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Distinct receptors mediate pituitary adenylate cyclase-activating peptide- and vasoactive intestinal peptide-induced relaxation of rat ileal longitudinal muscle

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Abstract

Relaxant responses to pituitary adenylate cyclase-activating peptide (PACAP)-27, PACAP-38 and vasoactive intestinal peptide (VIP) were examined in rat ileal longitudinal muscle. PACAP-27 was much more potent than PACAP-38 and VIP, with PACAP-38 and VIP being equipotent. The relaxation induced by each of the peptides was unaffected by pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M), tetrodotoxin (10^{-6} M) or atropine (10^{-6} M). Pretreatment with apamin (10^{-6} M) abolished the relaxations induced by PACAP-27, but not those induced by PACAP-38 or VIP. Pretreatment with neuropeptide Y (NPY) (10^{-7} M) inhibited relaxations induced by VIP, but not those induced by PACAP-27 or PACAP-38. No cross-desensitization between PACAP-27 and VIP could be revealed. In conclusion, distinct receptors mediate PACAP- and VIP- induced relaxations of rat ileal longitudinal muscle. At least three different types of receptors may exist: (1) a PACAP-27 preferring receptor coupled to apamin sensitive Ca^{2+}-dependent K^+ channels, (2) a PACAP specific receptor activated by both PACAP-27 and PACAP-38 but not by VIP and (3) a VIP specific receptor regulated by NPY by yet unknown mechanisms. © 1997 Elsevier Science B.V.

Keywords: PACAP (pituitary adenylate cyclase-activating peptide); VIP (vasoactive intestinal peptide); PACAP receptor; VIP receptor; Neuropeptide Y; Motor effect; Gastro-intestinal tract; Apamin

1. Introduction

Both pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) are considered important neurotransmitters within the enteric nervous system. Two molecular forms of PACAP exist, PACAP-38 and the C-terminally truncated peptide PACAP-27. The two peptides share a high degree of sequence homology with VIP, and thus PACAP belongs to the VIP superfamily of peptides (for a review see Arimura and Shioda, 1995). VIP-containing nerve fibres are numerous throughout the intestinal wall while PACAP-containing ones are less numerous (Sundler et al., 1991). The majority of VIP-containing enteric neurons have, in several species, been found to harbor also neuropeptide Y (NPY) (Ekblad et al., 1984). Interestingly, both the nitric oxide (NO)-generating enzyme NOsynthase and PACAP have also been described within subpopulations of VIP-containing enteric neurons (Sundler et al., 1991; Ekblad et al., 1994; Furness et al., 1995).

VIP is well established as an inhibitory neurotransmitter in the enteric nervous system; it acts by causing relaxation and hyperpolarization of gastrointestinal smooth muscle (for a review see Shuttleworth and Keef, 1995). PACAP also induces relaxant responses in gastrointestinal smooth muscle (Schworer et al., 1992; Katsoulis et al., 1993a, 1996; McConalogue et al., 1995; Shuttleworth and Keef, 1995). The relaxant responses evoked by both VIP and PACAP have been suggested to be dependent on or mediated by NO produced within smooth muscle (Murthy et al., 1995). In addition, VIP- and PACAP-induced contractions mediated via both cholinergic and non-cholinergic mechanisms have been reported in guinea-pig ileum (Katsoulis et al., 1992, 1993b).

The receptors for VIP and PACAP were first classified in binding studies and later by cloning of the encoding

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cDNAs (for a review, see Arimura and Shioda, 1995). Binding studies revealed the presence of PACAP type IA (PACAP-38 ≈ PACAP-27 ≫ VIP), type IB (PACAP-38 > PACAP-27 > VIP) and PACAP type II (PACAP-38 ≈ PACAP-27 ≈ VIP) receptors. Three distinct PACAP/VIP receptors were subsequently cloned (PACAP/VIP 1, 2 and 3). The PACAP/VIP1 receptor displays type I binding sites and is thus activated by both PACAP and VIP, with PACAP being much more potent than VIP. Intracellullary, this receptor activates both adenylyl cyclase and phospholipase C. PACAP/VIP2 and PACAP/VIP3 receptors display type II receptor binding sites. Both type II receptors activate adenylyl cyclase only. In the rat, the PACAP/VIP1 receptor has been shown to exist in several splice variants (Spengler et al., 1993). The different isoforms display similar potencies for PACAP-38 and PACAP-27 in the generation of cAMP whereas PACAP-38 is more effective than PACAP-27 in the production of inositol phosphates. Alternative splicing of the PACAP/VIP1 receptor has been demonstrated also in mice and humans (Pantoloni et al., 1996), suggesting that this may by a common phenomenon possibly related to differences in the functional properties of this receptor.

Beside the PACAP/VIP receptors identified by cloning techniques, an 'apamin-sensitive' PACAP receptor distinct from the three described has been suggested on the basis of functional studies (Schworer et al., 1992; Jin et al., 1994; McConalogue et al., 1995; Katsoulis et al., 1996).

A VIP specific receptor has not yet been cloned. Functional studies have, however, indicated the presence of such a receptor because of the lack of cross-desensitization with PACAP (Katsoulis et al., 1993a).

The aims of the present study were to elucidate the motor effects of PACAP-27, PACAP-38 and VIP in rat ileal longitudinal smooth muscle and their relative potencies. Cross-desensitization between PACAP and VIP was also tested. Furthermore, we examined the effect of PACAP and VIP after pretreatment with NPY, tetrodotoxin or apamin and after NOsynthase blockade with N^G-nitro-L-arginine methyl ester (L-NAME).

2. Materials and methods

45 female Spraque–Dawley rats (160–180 g) were used. The animals were anaesthetized in vaporized diethylether and killed by bleeding. The ileum was dissected out and placed in ice-cold Krebs solution with the following composition (in mM): NaCl 133.0, KCl 4.7, $CaCl_2 \times 2H_2O$ 2.5, $MgCl_2$ 1.0, $NaHCO_3$ 16.3, NaH_2PO_4 1.4 and glucose 7.8. Strips (10 mm long) from longitudinal smooth muscle with adherent myenteric ganglia were dissected out, using a dissection microscope. The strips were mounted in organ baths containing 5 ml of Krebs solution aerated with a mixture of 5% CO_2 and 95% O_2 and continuously recorded for isometric tension with a Grass FTO3C force-displacement transducer and registered on a Grass model 79D polygraph. Bath temperature was maintained thermostatically at 37°C and the pH was kept around 7.40 (range 7.35–7.45). The strips were given an initial passive load of 20–25 mN and allowed to equilibrate for 1 h before experimentation started. During this period the preparations gradually relaxed and the basal tension stabilized at 3–5 mN. Rhythmic spontaneous contractions of varying amplitude developed in all preparations.

The following drugs were used: Atropine sulphate, tetrodotoxin, acetylcholine, isoprenaline, forskolin, apamin, L-NAME, VIP, NPY, PACAP-38 and PACAP-27 were purchased from Sigma (St.Louis, MO, USA). Prostaglandin $F_{2\alpha}$ (Prostin) was from Upjohn (Kalamazoo, MI, USA). All substances were dissolved in 0.9% saline.

Experimentation started with the addition of acetylcholine (10^{-5} M) to the bath followed by wash out and 20 min recovery. The strips were then precontracted with prostaglandin $F_{2\alpha}$. When the response was stable, isoprenaline (10^{-5} M) was added and, after wash out, the strips were again allowed to recover. The prostaglandin $F_{2\alpha}$ concentration (10^{-8}–10^{-7} M) was chosen individually for each muscle strip in order to ensure that the contraction level was the same, relative to that elicited by acetylcholine (10^{-5} M), in all experiments. Concentration–response curves for PACAP-38, PACAP-27, VIP and forskolin were made with precontracted muscle strips. Precontraction of the muscle strips was achieved with a

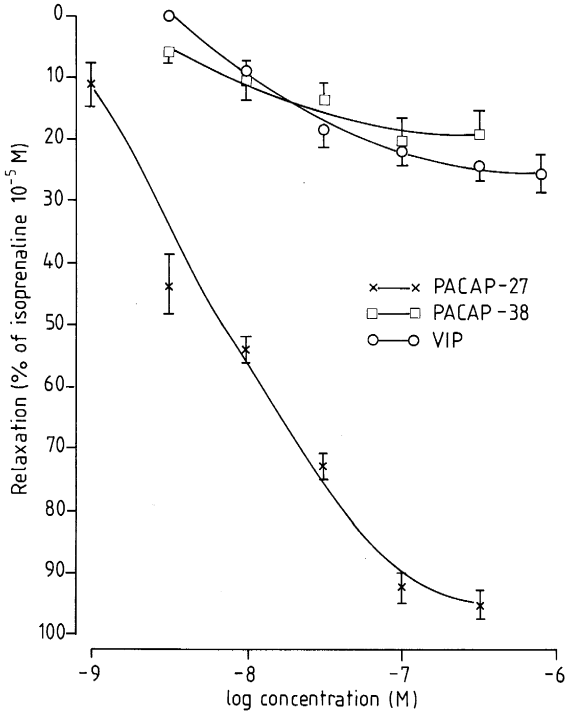

Fig. 1. Concentration–response curves showing the relaxatory effects of PACAP-27, PACAP-38 and VIP on rat ileal longitudinal muscle. Each value is the mean of 12–16 experiments. Vertical bars give S.E.M.

submaximal concentration of prostaglandin $F_{2\alpha}$. Single doses of the peptides or forskolin were tested one at a time and the strips were allowed to recover for 30 min between each dose. Each strip was exposed to 6–8 different concentrations of PACAP-38, PACAP-27 or VIP, always starting with the lowest dose. Tetrodotoxin (10^{-6} M), atropine (10^{-6} M), apamin (10^{-6} M), NPY (10^{-7} M), VIP (10^{-7} M), or PACAP-27 (10^{-7} M) (the latter two were used for studies of cross-desensitization between the two peptides) was added 5 min prior to precontraction. L-NAME (10^{-4} M) was added 30 min prior to precontraction. None of the substances used for pretreatment affected the baseline or the dose of prostaglandin $F_{2\alpha}$ needed to induce contraction.

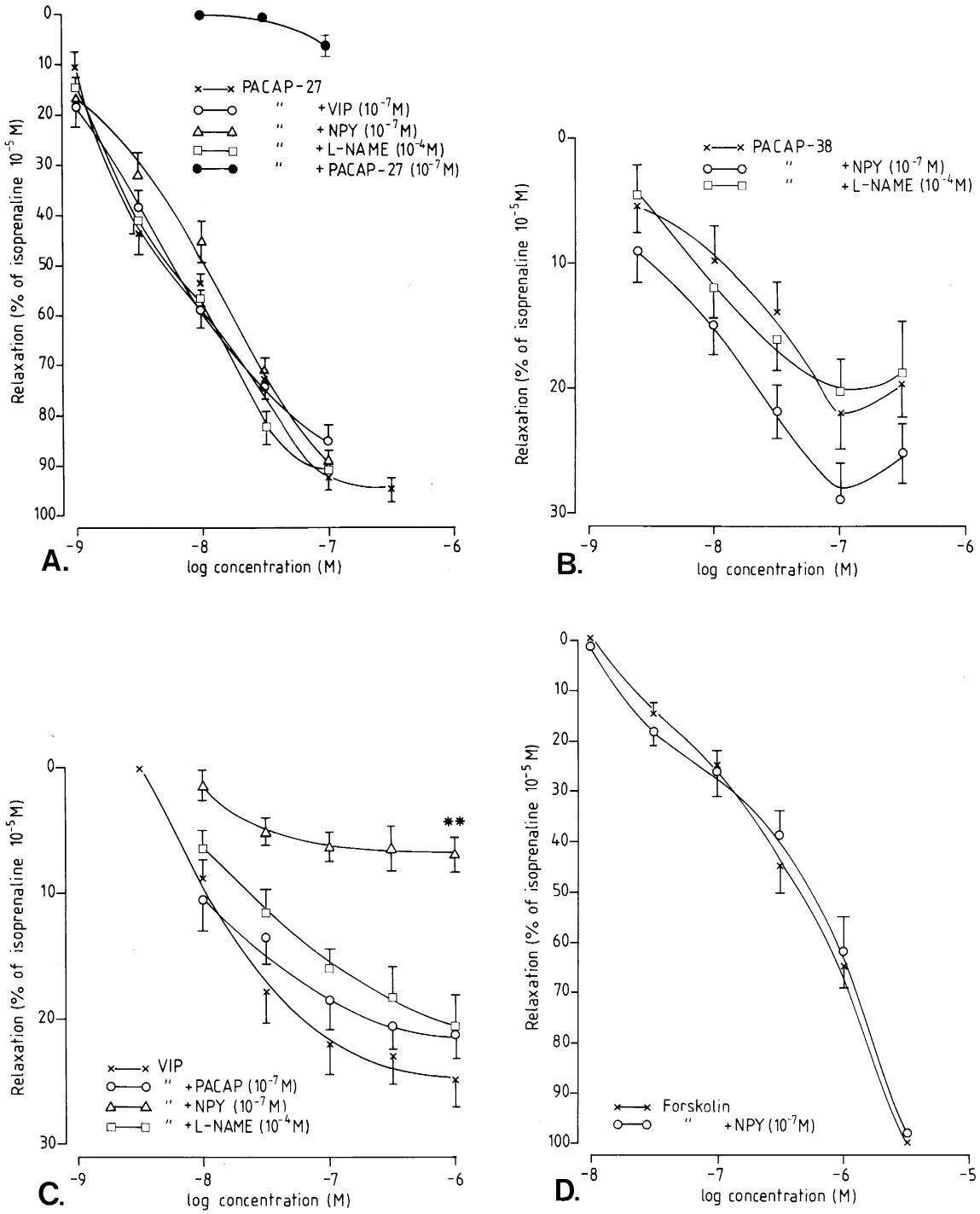

Fig. 2. Concentration–response curves showing the relaxatory effects of (A) PACAP-27, (B) PACAP-38, (C) VIP and (D) forskolin on rat ileal longitudinal muscle after pretreatment with L-NAME (A–C), NPY (A–D), VIP (A and C) and PACAP-27 (A and C). Each value is the mean of 12–18 experiments. Vertical bars give S.E.M. ** $P < 0.01$.

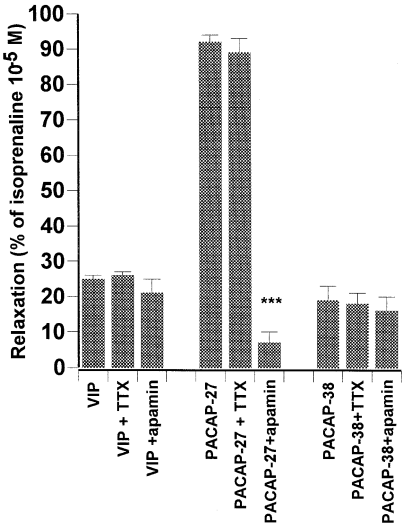

Fig. 3. Relaxatory effects of VIP (10^{-6} M), PACAP-27 (10^{-7} M) and PACAP-38 (10^{-7} M) in the presence of tetrodotoxin (TTX) (10^{-6} M) or apamin (10^{-6} M). Each value is the mean of 12–18 experiments. Vertical bars give S.E.M. *** $P < 0.001$.

Relaxant responses were expressed as percentages of the isoprenaline (10^{-5} M)-evoked relaxation in precontracted muscle strips. Statistical evaluation was by Student's *t*-test.

3. Results

VIP, PACAP-38 and PACAP-27 all caused concentration-dependent relaxations of the rat ileal longitudinal smooth muscle (Fig. 1). PACAP-27 was, however, much more potent than PACAP-38 and VIP. The relaxations induced by VIP, PACAP-38 or PACAP-27 were unaffected by pretreatment with L-NAME (10^{-4} M) (Fig. 2), tetrodotoxin (10^{-6} M) (Fig. 3) or atropine (10^{-6} M) ($n = 14$; not shown). Pretreatment with apamin (10^{-6} M) abolished the PACAP-27 induced relaxations while relaxations induced by VIP or PACAP-38 were unaffected (Fig. 3). Pretreatment with NPY (10^{-7} M) almost totally abolished the VIP-induced relaxations (Fig. 2C), but did not influence relaxations induced by PACAP-38 or PACAP-27 (Fig. 2A and B). No cross-desensitization between PACAP-27 and VIP could be revealed. Pretreatment with PACAP-27 (10^{-7} M) did not cause any shift of the VIP concentration–response curve (Fig. 2C) and pretreatment with VIP (10^{-7} M) did not alter the PACAP-27 concentration–response curve (Fig. 2A). Forskolin caused concentration-dependent relaxations that were unaffected by the pretreatment with NPY (10^{-7} M) (Fig. 2D). Isoprenaline (10^{-5} M) caused complete relaxation of the muscle strips.

4. Discussion

The present results suggest that different types of PACAP/VIP-receptors can mediate relaxations of rat ileal longitudinal muscle. This suggestion is based on the finding that PACAP-27 was a much more potent relaxant than PACAP-38 or VIP in this preparation, with PACAP-38 and VIP being equipotent. This indicates that a selective PACAP-27 receptor is present on rat ileal smooth muscle. High-affinity PACAP-27-preferring receptors have previously been suggested to mediate secretion in rat small intestine (Cox, 1992). In that study the PACAP-27 receptor was suggested to be located on submucous neurons, since the secretory response to PACAP-27 was blocked by the addition of tetrodotoxin. In the present study pretreatment with atropine or tetrodotoxin did not alter the relaxations induced by PACAP-27, suggesting that PACAP-27 acts directly on the smooth muscle, neither were the responses to PACAP-38 or VIP blocked by addition of tetrodotoxin or atropine. Both PACAP-27 and PACAP-38 have been found to elicit apamin-sensitive relaxations of guinea-pig gastric smooth muscle (Katsoulis et al., 1996) and taenia coli (Schworer et al., 1992; Jin et al., 1994; McConalogue et al., 1995). These data were interpreted to suggest the existence of a PACAP receptor distinct from the PACAP/VIP receptors identified by cloning techniques. However, divergent results have been presented (Katsoulis et al., 1993a). These authors found that PACAP-38-induced relaxations in rat ileum longitudinal muscle were unaffected by apamin. In a number of species apamin has been found incapable of changing the VIP-induced relaxation of intestinal smooth muscle (Costa et al., 1986; Ito et al., 1990; Jin et al., 1994). The present results on rat ileum longitudinal muscle agree with these observations, in that the relaxations induced by PACAP-38 or VIP could not be blocked by apamin. Interestingly, however, the PACAP-27 preferring receptor is probably coupled to apamin sensitive Ca^{2+}-dependent K^+ channels (Romey et al., 1984) since PACAP-27 induced relaxations were inhibited by apamin.

In accordance with previous results (Katsoulis et al., 1993a), our finding of a lack of cross-desensitization between PACAP-27 and VIP indicates the presence of both PACAP-specific receptor(s) and a VIP-specific receptor on rat ileal longitudinal smooth muscle cells. The presence of a VIP-specific receptor is further strengthened by the finding that pretreatment with NPY effectively inhibited the VIP-induced relaxation, while the relaxant responses to both PACAP-38 and PACAP-27 were unaffected. The mechanisms behind this inhibitory effect of NPY are unclear. NPY does not show any sequence homology with VIP, so there is probably no competitive receptor binding of NPY to the VIP receptor. Whether NPY affects the binding of VIP to its receptor or whether it acts intracellularly (e.g., as an adenylate cyclase inhibitor) needs further elucidation. NPY has been reported to inhibit cAMP-mediated relaxation elicited by β-adrenoceptor stimulation of rabbit tracheal smooth muscle via inhibition of the intracellular accumulation of cAMP (Tagaya et al., 1996). However, the finding in the present study that

addition of NPY affected neither the forskolin- nor the PACAP-38- or PACAP-27-induced relaxations suggests that the NPY-evoked inhibition of VIP-induced relaxation operates through a different mechanism. In vascular smooth muscle NPY has been reported to inhibit Ca^{2+}-activated K^+ channels (Xiong and Cheung, 1995). Whether NPY operates through this mechanism also in intestinal muscle is unknown. Large conductance Ca^{2+}-activated K^+ channels (BK channels) have been suggested to be important, not only in vascular, but also in intestinal smooth muscle (Carl et al., 1995). Recently cDNAs encoding two components (α- and β-subunits) of BK channels expressed in gastrointestinal smooth muscle were cloned (Vogalis et al., 1996). No enhancement of BK channel activity was, however, detected after cAMP stimulation.

Beside the PACAP-27 preferring receptor, an additional PACAP receptor may also be involved, since PACAP-38 elicited a relaxation, although weak, and since no cross-desensitization with VIP occurred. This postulated additional receptor probably belongs to the class of PACAP type1 receptor(s). However, PACAP/VIP 1 receptor mRNA has been found not to be expressed in the intestines (Hashimoto et al., 1993). In view of the known heterogeneity of this receptor, as yet undiscovered splice variants may operate here.

Both PACAP and VIP have been suggested to act via increased synthesis of NO in smooth muscle cells (Murthy et al., 1995). However, NOsynthase blockade by L-NAME did not influence the relaxant responses to PACAP or VIP in the present study, precluding that they are mediated via generation of NO.

In conclusion, the results indicate that PACAP/VIP receptors of different types mediate relaxation of rat ileal longitudinal muscle. The presence of a PACAP-27-preferring receptor is suggested. Activation of this receptor induces a pronounced relaxation which can be blocked by pretreatment with apamin. In addition, we present indications for the existence of yet another PACAP receptor. This receptor shows no cross-desensitization with VIP and thus probably belongs to the PACAP (type 1) receptors. Stimulation of this receptor evokes a weak relaxation. Finally, a VIP-selective receptor is suggested to be present. Activation of this causes a weak relaxation and there is no cross-desensitization with PACAP-27. The activity of the VIP-selective receptor is regulated by NPY via so far unknown mechanisms.

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