Role of Vasoactive Intestinal Polypeptide in the Adaptation of Intestinal Smooth Muscle Cells to Mechanical Distension

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ABSTRACT
Distension of the small intestine can play a role in the pathogenesis of various functional intestinal disorders. This study determined the role of vasoactive intestinal polypeptide (VIP) in the adaptive response of intestinal smooth muscle to acute and chronic distension of the ileum in vivo. Several in vitro experiments were performed to identify the mechanism of receptor regulation. Distension was applied by a balloon inflated with air in the ileum either during a single episode in anesthetized or repeatedly in conscious guinea pigs. Then, muscle cells were isolated by enzymatic digestion of the distended and nondistended adjacent ileal segments. In addition, in vivo experiments were performed on freshly dispersed cells for determination of mechanisms. Control cells maximally relaxed (Cmax) at 1 µM VIP (EC50 = 50 pM) and 100 µM isoproterenol (EC50 = 7 nM). Both acute and chronic distensions triggered a rightward shift of the concentration-response curves for VIP (Cmax = 100 µM, EC50 = 10 nM). A desensitization of the relaxing effect of VIP receptors was also observed when cells were preincubated for 30 min in vitro with VIP. By contrast, the relaxing effect of isoproterenol was affected neither by in vivo distension nor by in vitro incubation with isoproterenol. Desensitization of VIP receptors was prevented by in vitro incubation of cells with VIP plus a VIP antagonist ([d-P-Cl-Phe6,Leu17]VIP) and by intraluminal perfusion of the VIP antagonist during acute distention in vivo. Moreover, desensitization of VIP receptors did not occur after 30 min preincubation with either forskolin or 8-Bromo-cyclic AMP. These results indicate that mechanical distension of the ileum induces a homologous desensitization of VIP receptors on circular smooth muscle cells, which requires the occupation of its receptors by VIP.

Propulsion of the luminal content along the intestine during the digestive process depends on the coordination and activity of a number of reflexes. Indeed, content-induced distension of the intestine causes the release of various neurotransmitters and triggers both an ascending excitatory and a descending inhibitory reflexes (Grider and Makhlouf, 1986; Smith et al., 1990). However, changes in this motility pattern could play a role in the pathogenesis of various syndromes including functional digestive disorders, usually related to visceral neuropathies or myopathies of unexplained pathophysiology such as IBS. Abdominal distension and pain are the most frequent complaints of patients with IBS. A segmental dilatation of the intestine (Swenson and Rathouser, 1959) or an adynamic bowel (Kapila et al., 1975; Puri et al., 1977) were observed in some cases. An understanding of the mechanisms of IBS associated pain remains largely unclear but they might be related to changes in motility or tone of gut smooth muscle (Maxton et al., 1991).

VIP is an important NANC neuropeptide contained in motor neurons, which exerts an inhibitory effect on gut motility (Goyal et al., 1980). VIP relaxes smooth muscle cells of the gastrointestinal tract (Bitar and Makhlouf, 1982; Rekik et al., 1996). After interaction with its membrane receptor, VIP increases intracellular cAMP followed by production of cGMP via the nitric oxide pathway (Rekik et al., 1996). Previous studies have brought indirect evidence supporting the involvement of VIP in different pathological situations characterized by gut distension. Bojo et al. (1993) have suggested that VIP was the main mediator of gastric relaxation in response to colonic distension and painful stimulation. Similarly, You and Ma (1991) observed a marked increase in VIP level in portal blood after a 24-hr ligation of the ileum in the rabbit.

However, it is well known that mechanical distension of the intestine elicits active ion and water secretion (Caren et al., 1974; Harris et al., 1989). Various studies have provided evidence for a relationship between motility and secretion within the gastrointestinal wall (see Greenwood and Dawson, 1987 for review). Distension of the intestinal wall releases a number of neurotransmitters found in the intestinal wall and known to cause intestinal secretion (Schultzberg et

ABBREVIATIONS: cAMP, adenosine 3’5’-cyclic monophosphate; cGMP, guanosine 3’5’-cyclic monophosphate; CCK, cholecystokinin; CCK8, sulfated C-terminal octapeptide of CCK; Cmax, concentration of agonist inducing a maximal effect, EC50, effective concentration inducing a half-maximal effect; IBS, irritable bowel syndrome; NANC, nonadrenergic noncholinergic; PAF, platelet-activating factor; VIP, vasoactive intestinal polypeptide.
al., 1980). However, it has not yet been proven that these agents released by distension could also interact directly with muscle cells or nerves linked to them. VIP, one of these neurotransmitters, has been detected in the efferent nerves mediating distension-induced neuronal secretory reflex and in nerve endings of motoneurons in the submucosal plexus (Schulzke et al., 1995).

As regulatory mechanisms may occur at the cellular level in case of gut injury and as VIP is a relaxing neurotransmitter in the gut, we proposed that mechanical distension of the ileum could alter the responsiveness of intestinal smooth muscle cells to this relaxing agent. Therefore, we evaluated the effect of mechanical distension of the ileum on the relaxing effect of VIP and isoproterenol, a beta adrenergic agonist, on isolated smooth muscle cells from the circular layer of a distended and a nondistended (control) segments of the distal ileum in guinea pig. In this study we investigated the effects of ileal distension on VIP and isoproterenol receptors during ileal distension performed in two ways: 1) acute prolonged distension in anesthetized animals and 2) chronic and repeated distensions in conscious animals. Then, we completed this study by evaluating the effect of a prolonged incubation of cells with VIP or isoproterenol, in vitro, to determine the cellular events responsible for the observed changes.

Materials and Methods

Experiments were conducted on Dunkin-Hartley male guinea pigs (300–350 g). The animals were deprived of food for 24 hr before the experiments but had free access to water.

Acute distension in anesthetized animals. Anesthesia was induced with 2 g/kg i.p. urethane. After a midline laparotomy, the ileum was exteriorized and a balloon (length 5 cm) made from latex condom was inserted into the ileum at 5 cm proximal to the ileocecal junction. When the balloon is placed properly and not exteriorized at the back of the neck. After surgery, the animals were allowed to recover for 5 days, with free access to water and food.

From day 6 to day 11, chronic ileal distension was performed by inflating the balloon twice daily, at an interval of 7 hr. Inflation of the balloon was performed by connecting the external end of the polyethylene tube to a syringe and manometer assembly. A constant pressure of 30 mmHg (volume injected: 1 ml) was maintained for 1 hr in the balloon. At day 11, animals were killed. The distended and the distal nondistended segments of the ileum were removed and cell suspensions prepared as described below.

Experiments on fresh cells. Nonoperated anesthetized animals were killed by cervical dislocation and the ileum (5–10 cm orally from the ileocecal junction) was removed for preparation of dispersed circular smooth muscle cells.

Cell dispersion. Cell dispersion was achieved as previously reported (Bitar and Makhlouf, 1982; Botella et al., 1992; Rekik et al., 1996). After removal of the ileal segments, the circular muscle layer was separated from other layers and small muscle strips (3–4 cm) were incubated separately for two successive periods of 30 min at 31°C in a HEPES-PO4 buffer with the following composition [in mM] 132 NaCl, 5.4 KCl, 5 NaHPO4, 1 NaH2PO4, 1.2 MgSO4, 1 CaCl2, 25 HEPES, glucose 0.2% [w/v], bovine serum albumin 0.2% [w/v], pH 7.4, bubbled with 95% O2–5% CO2 and supplemented with antibiotics (penicillin G 100 IU/ml, streptomycin 50 μg/ml), in the presence of 0.25 IU/ml collagenase, 0.2 mg/ml pronase and 0.2 mg/ml soybean trypsin inhibitor. At the end of the second incubation, the medium was filtered and the partly digested muscle strips were washed four times with enzyme-free medium. These strips were then transferred into fresh, enzyme-free medium and left to stand for 20 min to allow the muscle cells to disperse spontaneously under very slow mechanical agitation. Cells were harvested through a 500-μm nylon filter. Only those cells that had dissociated spontaneously in enzyme-free medium were used for functional measurements. Viability tests (exclusion of trypan blue) showed that more than 85% of cells in suspension were viable at the time of contraction experiments.

Measurement of contractile response. Cell suspensions were usually assayed within 30 min of distension. Cell density of the suspension was adjusted to 250,000 cells/ml. The 250-μl aliquots of cell suspension were added to 250 μl of a solution containing the agent to be tested, thereby ensuring rapid mixing, and incubated for 30 sec at 31°C. The reaction was then interrupted by addition of glutaraldehyde to a final concentration of 2.5%.

In control experiments, 250 μl of the medium were substituted for the tested agent. To measure cell length, an aliquot of cells fixed with glutaraldehyde was placed on a Malassez slide and the length of the first 50 cells randomly encountered in successive microscopic fields was measured. Only intact cells on microscopic examination were measured. Cell length measurements were performed with a scale mask placed on a video screen. Magnification due to the video recording had first been calculated by comparison with length measurements obtained through the image splitting eye piece of the microscope, connected to a micrometer.

Experiments of relaxation. For relaxation experiments, cells were preincubated for 1 min in the presence of various concentrations of relaxing agents to be tested. Then, the contracting agent, CCK8 (10 nM) was added and the reaction stopped after 30 sec as described above.

Desensitization experiments. Freshly dispersed cells were incubated for 30 min at 31°C in the presence of the medium alone (HEPES-PO4 buffer, pH 7.4) or with VIP, isoproterenol or CCK8. At the end of this prolonged incubation, isolated smooth muscle cells were centrifuged (1000 rpm, 1 min, 20°C). The supernatant was removed and cells, resuspended in fresh medium. Contraction by CCK8 and relaxation by VIP or isoproterenol were then assayed as described above. All experiments were performed at 31°C as far as cell dispersion and contraction are usually assayed at this temperature.

Expression of results. The contractile response was defined as the decrease in the average cell length of a population of muscle cells exposed to a tested agent in comparison to controls. Cell contraction was expressed as the percentage decrease in cell length from control. The decrease in cell length was calculated using the following for-
mula: \( (L_0 - L_n) \times 100 \) where \( L_0 \) is the mean length of cells in resting state and \( L_n \), the mean length of treated cells.

In relaxation experiments, the degree of inhibition was expressed as the percentage decrease in the contractile response from the maximal response observed in the absence of inhibitors, taken as 100%.

Statistical analysis. Throughout this report, the data are expressed as the mean ± S.E.M., \( n \) refers to the number of experiments, each of those performed on samples from different animals. EC\(_{50}\) values were determined by linear regression analysis. Statistical evaluation was carried out using the Student’s \( t \) test, and the normality of the cell samples was assessed by the normal law test of Kolmogoroff. Values of \( P < .05 \) were considered statistically significant.

**Chemicals.** CCK8, VIP, Isoproterenol, VIP antagonist [(d-P-CI-Phe\(^6\),Leu\(^{17}\))VIP], Forskolin and 8-Bromo-cAMP were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase (CLS I) was purchased from Worthington Biochemical Corporation (Freehold, NJ). Pronase and soybean trypsin inhibitor were purchased from Boehringer Mannheim Ltd. (Meylan, France). Penicillin G and Streptomycin G were obtained from Specia (Paris, France).

**Results**

**Contraction of Isolated Smooth Muscle Cells by CCK8**

As previously shown, CCK8 contracted isolated intestinal smooth muscle cells in a concentration-dependent manner (Botella et al., 1992). Maximal contraction was obtained at 10 nM and corresponded to a cell shortening of 22.6 ± 3.42% of the length of control cells (table 1). The concentration of CCK8 inducing a half maximal contraction EC\(_{50}\) was 5 pM.

**Effect of VIP and Isoproterenol on CCK8-Induced Contraction**

When smooth muscle cells isolated from control animals (control cells) were incubated with increasing concentrations (ranging from 10 fM to 100 \( \mu \)M) of VIP or isoproterenol alone, the length of the cells was not affected. When cells were preincubated for 1 min with increasing concentrations of VIP (fig. 1A) or isoproterenol (fig. 1B) ranging from 10 fM to 100 \( \mu \)M, the CCK8-induced contraction was inhibited in a concentration-dependent manner. The concentration of VIP and isoproterenol inducing a half-maximal relaxation (EC\(_{50}\)) was 50 pM and 7 nM respectively. CCK8-induced contraction was abolished at 1 \( \mu \)M VIP and 100 \( \mu \)M isoproterenol.

**Influence of Ileal Mechanical Distension on the Relaxing Effect of VIP and Isoproterenol**

**Acute distension.** In smooth muscle cells isolated from the circular layer of the nondistended segment of the ileum, distal to the balloon, the characteristics of VIP- and isoproterenol-induced relaxations were similar to those observed in control cells, whether the balloon had been inflated or not. The maximal relaxation induced by VIP and isoproterenol was observed at 1 and 100 \( \mu \)M respectively. The concentration of VIP and isoproterenol inducing 50% of its maximal effect (EC\(_{50}\)) was 40 pM and 8 nM, respectively, and did not significantly differ from EC\(_{50}\) previously observed in control cells for these agents (table 2).

**Fig. 1.** Effect of VIP (A) and isoproterenol (B) on CCK8-induced contraction in smooth muscle cells isolated from the circular layer of control (nondistended) (■, ●) and 3 hr distended (□, ○) segments of guinea pig ileum. Cells were incubated with various concentrations of VIP and isoproterenol for 60 sec at 31°C. Then, CCK8 was added for 30 sec before cells were fixed by 2.5% glutaraldehyde. Values are the mean ± S.E.M. of six to nine separate experiments in cells from different animals.

<p>| TABLE 1 |
|--------------------------|--------------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th>Resting cell length and CCK8-induced contraction of intestinal smooth muscle cells from ileal segments submitted to various experimental conditions</th>
<th>Mean Length of Resting Cells (( \mu )M)</th>
<th>Mean Length of Cells Contracted by 10 nM CCK8 (( \mu )M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells from control animals</td>
<td>117.5 ± 7.8</td>
<td>90.9 ± 8.4</td>
</tr>
<tr>
<td>Cells from acutely distended segments</td>
<td>125.3 ± 9.1</td>
<td>99.2 ± 11.9</td>
</tr>
<tr>
<td>Cells from chronically distended segments</td>
<td>129.3 ± 8.4</td>
<td>101.8 ± 9.65</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. from six to nine experiments.
TABLE 2
Effects of VIP and isoproterenol on CCK8-induced contraction of intestinal smooth muscle cells isolated from control animals, nondistended or distended segments from animals submitted to acute or chronic distension

<table>
<thead>
<tr>
<th></th>
<th>VIP</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax EC50</td>
<td>Cmax EC50</td>
</tr>
<tr>
<td>Cells from control</td>
<td>1 µM 50 pM</td>
<td>100 µM 7 nM</td>
</tr>
<tr>
<td>Cells isolated from acute nondistended segments</td>
<td>1 µM 40 pM</td>
<td>100 µM 8 nM</td>
</tr>
<tr>
<td>Cells isolated from acute distended segments</td>
<td>100 µM 2 nM</td>
<td>100 µM 2 nM</td>
</tr>
<tr>
<td>Cells isolated from chronic nondistended segments</td>
<td>1 µM 75 pM</td>
<td>100 µM 3 nM</td>
</tr>
<tr>
<td>Cells isolated from chronic distended segments</td>
<td>100 µM 3 nM</td>
<td>100 µM 8 nM</td>
</tr>
</tbody>
</table>

Results from six to nine experiments.

P < .05 vs. nondistended segments and control animals, Student’s t test.

**Fig. 2.** Effect of VIP on CCK8-induced contraction in smooth muscle cells isolated from the circular layer of segments of guinea pig ileum distended for 3 hr. Distended segments were perfused with (o-P-CI-Phe6,Leu17) VIP (C) or saline (■) during distension period, then, isolated cells were incubated with various concentrations of VIP. 60 sec at 31°C. CCK8 was added for 30 sec before cells were fixed by 2.5% glutaraldehyde. Values are the mean ± S.E.M. of six to nine separate experiments in cells from different animals.

In Vitro Studies

**Desensitization of VIP and isoproterenol receptors in vitro.** As previously shown (Jeanneton et al., 1994), preincubation of cells for 30 min at 31°C in the medium alone did not alter the characteristics of CCK8-induced contraction, which did not significantly differ from the contraction observed in control cells.

After preincubation of the cells with 100 µM VIP for 30 min, a rightward shift of the concentration-response curve was observed for VIP (EC50 = 8 nM) but not for isoproterenol (EC50 = 9 nM) as compared to concentration-response curves obtained in control cells (fig. 3). Preincubation of cells with 100 µM isoproterenol for 30 min, did not alter the response to VIP or to isoproterenol.

**Effect of VIP after prolonged incubation of cells with VIP plus VIP antagonist.** We have previously shown that VIP antagonist [(o-P-CI-Phe6,Leu17) VIP] inhibited in a concentration-dependent manner VIP-induced relaxation in smooth muscle cells isolated from the circular layer of guinea pig ileum (Botella et al., 1994).

When smooth muscle cells isolated from control animals were incubated for 10 min in the sole presence of 100 µM VIP antag-
experiments in cells from different animals. Freshly dispersed cells were incubated for 60 sec at 31°C with various concentrations of VIP, then CCK8 was added for 30 sec before cells were fixed. After preincubation with VIP alone or with VIP plus (D-P-Cl-Phe6,Leu17) VIP, cells were centrifuged (1000 rpm, 1 min, 20°C), the supernatant was removed, cells were resuspended in fresh medium and incubated with various concentrations of VIP for 60 sec at 31°C, then 10 nM CCK8 was added for 30 sec and cells were fixed by 2.5% glutaraldehyde. Values are the mean ± S.E.M. of six separate experiments in cells from different animals.

Fig. 3. Effect of VIP on cells preincubated for 30 min with 1 μM of VIP on cells preincubated for 30 min with both 1 μM VIP plus 1 μM (D-P-Cl-Phe6,Leu17) VIP. Freshly dispersed cells were incubated for 60 sec at 31°C with various concentrations of VIP (■), then CCK8 was added for 30 sec before cells were fixed. After preincubation with VIP alone (○) or with VIP plus (D-P-Cl-Phe6,Leu17) VIP (□), cells were centrifuged (1000 rpm, 1 min, 20°C), the supernatant was removed, cells were resuspended in fresh medium and incubated with various concentrations of VIP for 60 sec at 31°C, then 10 nM CCK8 was added for 30 sec and cells were fixed by 2.5% glutaraldehyde. Values are the mean ± S.E.M. of six separate experiments in cells from different animals.

Effect of VIP on circular smooth muscle cells. Cells were isolated from control animals were incubated for 15 min with increasing concentrations (10 μM to 100 μM) of forskolin or 8-bromo-cAMP alone, the length of the cells was not affected. However, when cells were incubated for 15 min with increasing concentrations of forskolin or 8-bromo-cAMP, the CCK8-induced contraction was inhibited in a concentration-dependent manner. By contrast, when cells were preincubated with both 100 μM of VIP antagonist and 100 μM of VIP for 30 min before assaying the relaxing effect of VIP, VIP inhibited the CCK8-induced contraction in a concentration-dependent manner. The characteristics of the concentration-response curve for VIP were similar to that obtained in control cells, with a maximal relaxation observed at 1 μM VIP and an EC50 value of 70 pM (fig. 3). Thus, the presence of the VIP antagonist prevented the desensitizing effect of VIP itself.

Fig. 4. Effect of VIP and forskolin on cells preincubated for 30 min with 100 μM of forskolin. Freshly dispersed cells isolated from the circular layer of guinea pig ileum were incubated with various concentrations of VIP for 60 sec (■) or forskolin for 15 min (●) at 31°C, then CCK8 was added for 30 sec before cells were fixed. Cells were preincubated for 30 min at 31°C with 100 μM of forskolin, then centrifuged (1000 rpm, 1 min, 20°C), the supernatant was removed, cells were resuspended in fresh medium and incubated with various concentrations of VIP (○) or forskolin (△) for 60 sec. Then 10 nM CCK8 was added for 30 sec and cells were fixed by 2.5% glutaraldehyde. Values are the mean ± S.E.M. of six separate experiments in cells from different animals.

We demonstrate that a mechanical distension of the ileum induces a desensitization of the effect of the NANC relaxing mediator VIP in circular smooth muscle cells. This desensitization occurs after a prolonged single session of distension as well as after chronic repeated distension of the ileum. Moreover, incubation of freshly isolated smooth muscle cells with VIP in vitro also induces desensitization of the effect of VIP.

The characteristics of desensitization for the relaxing effect of VIP were similar whatever the experimental conditions inducing it. They were also similar to the characteristics of the homologous desensitization of PAF receptors that we observed on these cells in vitro (Jeanneton et al., 1994) and after experimental ileitis induced by chemical agents (Jeanneton et al., 1995). Indeed, the potency of VIP was altered as shown by the right shift of the concentration-response curve although its efficacy remained unchanged because VIP was still able to fully relax smooth muscle cells. We characterized receptor desensitization by demonstrating that the various experimental conditions induced a 2 log M rightward shift of the VIP concentration-response curve. The EC50 and the Cmax were increased by 100 times and that this effect was reversed by the VIP antagonist in the in vivo and in vitro experiments. The relaxing effect of mediators or drugs on isolated smooth muscle cells is usually measured as an inhibition of cell contraction induced by a contracting agent such as CCK. We carefully controlled in a set of preliminary experiments that distension of the ileum did not result in cell contraction induced by CCK8 was not modified. Similarly, prolonged incubation of cells with 100 μM of VIP antagonist for 30 min modified neither the resting cell length nor the CCK8-induced cell contraction. By contrast, when cells were preincubated with both 100 μM of VIP antagonist and 100 μM of VIP for 30 min before assaying the relaxing effect of VIP, VIP inhibited the CCK8-induced contraction in a concentration-dependent manner. The characteristics of the concentration-response curve for VIP were similar to that obtained in control cells, with a maximal relaxation observed at 1 μM VIP and an EC50 value of 70 pM (fig. 3). Thus, the presence of the VIP antagonist prevented the desensitizing effect of VIP itself.

Effect of VIP after prolonged incubation of cells with forskolin and 8-bromo-cAMP. When smooth muscle cells isolated from control animals were incubated for 15 min with increasing concentrations (10 μM to 100 μM) of either forskolin or 8-bromo-cAMP alone, the length of the cells was not affected. However, when cells were incubated for 15 min with increasing concentrations of forskolin or 8-bromo-cAMP, the CCK8-induced contraction was inhibited in a concentration-dependent manner. The CCK8-induced contraction was abolished at 1 μM forskolin or 8-bromo-cAMP (figs. 4 and 5). The EC50 was 70 and 97 pM for forskolin and 8-bromo-cAMP respectively.

When cells were incubated with increasing concentrations of VIP after pretreatment for 30 min at 31°C with 100 μM of forskolin or 8-bromo-cAMP, the concentration-response curve of VIP did not significantly differ from that obtained in cells from control animals. Cmax and EC50 observed under these conditions were similar to that obtained in control cells.

Discussion

We demonstrate that a mechanical distension of the ileum induces a desensitization of the effect of the NANC relaxing mediator VIP in circular smooth muscle cells. This desensitization occurs after a prolonged single session of distension as well as after chronic repeated distension of the ileum. Moreover, incubation of freshly isolated smooth muscle cells with VIP in vitro also induces desensitization of the effect of VIP.

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damage and thereby in an altered reactivity to tested agents. From several observations, we conclude that the viability and contractility of cells isolated from a distended ileal segment were not modified: 1) cell viability characterized by trypan blue exclusion was not altered; 2) length of resting smooth muscle cells obtained from ileal segments submitted to various in vitro distension protocols or in vitro prolonged incubation did not change significantly (see table 1); 3) cell contraction induced by CCK was not altered when cells from distended segments were compared to those obtained from nondistended segments or from control animals; 4) relaxation by isoproterenol was not affected by distension, showing that the ability of cells to relax was not altered. Abdominal surgery for placement of the distending balloon also did not influence the capability of cells to contract nor their viability. Indeed results were quite similar when comparing the cells obtained from non-distended ileal segments of operated animals with cells from nonoperated ones.

Desensitization of the effect of VIP occurred in different experimental conditions: incubation in vitro, acute and prolonged distension, chronic and repeated distensions. There may be multiple mechanisms underlying this desensitization. Experiments in vitro suggest that desensitization could result from a down-regulation of VIP receptors that could be triggered by a long-lasting occupation of these receptors and a sustained stimulation of the cells. Binding of VIP to its receptor seems a necessary condition to trigger desensitization because this desensitization was inhibited after occupancy of the receptor by its specific antagonist. Similarly, we did not observe desensitization of VIP receptors when cells were incubated in the presence of forskolin, which directly stimulates adenylate cyclase or in the presence of 8-bromo-cAMP, an analog of cAMP. However, both agents were able to relax the smooth muscle cells in the same experimental conditions. In vitro, desensitization of VIP receptors seems to be homologous. Desensitization after a relatively short period of time, precludes a decrease in synthesis of receptors by the cell. Internalization of receptors or modification of their affinity for their natural ligands could be responsible for this desensitization. Desensitization of the effect of biological agents by internalization of their receptors has been observed for catecholamines on astrocytoma cells (Perkins et al., 1984). Rapid desensitization could also result from changes in affinity of the receptors due to modifications of their binding sites to the Gs protein (Hausdorff et al., 1990). For instance, adrenergic receptors can be desensitized by phosphorylation by protein kinase A that is itself stimulated by cAMP (Leffkowitz et al., 1990). The consequence of these changes could be an impaired coupling of the receptors to the intracellular signalling pathway. Enhanced activity of phosphodiesterases and subsequent impaired intracellular activity of cAMP could also occur following a long standing stimulation by agonists (Harden, 1983). It is noteworthy to observe that all these desensitizing processes are triggered by the agonists of the receptor themselves. However, it has been shown that cGMP was also an important pathway of intracellular signalling of VIP-induced relaxation in smooth muscle cells (Jin et al., 1993; Rekik et al., 1996). Some authors have shown that the two intracellular pathways—cAMP and cGMP—could be triggered in parallel by VIP (Jin et al., 1993) although we have shown that VIP activated sequentially the cAMP-dependent pathway and then, the cGMP pathway (Rekik et al., 1996). In view of these results, one may not rule out a role for cGMP-dependent steps in VIP-induced relaxation in the mechanisms regulating VIP receptors during distension. However, the requirement for VIP receptor occupancy by the agonist suggests that the key step for receptor regulation takes place in the initial part of the cascade of cellular events leading to cell relaxation. Further investigations are nevertheless needed to confirm this hypothesis.

Mechanisms of desensitization occurring after either acute or chronic distension of the ileum in vivo are more difficult to determine. Different levels of control may all be involved in the adaptation of the gut to distension (Mayer and Raybould, 1990). In this study, the desensitization effect observed by the long period of distension of the intestine seems to be compatible with mechanisms of regulation affecting expression and synthesis of receptors by the smooth muscle cells. Indeed, it has been shown in several cell types that pathological conditions or prolonged stimulation of the cells might result in down regulation of the receptors due to decreased synthesis. In cardiac muscle from patients with heart failure (Bristow et al., 1982; Bristow et al., 1984) or from rats chronically infused with isoproterenol (Cohen and Schenck, 1987), a selective reduction in responsiveness of beta-adrenergic receptors has been observed. In this study, NANC nerve pathways could be involved in the adaptive mechanisms (Mayer and Raybould, 1990). Our results may at least partially support this hypothesis. Indeed, we evaluated the response of smooth muscle cells from the distended and non-distended segments of the ileum to isoproterenol, a beta adrenergic agonist as we did for VIP. We did not observe any desensitization of beta adrenergic receptors in these experiments. However, desensitization of beta-adrenergic receptors occurs and has been
extensively studied in many cell types (Lefkowitz et al., 1990). Failure to observe it in the present experiments suggests that the adrenergic pathway is not sensitive to desensitization and that it could be less or not involved in the adaptive mechanisms of the gut to distension. Indeed, desensitization of receptors is mainly the consequence of a prolonged and strong stimulation by their respective ligands. Desensitization of VIP receptors could thus be the consequence of an overstimulation of smooth muscle by large amounts of this neuromediator released during distension. This assumption is reinforced by the observation that the VIP antagonist prevented cells from desensitization of the VIP effect when it was perfused intraluminally during the whole distension period. It seems thus that in vivo, desensitization of the effect of VIP is also triggered by homologous mechanism. Moreover, it seems to be quite specific because beta adrenergic receptors were not affected by distension.

Taken together, these observations suggest that VIP is important in the adaptive response of smooth muscle to intestinal distension. The absence of influence of distension on beta adrenergic receptors indicate that the NANC adrenergic pathway could be the main pathway mediating this adaptation. This is in agreement with the observations that the secretory reflexes triggered by distension are mediated by VIP (Caren et al., 1974; Harris et al., 1989; Schulzke et al., 1995). Moreover, previous studies have shown that VIP is one of the main neurotransmitters involved in the control of gastric and intestinal relaxation in physiological conditions (Grider, 1994; Grider and Makhlof, 1990). In some pathological conditions, the role of VIP has also been recognized and several studies have shown that VIP-containing nerves are less numerous or even absent from the myenteric plexus of the lower esophageal sphincter in patients with achalasia (Aggestrop et al., 1983), from the pylorus in children with hypertrophic stenosis (Wattchow et al., 1987) and from the colon of patients with Hirschprung’s disease (Larsson et al., 1983). It is noteworthy to observe that these conditions are all characterized by a defective relaxation of the affected segments of the digestive tract. Finally, it has been shown that levels of VIP were increased in blood from the portal vein in dogs with small bowel obstruction (Basson et al., 1989). One may thus assume that distension of the gut could be responsible for an increase in VIP synthesis or release at the myenteric plexus level and thus that desensitization of receptors could be a protective mechanism against an exaggerated stimulation of the cells by relaxing agents.

Because desensitization of VIP receptors occurred in chronic conditions, these data could be relevant to functional bowel disorders, where abdominal distension could play a pathophysiological role.

**References**


