Region-specific antiproliferative effect of VIP and PACAP-(1—38) on rabbit enteric smooth muscle

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Van Assche, Gert, Inge Depoortere, Rita de Vos, Karel Geboes, Jozef J. Janssens, Stephen M. Collins, and Theo L. Peeters. Region-specific antiproliferative effect of VIP and PACAP-(1—38) on rabbit enteric smooth muscle. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G303–G310, 1999.—The ability of neuropeptides to modulate enteric smooth muscle proliferation was examined in primary explant cultures of rabbit gastric antrum and colon smooth muscle. Cell proliferation was determined by [3H]thymidine incorporation measurements and cell counting. Subcultured rabbit antrum and colon myocytes (passages 2–6) preserved a smooth muscle phenotype, as verified by immunohistochemistry for α-smooth muscle actin and electron microscopy. Both vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide (1–38) (PACAP-(1—38)) concentration dependently (10⁻¹⁰ to 10⁻⁶ M) inhibited the serum-induced [³H]thymidine incorporation (in colon, 48.2 ± 5.8 and 55.6 ± 9.3% of control with 10⁻⁶ M VIP and 10⁻⁷ M PACAP-(1–38)) and inhibited increase in cell numbers in cultures derived from the colon but not in those from the antrum. Effects of VIP and PACAP-(1—38) were mimicked by forskolin (10⁻⁷ to 10⁻⁶ M) but not by 8-bromo-cGMP, whereas theophylline enhanced the effects of VIP. Inhibition of nitric oxide synthase with N⁶-nitro-arginine methyl ester (10⁻⁴ M) did not alter the effects of VIP. Substance P, motilin, calcitonin gene-related peptide, and somatostatin had no effect. A single class of 125I-labeled VIP binding sites was found in antrum and colon myocyte cultures with an equal affinity for VIP and PACAP-(1—38) (dissociation constant (Kd) in antrum = 3.4 ± 0.8 nM for VIP and 2.0 ± 1.0 nM for PACAP-(1—38); Kd in colon = 2.0 ± 1.0 nM for VIP and 2.8 ± 1.6 nM for PACAP-(1—38)). Density of binding sites in the antrum was higher than in the colon. In disease states such as inflammatory bowel disease, inhibition of myocyte proliferation by VIP and PACAP may serve to control smooth muscle hyperplasia in the colon but not in the antrum.

enteric neuropeptides; smooth muscle pathophysiology; signal transduction; neuropeptide receptors

IN THE NORMAL muscularis propria, enteric myocytes have a “contractile” phenotype and a low rate of proliferation and secretion. In contrast, smooth muscle hyperplasia and hypertrophy are prominent histological features of acute and chronic enteric inflammation and may reflect phenotypic transformations. For example, in Crohn’s disease, smooth muscle cells of the muscularis mucosae show a striking proliferation and secrete copious amounts of collagen (9, 14). Enteric smooth muscle cells in primary culture also transform phenotypically and secrete a diversity of proteins such as collagen and cytokines (13, 17).

The factors controlling enteric smooth muscle proliferation remain largely unknown. Cytokines, such as interleukin-1, tumor necrosis factor-α, and transforming growth factor-β, have mitogenic effects on enteric smooth muscle in culture (28, 42), whereas transforming growth factor-β inhibits smooth muscle proliferation (30).

It is becoming increasingly evident that interactions between enteric nerves and smooth muscle cells influence the course of the inflammatory reaction in the deeper layers of the gut wall (5). Recently, we have submitted evidence that enteric neuropeptides enhance interleukin-1-induced interleukin-6 secretion by enteric smooth muscle cells in primary cultures (39). Therefore, we hypothesized that neuropeptides may modulate the proliferation of smooth cells in the muscle layers. Indeed, neuropeptides have been shown to regulate cell growth and differentiation at other sites of the body. For instance, vasoactive intestinal polypeptide (VIP) has trophic and mitogenic effects on embryonic neural tissue (2), but at the same time the peptide inhibits the mitosis of pancreatic tumors (31), colon adenocarcinoma cells (38), and murine Peyer’s patch lymphocytes (33). VIP and the related peptide, pituitary adenylate cyclase-activating peptide, inhibit the proliferation of vascular and bronchial smooth muscle cells in primary culture (16, 22, 26), and substance P and somatostatin modulate vascular smooth muscle proliferation (29, 15). The aim of the present study, therefore, was to explore the role of enteric neuropeptides in the control of smooth muscle proliferation using a model of mammalian enteric myocytes in primary culture.

MATERIALS AND METHODS

Enteric smooth muscle culture. New Zealand White rabbits of either sex (2.5–3.0 kg) were euthanized, and the gastric antrum and 10 cm of the distal colon were removed and rinsed. The mucosa and submucosa of the antrum were removed by sharp dissection, and the serosa was removed by peeling under a stereoscopic microscope. The colonic segment was cleared of mucosa by scraping with a scalpel blade, and the submucosa and serosa were peeled off. Samples of the dissected muscle layers were rapidly frozen and hematoxylin-eosin-stained sections were examined for the extent of dissection using light microscopy. Explant cultures were prepared as described by Kahn et al. (17). Briefly, fragments of the muscle layers were explanted in 60-mm culture dishes (Corn-
ing, NY) and grown in DMEM (GIBCO BRL, Grand Island, NY) with 100 U/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B (GIBCO BRL) and supplemented with 10% fetal bovine serum (FBS; GIBCO BRL). The primary cultures were incubated in a 5% CO2 incubator at 37°C, and the medium was changed twice weekly. When cultures reached confluency, cells were dissociated with 0.125% trypsin-EDTA (GIBCO BRL), centrifuged at 500 rpm for 5 min and replated at the desired density. Cells were used between passages 2 and 5. For cell counts, trypsinized cells in suspension were diluted 1:4 with trypan blue (0.4%) and loaded into a Burker chamber. Viability, as judged by trypan blue exclusion, was always preserved in more than 90% of trypsinized cells. Cells for immunohistochemistry were seeded in 24-well plates (Corning) on Thermopan inserts (Nunc, Naperville, IL). Inserts with confluent cells attached were removed from medium and soon after were fixed in acetone. Immunohistochemistry was performed on cell cultures and on cryostat sections from snap-frozen (−80°C) undissected tissue samples using a three-step indirect immunoperoxidase method with monoclonal antibodies against α-smooth muscle actin (1/400; Sigma, St. Louis, MO), desmin (1/10; Boehringer, Mannheim, Germany), vimentin (1/20; Amersham, Little Chalfont, UK), and neuron-specific enolase (NSE; 1/10; both from Monosan, Uden, The Netherlands). Cells for transmission electron microscopy were seeded in six-well plates, mobilized with 0.125% trypsin, and pelleted by centrifugation at 2000 g for 10 min. Pellets were fixed in 2% glutaraldehyde, postfixed in osmium tetroxide, embedded in epon, and further processed for light (semithin sections) and electron microscopy (ultrathin sections).

Proliferation studies. Cells were seeded in 24-well plates at a density of 5 × 104 per well and cultured for 48 h in DMEM containing 10% FBS. Subconfluent cells were then growth arrested by exposure to serum-free DMEM for 24 h. The medium was subsequently replaced by either 1 ml of DMEM containing 2% FBS or DMEM alone (control). Neuropeptides at different concentrations were added to the medium, and cells were incubated for another 24 h; 0.3 µCi [3H]thymidine (sp act 15 Ci/mmol; Amersham) was added for the final 18 h of incubation. Cells were subsequently dehydrated by adding 1 ml of ice-cold methanol for 1 min and washed twice with 10% TCA. Cells were lysed with 1% SDS in 0.3 M NaOH, lysates were aspirated, and the retained radioactivity was determined in a liquid scintillation counter (Tri-Carb 1500; Packard, Meriden, CT). The following peptides were tested in the proliferation studies: porcine VIP (which is identical to rabbit VIP (19)) (Biogenesis, Poole, UK), ovine pituitary adenylate cyclase activating peptide-(1—38) [PACAP-(1—38); Novabiochem, Laufelfingen, Switzerland], [Ac-Tyr1,D-Phe2]growth hormone releasing factor-(1—29)-amide [Ac-Tyr1,D-Phe2]GRF-(1—29)-NH2; Peninsula, Belmont, CA), substance P (19), motilin; Eurogentec, Namur, Belgium), somatostatin (UCB Bioproducts, Braine-l’Alleud, Belgium), and [125I]-VIP from NEN (sp act = 2,000 Ci/mmol). The detection limit of the assay was 20 pg/ml. Interassay variation was <10%.

Data analysis. The modulation of [3H]thymidine incorporation by neuropeptides and other compounds is expressed as a percentage of the response to 2% FBS. Radlig-Ligand software (Elsevier-Biosoft, Cambridge, UK) was used for the analysis of [125I]-VIP binding data.

All data are expressed as means ± SE. Statistical analysis was performed using Student’s t-test when comparing two groups and one-way ANOVA, followed by Student’s t-test or Student-Newman-Keuls test, when comparing three or more groups. Statistical significance was accepted at a P value of <0.05.

RESULTS

Characterization of enteric myocytes. Cultures were inspected at least twice weekly for growth and microscopic appearance. Cells in primary cultures were spindle shaped and displayed a hill-and-valley growth pattern. Subcultured cells had a heterogeneous appearance at low density, but confluent cells were spindle shaped and also adopted a hill-and-valley appearance (Fig. 1A). More than 95% of confluent cells from colon and antrum (passages 2–4) stained intensely for α-smooth muscle actin, but staining for vimentin, desmin, NSE, and glial fibrillary acidic protein was not observed. In control tissue sections from rabbit colon and antrum, the α-smooth muscle actin and desmin immunoreactivity was restricted to the muscularis mucosae, muscularis propria, and vascular tunica media. The appearance of confluent colonic and antral cells (passage 3) using electron microscopy was characterized by the presence of a discontinuous basement membrane around individual cells, by caveolae of the plasma membrane, and by cytoplasmatic actin filaments (Fig. 1B).

Effects of neuropeptides on [3H]thymidine incorporation. [3H]thymidine was incorporated in cultured myocytes from rabbit antrum and colon to an equal extent (1,152 ± 403 and 1,131 ± 235 dpm/g protein for antrum and colon, respectively). About 80% was specifically incorporated into DNA because addition of cystine...
arabinoside (10⁻⁶ M), to inhibit DNA biosynthesis, reduced thymidine incorporation to 19.0 ± 3.1 and to 19.9 ± 5.5%, respectively, of the control values obtained in 2% FBS. When serum-free DMEM was added to the growth-arrested myocytes, [³H]thymidine incorporation was only 16.8 ± 8.7% (antrum) and 8.2 ± 3.3% (colon) of the incorporation obtained with 2% FBS-containing medium.

Exposure of growth-arrested colon myocytes to DMEM-2% FBS containing the neuropeptides VIP and PACAP-(1—38) resulted in a concentration-dependent inhibition of thymidine incorporation (Fig. 2A). Maximal suppression was ~50% of the control value, and half-maximum inhibition was estimated to occur at 1 nM for both peptides (EC₅₀ = 10⁻⁹.05 and 10⁻⁸.96 M for VIP and PACAP-(1—38), respectively). The VIP receptor antagonist [Ac-Tyr¹,D-Phe²]GRF-(1—29)-NH₂, at a concentration of 10⁻⁵ M, did not alter the inhibitory effects of VIP (10⁻⁸.⁵ M) (VIP: 55.1 ± 7.7; VIP plus antagonist: 61.8 ± 10.6% of control [³H]thymidine incorporation; n = 4).

In myocytes obtained from antral muscle layers, the thymidine incorporation in DMEM-2% FBS was not affected by VIP and PACAP-(1—38) (10⁻¹⁰ to 10⁻⁶ M) under identical conditions (Fig. 2B).

As can be seen in Table 1, substance P (10⁻⁶ M), somatostatin (10⁻⁴.⁵ M), and [nle¹³]po-motilin (10⁻⁶ M) did not influence serum-induced [³H]thymidine incorpo-
incorporation of [3H]thymidine in cultured myocytes

Table 1. Effect of several neuropeptides on the cell counts were monitored for 3 more days. VIP (10 \(10^{-8}\) to 10 \(10^{-6}\) M) inhibited the [3H]thymidine incorporation of colon but not of antrum myocytes. This effect was preserved for 3 days (Fig. 3). In all conditions, more than 90% of the trypsinized cells excluded trypan blue.

Transduction pathway for the effect of VIP. The adenylate cyclase-stimulating compound forskolin concentration dependently (10 \(10^{-8}\) to 10 \(10^{-6}\) M) inhibited the [3H]thymidine incorporation of colon but not of antrum myocytes. On the contrary, 8-BrGMP (10 \(10^{-4}\) M) was without effect and did not interfere with the effects of VIP (10 \(10^{-7}\) M). Also, inhibition of nitric oxide synthase with L-NAME (10 \(10^{-3.5}\) M) did not change the effects of VIP (Table 2).

Inhibition of phosphodiesterase activity with theophylline (10 \(10^{-5}\) M) had no effect by itself, but the combined administration of VIP (10 \(10^{-7}\) M) with 10 \(10^{-5}\) M theophylline significantly enhanced the effects of the peptide (Table 2).

Degradation of VIP by smooth muscle membranes. Incubation of VIP (289.5 \(21.5\) ng/ml) in the cell culture medium for 6 or 24 h at 37°C did not result in VIP degradation (89.4 \(5.1\)% of control at 24 h; not significant, n = 4). In the presence of either antral or colonic smooth muscle cells, no significant decrease was detected after 6 h (87.3 \(5.9\) and 92.7 \(4.6\)% of control VIP levels for antrum and colon myocytes; n = 4), but after 24 h partial degradation was evident (70.5 \(11.5\) and 67.7 \(8.9\)% of control in antrum (P < 0.05) and colon (P < 0.02), respectively; n = 4).

Binding of 125I-labeled VIP to smooth muscle membranes. Specific 125I-labeled VIP binding was observed intact myocytes of both antrum and distal colon. Nonspecific binding, in the presence of 10 \(10^{-6}\) M VIP, was 36.0 \(5.7\) (antrum) and 34.2 \(5.6\)% (colon) of the total binding. These values compare with the previously reported nonspecific binding in myocytes from rabbit colon and guinea pig antrum (4, 10). Analysis of the displacement studies in colon and antrum myocytes demonstrated the presence of a single binding site in both preparations, with a dissociation constant of 3.4 ±

**Fig. 3. Effects of VIP and PACAP-(1–38) on cell counts of myocytes cultured from explants of the rabbit colon (A) and antrum (B). Neuropeptides were added, and FBS content was decreased to 2% 24 h after seeding (arrows). \(P < 0.05\), \#first time point with \(P < 0.01\); n = 5.**
Table 2. Effect of agents affecting intracellular transduction pathways on the incorporation of [3H]thymidine by cultured colon myocytes

<table>
<thead>
<tr>
<th>Agent</th>
<th>[3H]thymidine incorporation, % of control</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon myocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-7} M VIP</td>
<td>53.1 ± 13.9†</td>
<td>5</td>
</tr>
<tr>
<td>Forskolin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^{-3} M</td>
<td>89.6 ± 10.1</td>
<td>3</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>36.9 ± 4.3†</td>
<td>3</td>
</tr>
<tr>
<td>10^{-6} M</td>
<td>26.0 ± 18.3*</td>
<td>4</td>
</tr>
<tr>
<td>10^{-4} M B-BrcGMP</td>
<td>110.0 ± 17.3</td>
<td>3</td>
</tr>
<tr>
<td>10^{-4} M B-BrcGMP + 10^{-7} M VIP</td>
<td>45.5 ± 3.8†</td>
<td>3</td>
</tr>
<tr>
<td>10^{-4} M L-NAME + 10^{-7} M VIP</td>
<td>99.0 ± 4.9</td>
<td>3</td>
</tr>
<tr>
<td>10^{-4} M L-NAME + 10^{-7} M VIP</td>
<td>60.5 ± 9.1*</td>
<td>4</td>
</tr>
<tr>
<td>10^{-3} M Theophylline + 10^{-7} M VIP</td>
<td>101.0 ± 3.9†</td>
<td>3</td>
</tr>
<tr>
<td>10^{-5} M Theophylline + 10^{-7} M VIP</td>
<td>35.1 ± 11.3†</td>
<td>4</td>
</tr>
<tr>
<td>10^{-6} M Forskolin</td>
<td>87.1 ± 15.0</td>
<td>4</td>
</tr>
</tbody>
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Antrum myocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antrum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd for VIP, nM</td>
<td>3.4 ± 0.8</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Kd for PACAP-(1—38), nM</td>
<td>2.0 ± 1.0</td>
<td>2.8 ± 1.6</td>
</tr>
<tr>
<td>Bmax, fmol/mg protein</td>
<td>170.1 ± 39.6*</td>
<td>82.4 ± 21.4</td>
</tr>
<tr>
<td>Bmax, sites/cell</td>
<td>64.1 ± 11.9 × 10^2</td>
<td>17.9 ± 7.8 × 10^2</td>
</tr>
</tbody>
</table>

*Values are means ± SE of 4 independent experiments. †P < 0.05, ‡P < 0.01, different from control thymidine incorporation; †P < 0.05 different from inhibition with 10^{-7} M VIP alone.

Table 3. Parameters of [125I]-labeled VIP binding to intact enteric myocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antrum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd for VIP, nM</td>
<td>3.4 ± 0.8</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Kd for PACAP-(1—38), nM</td>
<td>2.0 ± 1.0</td>
<td>2.8 ± 1.6</td>
</tr>
<tr>
<td>Bmax, fmol/mg protein</td>
<td>170.1 ± 39.6*</td>
<td>82.4 ± 21.4</td>
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<tr>
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<td>64.1 ± 11.9 × 10^2</td>
<td>17.9 ± 7.8 × 10^2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 independent experiments. Kd, dissociation constant; Bmax, density of binding sites. *P < 0.05, †P < 0.01, antrum had significantly more binding sites than colon.

DISCUSSION

The results of the present study provide the first evidence that the enteric neuropeptides VIP and PACAP inhibit the proliferation of colon but not of antrum myocytes in culture. Moreover, we have shown that specific VIP receptors are expressed in primary cultures of both rabbit antrum and colon myocytes.

Cultures of rabbit enteric myocytes have been described before (10, 42), but, to the best of our knowledge, this is the first report on primary and secondary cultures of rabbit antrum and colon myocytes using an explant technique. Therefore, extensive characterization of the cultures was performed. The hill-and-valley growth pattern, shape, and ultrastructural appearance of confluent cells were typical for smooth muscle cells.

The presence of α-smooth muscle actin and the absence of vimentin staining, even after being subcultured, indicated that the cells preserved the phenotype of smooth muscle cells and not of myofibroblasts, which have been described to contain smooth muscle α-actin but invariably coexpress vimentin (32). Also, the absence of staining for glial fibrillary acidic protein and for NSE excluded the contamination of the cultures by a significant amount of enteric glial cells or enteric nerves. It is also worth mentioning that both rabbit...
antrum and colon myocytes in culture responded to agonists, such as ACh and motilin, with elevations of intracellular calcium (40). Nevertheless, the cultured myocytes were phenotypically transformed, since desmin staining was not observed, whereas smooth muscle in control tissue sections stained intensely for this filament. Phenotypic transformation of smooth muscle cells in primary culture is a general observation (3). The myocytes transform from a strictly contractile cell type to cells with secretory capacities. This transformation is also observed in most pathological conditions characterized by smooth muscle hyperplasia. In strictures caused by inflammatory bowel disease, phenotypically transformed myocytes are the principal cell type of the thickened gut wall and they are responsible for collagen deposition (14). Therefore, primary cultures of enteric myocytes appear to be a suitable model to study the regulation of smooth muscle proliferation.

Inhibition of myocyte growth by VIP and PACAP has been previously demonstrated in cultured myocytes from rat aorta and guinea pig bronchi with a similar potency as observed in the present study (22, 26). However, the antiproliferative effect of neuropeptides in the gut is region specific, since VIP and PACAP had an effect on myocytes of colonic but not of antral origin. Differences in phenotype were not observed between antrum and colon myocytes in culture and cannot account for this observation.

VIP is rapidly degraded by homogenized antral smooth muscle membranes (19), although it is not clear whether this is caused by a membrane-bound enzyme or by cytosolic hydrolases. The absence of an effect of VIP on antral myocytes could therefore have been caused by the rapid degradation of VIP. However, we found that VIP is only slightly degraded by intact colon and antrum myocytes. The stability of VIP in the presence of colon myocytes is further confirmed by the fact that with these cells VIP and PACAP effects on [3H]thymidine incorporation were preserved for 24 h and the reduction in cell numbers was preserved for 3 days.

The effects of substance P, motilin, and somatostatin, which have been shown to modulate cell growth in vitro in other systems, were explored. Substance P had no effect in contrast to previous observations of mitogenic effects in vascular and bronchial cultured myocytes (15, 25). Similarly, motilin, which was reported to enhance lymphocyte proliferation, and somatostatin, which inhibited vascular smooth muscle proliferation in allografts (15, 35), were without effect. The expression of specific receptors for these peptides was not further investigated; therefore, their lack of effect may be due to the lack of receptor expression.

The second messenger pathway involved in the observed effect of VIP and PACAP was further investigated, since modulation of myocyte proliferation by cAMP and cGMP in vitro has been reported in rat and rabbit aortic myocytes. (6, 18, 32). In the present study, the effects of VIP and PACAP were mimicked by the adenylyl cyclase activator, forskolin, and enhanced by the phosphodiesterase inhibitor, theophylline. These results support a role for the cAMP-protein kinase A-dependent pathway, whereas the negative results with 8-Br cGMP, in a concentration known to modulate vascular smooth muscle proliferation (11), exclude the cGMP-protein kinase G pathway. The latter transduction mechanism was also rejected on the basis of the negative results obtained with the nitric oxide synthase inhibitor L-NAME. Nitric oxide is believed to be the downstream intermediary of mitogenic cytokines, such as interleukin-1, responsible for the activation of guanylate cyclase in vascular smooth muscle (8). VIP and PACAP are known to activate membrane-bound nitric oxide synthase in rabbit gastric smooth muscle (23). However, the cAMP-protein kinase A pathway appears to be the sole second messenger system involved in the VIP modulation of myocyte proliferation.

Serum-starved smooth muscle cells in culture become nonproliferative and are blocked in the late G0 phase of the cell cycle (17). The antiproliferative effects of both VIP and PACAP, however, do not seem to be cell cycle specific, since inhibition of the [3H]thymidine incorporation by VIP was present in both growth-arrested cells and in cells that had already been exposed to the mitogenic action of FBS. VIP was most effective at inhibiting thymidine incorporation when added 6 h after FBS (2%), indicating that the myocytes were more sensitive after the cell cycle had been reactivated. Furthermore, VIP and PACAP were effective in reducing cell numbers when administered 24 h after passage in medium with 10% serum.

VIP and PACAP have a very similar profile of biological activity, and their receptors are commonly classified as “classical” VIP receptors, which have an equal affinity for VIP and PACAP receptors and PACAP receptors, which bind PACAP preferentially (34). In colon myocytes, we characterized a single binding site with equal affinity for both peptides. Moreover, the concentration dependence for the antiproliferative effect is similar for VIP and PACAP and correlates well with the binding affinity. Therefore, the antiproliferative effects of these neuropeptides appear to be mediated through a classical VIP receptor. Its affinity correlates well with the high-affinity VIP binding site described in rabbit colon myocytes (10). Two subtypes of the VIP receptor, VIP1 and VIP2, have been reported in other species, but in rabbits only limited information became recently available. In freshly isolated rabbit gastric myocytes, VIP receptors were demonstrated (24); with the use of Northern blot analysis, using primers based on the rat VIP receptor subtypes, only VIP2 receptors appear to be present (37). It is tempting to speculate that the differences in responses of antrum and colon are due to VIP receptor subtypes; however, more detailed studies of VIP receptors in the rabbit are needed to clarify this point. The fact that the GRF analog, [Ac-Tyr1,D-Phe2]GRF-(1—29), a VIP antagonist in the rat pancreas (41) and rat vascular smooth muscle (26), was ineffective in our study in the proliferation as well as in the binding experiments may indicate species differences between the rat and rabbit VIP receptors.
Interestingly, more VIP receptors were found in the antrum than in the colon myocytes, despite the absence of antiproliferative effects. Also, receptor downregulation by VIP was similar in antrum and colon myocytes. Therefore, downregulation cannot account for the absence of an effect in antrum myocytes. Because forskolin did not alter the thymidine incorporation of antrum myocytes, we suggest that the growth of antrum myocytes is insensitive to increases in cAMP despite the presence of a functional VIP receptor.

Neuropeptide-mediated antiproliferative effects may be of importance in disease states. For example, hypertrophy of the muscle layers has been documented in Crohn’s colitis (9) and is accompanied by an increase in the number and size of VIPergic nerves and of the VIP and PACAP content of the gut wall (1, 27). Because our results indicate that VIP and PACAP are putative endogenous inhibitors of smooth muscle proliferation in the mammalian colon, we propose that both neuropeptides serve to control unlimited muscle hyperplasia. The antiproliferative effects of VIP and PACAP illustrate the importance of the interactions between enteric nerves and smooth muscle as active participants of the inflammatory reaction in the gut. Because the observed effects were region specific for the colon, further studies will be required to outline the role of enteric nerves in controlling smooth muscle hyperplasia at different levels of the gastrointestinal tract.

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REFERENCES


