIP-(10–28) and PACAP-(6–38). Panels A and B show the effect of both putative antagonists on the IJP elicited by electrical-field stimulation (5, 10, 12, 15, 17, 20 and 25 V; pulse duration 0.3 ms, frequency 20 Hz, train duration 100 ms). Panel C shows the effect of both putative antagonists on the mechanical activity in the control, in the presence of VIP-(10–28) and PACAP-(6–38). Electrical-field stimulation (10 V, pulse duration 0.3 ms, frequency 20 Hz, train duration 100 ms) one stimulus each 0.5 s for at least 2 min. Similar results were obtained when 15 and 25 V were applied. Recordings obtained from three different tissues.

3.4. Effect of L-NOARG on VIP and PACAP response

In order to check if the inhibitory response to VIP and PACAP was related to activation of NO pathways, we tested the effect of both peptides in the presence of L-NOARG, a NO synthase inhibitor. L-NOARG (1 mM, $n = 5$), increased the amplitude (3.7 ± 1.0 vs. 5.2 ± 1.1 g; $P < 0.05$) and frequency of contractions (0.44 ± 0.05 vs. 0.72 ± 0.04 contractions/min; $P < 0.01$) but did not modify their duration. L-NOARG did not modify the inhibitory effects of VIP and PACAP (0.1 μM, $n = 4$) on the spontaneous contractions (Fig. 4A and B).

3.5. Effect of VIP-(10–28) and PACAP-(6–38) on the VIP- and PACAP-induced relaxations

Addition to the bath of either the VIP receptor antagonist, VIP-(10–28) (1 μM, $n = 5$), or the PACAP receptor antagonist, PACAP-(6–38) (1 μM, $n = 6$), did not modify the characteristics of the spontaneous mechanical activity (Fig. 4E and F). At a higher concentration (3 μM), both VIP-(10–28) ($n = 4$) and PACAP-(6–38) ($n = 5$) increased the amplitude of spontaneous cyclic contractions (3.2 ± 0.4 vs. 3.4 ± 0.4 g; $P < 0.05$ and 3.0 ± 0.6 vs. 4.4 ± 1.1 g; $P < 0.05$, respectively), but did not modify either the duration or the frequency (Fig. 4C, D, G and H). However, VIP-(10–28) (1–3 μM, $n = 4$) and PACAP-(6–38) (1–3 μM, $n = 4$) failed to block the VIP- and PACAP-induced relaxation, respectively (0.1 μM, $n = 4$) (Fig. 4C and D). In order to check if the dose of the agonists was too high, we used the agonists at 0.01 μM. However, at this concentration, VIP-(10–28) and PACAP-(6–38) (1–3 μM, $n = 4$) failed to block the VIP- and PACAP-induced relaxation (Fig. 4E, F, G and H).

In the presence of VIP-(10–28) (1 μM), PACAP (0.1 μM, $n = 4$) abolished the cyclic mechanical activity. VIP (0.1 μM, $n = 4$) also abolished spontaneous cyclic contractions in the presence of PACAP-(6–38) (1 μM). The combination of VIP-(10–28) and PACAP-(6–38) (1 μM) did not modify either the VIP-induced relaxation or the PACAP one (both 0.1 μM, $n = 4$) (data not shown).

3.6. Effect of VIP-(10–28) and PACAP-(6–38) on the IJP and relaxation induced by electrical-field stimulation

Neither VIP-(10–28) (3 μM) nor PACAP-(6–38) (3 μM) modified the IJPs elicited by electrical-field stimulation (both $n = 6$; Fig. 5A and B). The fast and the slow components of the IJPs were still recorded in presence of both putative antagonists (ANOVA: $P > 0.05$). Accordingly, an inhibitory effect on the spontaneous mechanical activity induced by electrical-field stimulation (10, 15 and 25 V) was still recorded in the presence of both putative antagonists ($n = 3$) (Fig. 5C).

4. Discussion

This study showed that VIP and PACAP inhibit the spontaneous mechanical activity due to cyclic depolarization of the circular muscle of the rat colon (Plujà et al., 1999). In contrast to the results found with mouse colon (Lyster et al., 1995), tetrodotoxin increases phasic contractions, suggesting that cyclic depolarizations are myogenic in the rat colon. In the presence of tetrodotoxin, both VIP and PACAP abolished the mechanical activity, suggesting an effect on muscle. This result is very similar to the inhibition of spontaneous activity found in the longitudinal muscle of the human sigmoid colon (Schwörer et al., 1993).

Even in the presence of tetrodotoxin, VIP and PACAP hyperpolarized the smooth muscle. PACAP-induced hyperpolarization had been demonstrated in the guinea-pig colon and taenia caecum and rat distal colon (McConalogue et al., 1995b; Kishi et al., 1996; Zagorodnyuk et al., 1996). Moreover, in the guinea-pig taenia caecum, PACAP increases membrane conductance (McConalogue et al., 1995b). The hyperpolarization induced by VIP and PACAP prevents the cell from reaching the threshold potential that allows L-type Ca^{2+} channel opening. This results in a blockade of spontaneous mechanical activity.

Apamin increases the frequency and amplitude of spontaneous contractions. This result suggests that at least one of the spontaneously released inhibitory transmitters activates small-conductance Ca^{2+}-activated K^+ channels. However, as we now showed, apamin failed to block PACAP- and VIP-induced inhibition of cyclic mechanical activity. In contrast, apamin blocked the hyperpolarization induced by PACAP but not that due to VIP. This result suggests that PACAP causes hyperpolarization through small-conductance Ca^{2+}-activated K^+ channels but relaxation can still be observed when apamin is added previously to the organ bath. A similar result has been reported for the guinea-pig colon (Zagorodnyuk et al., 1996).

In contrast to what we found with PACAP, apamin did not modify the VIP response (hyperpolarization and inhibition of spontaneous activity). This result shows that VIP hyperpolarizes the smooth muscle through an alternative pathway. This hyperpolarization causes inhibition of cyclic activity. Large-conductance Ca^{2+}-activated K^+ channels might be involved in the VIP response (Kishi et al., 1996). However, in the rat proximal colon the inhibitory effect of VIP on cyclic spontaneous activity is not blocked by charybdotoxin (data not shown) but we cannot rule out the possibility that charybdotoxin could block or decrease the hyperpolarization induced by VIP.

Suramin has been used to block ATP responses in several studies (Den Hertog et al., 1989; Fernández et al., 1998; Plujà et al., 1999). However suramin reduces the PACAP-induced hyperpolarization in the guinea-pig taenia caecum and colon (McConalogue et al., 1995b; Zagorodnyuk et al., 1996). These results could suggest that suramin is not selective for discriminating between ATP and PACAP/VIP responses (Zagorodnyuk et al., 1996) or alternatively that PACAP/VIP induce ATP release from nerve endings. Accordingly, in this study we show that both VIP- and PACAP-induced hyperpolarizations were blocked by suramin. However, suramin did not block the effect of PACAP or VIP on spontaneous mechanical activity. These results suggest that suramin does not discriminate between PACAP and VIP responses.

According to the results described above, several receptors or mechanisms (one receptor coupled to different intracellular pathways) might be involved in the VIP/PACAP response: (1) a PACAP receptor or pathway activating apamin-sensitive channels, which is sensitive to

suramin, (2) a VIP receptor or pathway sensitive to suramin, that does not activate apamin-sensitive K^+ channels and (3) a VIP/PACAP receptor or pathway perhaps coupled to adenylate cyclase, which is apamin/suramin insensitive. The possibility that VIP/PACAP induce ATP release needs further studies.

It has been shown that NO synthase inhibitors decrease the relaxant effect of VIP and PACAP in dispersed smooth muscle cells from the rat colon (Grider et al.,1994). However, we did not find a direct relationship between VIP/PACAP and NO in our study. NO is probably not involved in the VIP- and PACAP-induced relaxation in the rat gastrointestinal tract (Kishi et al., 1996; Ekblad and Sundler, 1997). As was reported for the rat, NO synthase inhibitors did not block spontaneous cyclic contractions in the longitudinal muscle of the human sigmoid colon (Schwörer et al., 1993).

VIP and PACAP receptor antagonists cause a mild or moderate increase in spontaneous contractions, suggesting that VIP/PACAP might to some extent be released endogenously. PACAP-(6–38) and VIP-(10–28) have been used to block the relaxant phase of the peristaltic reflex (Grider et al., 1994). However, in our study, PACAP-(6–38) and VIP-(10–28) did not modify the relaxant effect of either VIP or PACAP-27. Moreover PACAP-(6–38) and VIP-(10–28) did not modify the IJP and relaxation induced by electrical-field stimulation. Further studies are needed when selective antagonists become available.

In the rat proximal colon, the fast component of the IJP is apamin- and suramin-sensitive (Kishi et al., 1996; Plujà et al., 1999). Based on our results, we cannot rule out the possibility that PACAP and/or ATP are involved in the fast component of the IJP: both ATP (Plujà et al., 1999) and PACAP cause a hyperpolarization sensitive to suramin and apamin. The second component of the IJP, which is suramin/apamin-insensitive and inhibited by NO synthase inhibitors (Plujà et al., 1999), is probably not VIP-mediated because, according to our study, suramin abolishes the hyperpolarization induced by VIP. VIP and PACAP might be involved in the descending relaxation, using mechanisms independent of membrane potential and probably activating adenylate cyclase, which are apamin and suramin insensitive. This ''pharmaco-mechanical'' coupling, which might be independent of membrane potential, has been reported on from recent studies (Suthamnatpong et al., 1994; Bayguinov and Sanders, 1998; Plujà et al., 1999).

Acknowledgements

This work was supported by grant PM95-0121, Dirección General de Investigación Científica y Técnica (DGICYT) and a personal grant from Universitat Autònoma de Barcelona (Lídia Plujà).

References

Bayguinov, O., Sanders, K.M., 1998. Dissociation between electrical and mechanical responses to nitrergic stimulation in the canine gastric fundus. J. Physiol. 509, 437–448.

Börjesson, L., Nordgren, S., Delbro, D.S., 1997. DMPP causes relaxation of rat distal colon by a purinergic and a nitrergic mechanism. Eur. J. Pharmacol. 334, 223–231.

Den Hertog, A., Nelemans, A., Van den Akker, J., 1989. The inhibitory action of suramin on the P2-purinoceptor response in smooth muscle cells of guinea-pig taenia caeci. Eur. J. Pharmacol. 166, 531–534.

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. 334, 61–66.

Fernández, E., Guo, X., Vergara, P., Jiménez, M., 1998. Evidence supporting a role for ATP as non-adrenergic non-cholinergic inhibitory transmitter in the porcine ileum. Life Sci. 62 (15), 1303–1315.

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., 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.

Katsoulis, S., Clemens, A., Schwörer, H., Creutzfeldt, W., Schmidt, E., 1993. Pituitary adenylate cyclase activating polypeptide (PACAP) is a potent relaxant of the rat ileum. Peptides 14 (3), 587–592.

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.

Lyster, D.J.K., Bywater, R.A.R., Taylor, G.S., 1995. Neurogenic control of myoelectric complexes in the mouse isolated colon. Gastroenterology 108, 1371–1378.

McConalogue, K., Furness, J.B., Vremec, M.A., Holst, J.J., Tornøe, K., Marely, P.D., 1995a. Histochemical, pharmacological, biochemical and chromatographic evidence that pituitary adenylyl cyclase activating peptide is involved in inhibitory neurotransmission in the taenia of the guinea-pig caecum. J. Auton. Nerv. Syst. 50, 311–322.

McConalogue, K., Lyster, D.J.K., Furness, J.B., 1995b. 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.

Miyata, A., Arimura, A., Dahl, R.R., Minamino, N., Uehara, A., Jiang, L., Culler, M.D., Coy, D.H., 1989. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem. Biophys. Res. Commun. 164, 567–574.

Mungan, Z., Rossowski, W.J., Coy, D.H., 1991. Pituitary adenylate cyclase activating polypeptide relaxes gastrointestinal smooth muscles in rat. Clin. Res. 39, 236A.

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.

Plujà, L., Fernández, E., Jiménez, M., 1999. Neural modulation of the cyclic electrical and mechanical activity in the rat colonic circular muscle: putative role of ATP and NO. Br. J. Pharmacol. 126, 883–892.

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, 385–392.

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.

Schwörer, H., Clemens, A., Katsoulis, S., Köhler, H., Creutzfeldt, W., 1993. Pituitary adenylate cyclase-activating peptide is a potent modulator of human colonic motility. Scand. J. Gastroenterol. 28, 625–632.

Shuttleworth, C.W.R., Keef, K.D., 1995. Roles of peptides in enteric neuromuscular transmission. Regul. Pept. 56, 101–120.

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 (2), 439–454.

Suthamnatpong, N., Hosokawa, M., Takeuchi, T., Hata, F., Takewaki, T., 1994. Nitric oxide-mediated inhibitory response of rat proximal colon: independence from changes in membrane potential. Br. J. Pharmacol. 112, 676–682.

Teng, B., Murthy, K.S., Kuemmerle, J.F., Grider, J.R., Makhlouf, G.M., 1998. Selective expression of vasoactive intestinal peptide $(VIP)_2$ /pituitary adenylate cyclase-activating polypeptide $(PACAP)_3$ receptors in rabbit and guinea pig gastric and tenia coli smooth muscle cells. Regul. Pept. 77, 127–134.

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.