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Vasoactive Intestinal Polypeptide as Possible Mediator of Relaxation in the Rat Gastric Fundus

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Abstract—Relaxations were induced in longitudinal muscle strips of the rat gastric fundus by stimulation of non-adrenergic non-cholinergic (NANC) neurons and by administration of vasoactive intestinal polypeptide (VIP) or isoprenaline. The effect of antiserum against vasoactive intestinal polypeptide (VIP antiserum) and of control serum on these relaxations was investigated. Incubation with VIP antiserum (dilution 1/50) for 1 h almost completely prevented the relaxation by VIP. It partially prevented the relaxation evoked by electrical stimulation while the relaxation induced by isoprenaline was not influenced. Control serum decreased the VIP- and stimulation-induced relaxations much less than did VIP antiserum. In addition, the effect of the putative VIP antagonist (4Cl-D-Phe^6, Leu^{17}) VIP was studied on the relaxations induced by NANC neuron stimulation and by VIP. The VIP antagonist ($3 \cdot 10^{-5}$ M, incubation time 10 min) had a relaxatory effect itself but had no influence on either VIP- or stimulation-induced relaxations. The results with VIP antiserum confirm the involvement of VIP in the inhibitory NANC neurotransmission of the rat gastric fundus.

Non-adrenergic non-cholinergic (NANC) neurons are ubiquitous in the body. In contrast to the (nor)adrenergic and cholinergic innervations, more than one neurotransmitter seems to be involved in NANC neurotransmission. With regard to the inhibitory NANC neurotransmission in the gastrointestinal tract, evidence has been presented that polypeptides could be involved. In the rat gastric fundus, vasoactive intestinal polypeptide (VIP) seems a reasonable candidate as inhibitory NANC neurotransmitter. Indeed, VIP-like immunoreactivity has been found in nerve cell bodies and axons in the rat stomach (Schultzberg et al 1980; Sundler et al 1980); furthermore, we have previously shown that in-vitro VIP mimics the relaxation induced by NANC neuron stimulation (Lefebvre 1986) and α-chymotrypsin and trypsin partially inhibit the relaxation induced by NANC neuron stimulation (De Beurme & Lefebvre 1987).

To further establish the involvement of VIP in the inhibitory NANC neurotransmission in the rat gastric fundus, we have now studied the effect of VIP antiserum and of a putative VIP antagonist (4Cl-D-Phe^6, Leu^{17}) VIP, on the relaxation induced by exogenous administration of VIP and by stimulation of the NANC neurons. Our results with VIP antiserum provide further support for the hypothesis that VIP is involved in the NANC relaxation of the rat gastric fundus. A preliminary account of this work has been presented (De Beurme et al 1987).

Materials and Methods

Rats (either sex, 130–210 g) were reserpinized (5 mg kg^{-1} intraperitoneally) 24 h before death and were fasted from then on. Two longitudinal muscle strips (15 mm long × 3 mm wide) of the gastric fundus were prepared as described by Vane (1957). The strips were mounted under a load of 1 g between 2 parallel platinum electrodes (48 mm long × 6 mm wide) in 18 mL organ baths containing Krebs solution (composition in mM: NaCl 118·5, KCl 4·8, $CaCl_2$ 1·9, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25·0 and glucose 10·1) at 37 °C, bubbled with 95% O_2 and 5% CO_2. The Krebs solution also contained atropine 10^{-6} and 5-HT $3 \cdot 10^{-6}$ M. Strips were equilibrated for 1 h, with rinsing every 15 min, and changes in length were recorded auxotonically (Harvard heart-smooth muscle transducer) on a Beckman Type R Dynograph Recorder. After the equilibration period, strips were rinsed every 5 min in between drug administration and/or periods of electrical stimulation. Electrical stimulation (supramaximal voltage, 5 Hz, duration 0·1 or 1 ms) of the NANC neurons was performed using a S88 Grass stimulator and a constant current unit.

Influence of VIP antiserum

The effect of VIP antiserum, raised in rabbits against porcine VIP, was studied on relaxatory responses obtained by electrical stimulation of the NANC neurons (ES), or by exogenous administration of VIP or isopropylnoradrenaline (ISO); only one relaxant stimulus was studied per strip. The following protocol was used.

An initial ES (supramaximal voltage, 5 Hz, 1 ms) was applied during 5 min. After tone had recovered, the relaxation in response to either ES (supramaximal voltage, 5 Hz, 0·1 ms during 10 min) or exogenous administration of VIP (10^{-8} M, contact time 5 min) or ISO ($3 \cdot 10^{-8}$ M, contact time 10 min) was studied and measured at the end of the stimulation period or the contact time. After the original tone was restored, VIP antiserum was added to a final dilution of 1/50 and left in contact with the strip for 1 h. The response to either ES, VIP or ISO was then studied again, while VIP antiserum was still present. The effect of control serum on the relaxatory responses to VIP, ES or ISO was studied in parallel strips in a similar way.

Changes of tone at the end of the 1 h incubation with the

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sera were expressed as a percentage of the maximal relaxatory response obtained by the stimulus under study, applied before addition of the sera. The relaxatory responses induced by ES, VIP or ISO in the presence of either VIP antiserum or control serum were measured against this changed base line tension and were expressed as a percentage of the maximal relaxation obtained before addition of the sera.

Influence of (4Cl-D-Phe⁶, Leu¹⁷)VIP

The influence of (4Cl-D-Phe⁶, Leu¹⁷)VIP was studied on relaxations induced by either VIP or ES in parallel strips, using the following protocol. An initial ES (supramaximal voltage, 5 Hz, 1 ms) was performed during 5 min. After tone was restored, 10^{-9} M and 10^{-8} M VIP were administered cumulatively. After rinsing until the tone was restored, $3{\cdot}10^{-5}$ M (4Cl-D-Phe⁶, Leu¹⁷)VIP was added and left in contact with the strip for 10 min. The response to either VIP (10^{-9} and 10^{-8} M, in a cumulative way) or ES was then studied again, in the presence of the VIP antagonist. The relaxation induced by (4Cl-D-Phe⁶, Leu¹⁷)VIP itself after 10 min of contact was expressed as a percentage of the maximal relaxation induced by 10^{-8} M VIP or by ES before addition of the VIP antagonist.

Drugs used

Atropine sulphate (Merck, Brussels, Belgium), (±)-isopropylnoradrenaline hydrochloride (Winthrop, Brussels, Belgium), reserpine (Aldrich Chemie, Brussels, Belgium), 5-hydroxytryptamine creatinine sulphate (5-HT), vasoactive intestinal polypeptide (VIP) (Sigma, St. Louis, USA) were used. For isoprenaline, commercially available ampoules were used. For reserpine, a stock solution was prepared from powder (5 mg mL^{-1} dissolved in 10% ascorbic acid). For VIP, a stock solution was prepared by dissolving 1 mg of VIP in 16.6 mL of distilled water. The stock solution was kept deep frozen in fractions of 1 mL; further dilutions were made the day of the experiment. On the day of the experiment, drugs were kept on ice, and added to the bath in a volume of 0·1 mL.

VIP antiserum, raised against porcine VIP in rabbits and control rabbit serum were obtained from Prof. Vande Sande (Laboratory for neuroendocrinology and immunological biotechnology, Institute of Zoology, Catholic University of Leuven, KUL). With the immunospotting technique, the VIP antiserum in a dilution of 1/20 did not show cross reactivity with PHI, glucagon, secretin and GIP. In preliminary experiments, we showed that the VIP antiserum in a final dilution of 1/500 was able to antagonize VIP 10^{-8} M; this corresponds to 1 mL of undiluted VIP antiserum blocking 16·6 μg VIP. (4Cl-D-Phe⁶, Leu¹⁷) VIP was a gift of Dr. J. Rivier (The Salk Institute, Laboratories for Peptide Biology, San Diego, California). A solution was prepared from powder dissolved in distilled water. The VIP antagonist was added to the bath in a volume of 0·1 mL.

Statistical analysis

Results are expressed as means with their standard error (s.e.m.). Responses obtained in the presence of VIP antiserum were compared with those in the presence of control serum by use of the two sample rank test.

Results

Influence of VIP antiserum

The relaxations induced by ES (supramaximal voltage, 5 Hz, 0·1 ms), by VIP (10^{-8} M) and by ISO ($3{\cdot}10^{-8}$ M) at the beginning of the experiments were comparable with the relaxation induced by the initial ES (1 ms); these relaxations were 93 ± 8% (n = 10), 94 ± 6% (n = 10) and 109 ± 14% (n = 9 as in one of the experiments ES was not possible). During the incubation with VIP antiserum, and with control serum, basal tension increased by 9 ± 14·5 and 4 ± 10·8%, respectively.

Examples of actual experiments with VIP antiserum are shown in Fig. 1 and the mean results of the experiments with VIP antiserum and control serum are given in Fig. 2. Incubation with VIP antiserum (final dilution 1/50) antagonized almost completely the relaxation induced by VIP while incubation with control serum induced only a small reduction; the relaxation in the presence of control serum was significantly higher than that in the presence of VIP antiserum ($P < 0{\cdot}01$). ES in the presence of VIP antiserum induced an initial relaxation, which on average was 65 ± 27% of the relaxation obtained in the absence of VIP antiserum; during the 10 min stimulation period however, tone recovered so that the relaxation at the end of the stimulation period was only 12 ± 10%. After incubation with control serum, ES induced initially, like in the presence of VIP antiserum, a relaxation of about 71 ± 6%; recovery of tone during the 10 min stimulation period was however small, so that the relaxation after 10 min was significantly more pronounced than the relaxation after 10 min in the presence of VIP antiserum ($P < 0{\cdot}05$). The relaxation induced by isoprenaline was not influenced by VIP antiserum and control serum.

Influence of (4Cl-D-Phe⁶, Leu¹⁷)VIP

$3{\cdot}10^{-5}$ M (4Cl-D-Phe⁶, Leu¹⁷) VIP induced a relaxation on its own (31 ± 17% of the VIP-induced response, n = 3; 53 ± 12% of the ES-induced response, n = 3). The relaxation induced by the VIP antagonist itself was more pronounced than that induced by the prior addition of VIP 10^{-9} M. Therefore, only the influence on the relaxation induced by VIP 10^{-8} M was evaluated. When measured against the tension present just before addition of (4Cl-D-Phe⁶, Leu¹⁷) VIP, the VIP (10^{-8} M)- and ES-induced relaxation was not different from that obtained before addition of the antagonist (111 ± 11%, n = 3 and 112 ± 13%, n = 3, respectively). Two representative experiments are shown in Fig. 3.

Discussion

These experiments were performed to further investigate the possible role of VIP as the inhibitory NANC neurotransmitter in the rat gastric fundus. The influence of rabbit VIP antiserum and that of a putative VIP antagonist, (4Cl-D-Phe⁶, Leu¹⁷)VIP, were studied on relaxations induced by administration of VIP or isoprenaline and by electrical stimulation of the NANC neurons. Inhibition of the neurally induced NANC relaxation by VIP antiserum has been found in several gastrointestinal smooth muscle preparations including the opossum lower oesophageal sphincter (Goyal

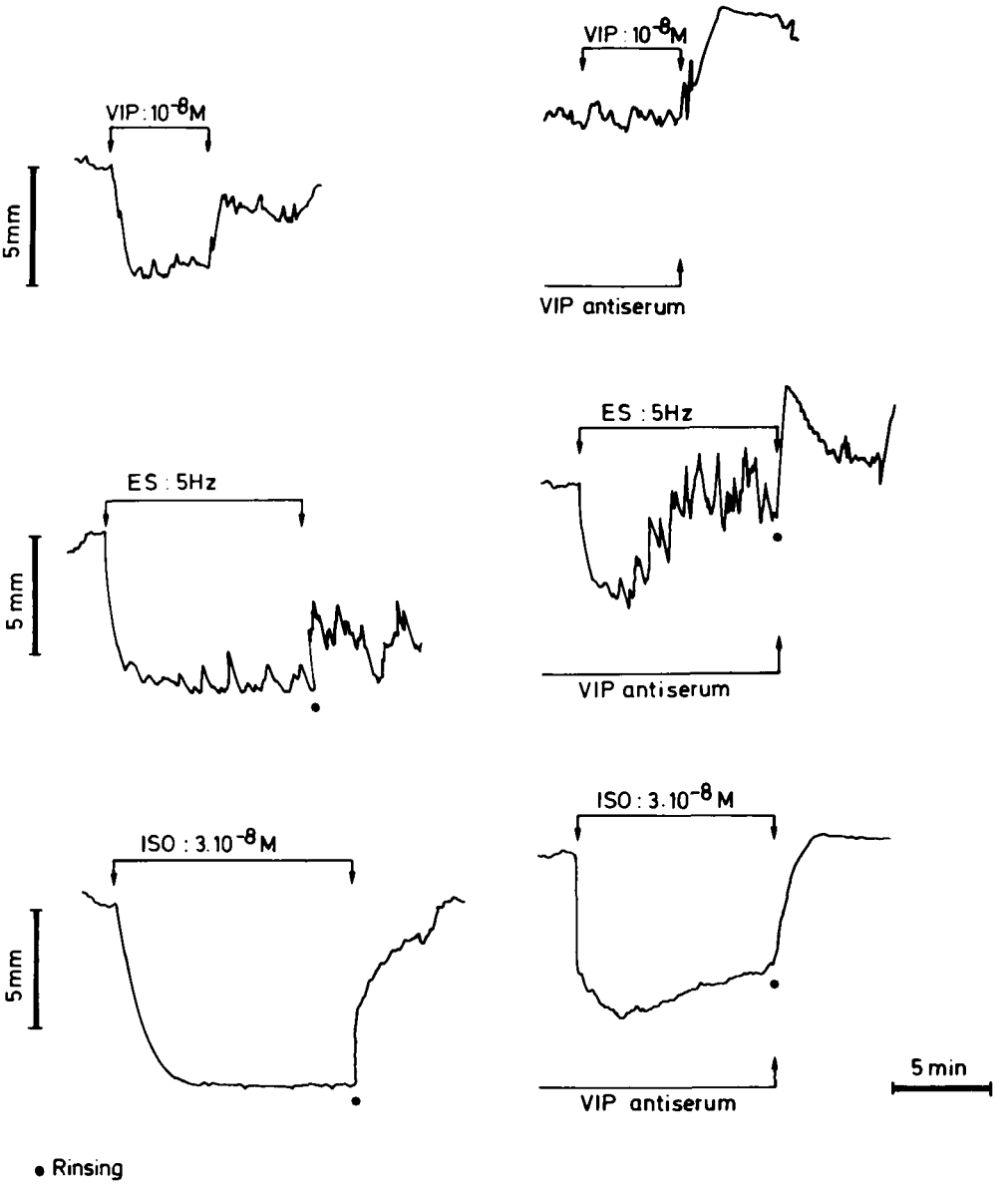

FIG. 1. Longitudinal fundus strip of the rat. Relaxation in response to vasoactive intestinal polypeptide (VIP, 10^{-8} M), electrical stimulation of the NANC neurons (ES, supramaximal voltage, 5 Hz, 0·1 ms) and isoprenaline (ISO, $3{\cdot}10^{-8}$ M) before (left panel) and after (right panel) addition of VIP antiserum (dilution 1/50, incubation time 1 h).

et al 1980), the dog gastric muscularis mucosae (Angel et al 1983), the cat lower oesophageal sphincter (Biancani et al 1984), the rabbit internal anal sphincter (Biancani et al 1985), the guinea-pig taenia coli (Grider et al 1985a) and the guinea-pig stomach (Grider et al 1985b). In our preparation, we observed in preliminary experiments that VIP antiserum, in the maximal concentration (dilution 1/50) that we could add because of cost, had no effect on the relaxation induced by stimulation at supramaximal voltage, 1 ms duration and 5 Hz frequency. It has been shown that the antagonism of the neurally induced NANC relaxation by VIP antiserum is inversely related to the intensity of stimulation, even when considering frequency as duration (Biancani et al 1985; Grider et al 1985b). Therefore, the duration of stimulation was decreased to 0·1 ms. Although the amplitude of the relaxation induced by stimulation at 0·1 ms was only slightly lower than that induced by stimulation at 1 ms in the beginning of the experiment, VIP antiserum in a concentration inhibiting almost completely the relaxation induced by exogenous VIP, also antagonized the stimulation-induced relaxation at 10 min to a high degree. This effect of VIP antiserum does not seem to be non-specific, as the isoprenaline-induced relaxation, which is due to interaction with postsynaptic β-adrenoceptors (Lefebvre et al 1984), was not influenced. In addition, control serum influenced the VIP- and ES-induced relaxation much less than the VIP antiserum did. However, electrical stimulation induced initially a relaxation with an amplitude of 65% of the relaxation obtained in the absence of VIP antiserum. This initial relaxation might be induced by an initial release of VIP,

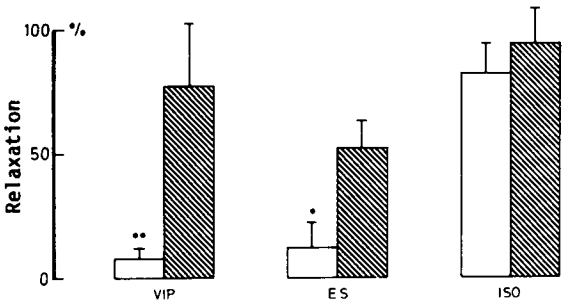

FIG. 2. Longitudinal fundus strip of the rat. Relaxation induced by vasoactive intestinal polypeptide (VIP, 10^{-8} M), by electrical stimulation of the NANC neurons (ES, supramaximal voltage, 5 Hz, 0·1 ms) and by isopenaline (ISO), in the presence of VIP antiserum (open bars) or control serum (dashed bars). Values were measured at the end of the stimulation period or contact time and are expressed as a percentage of the maximal response obtained before addition of VIP antiserum or control serum. Each value represents the mean ±s.e.m. of 5 observations. $*P<0.05$, $**P<0.01$: significantly different from the value in the presence of control serum.

which escapes inactivation by the antibodies, as it has been shown that the antiserum may be slow in binding VIP (Pandian et al 1982).

The fact that even after 10 min some degree of relaxation persists in the presence of VIP antiserum could be due to release of another substance together with VIP during neuron stimulation. Co-distribution, shown in the feline gastrointestinal tract (McGregor et al 1984), and co-release, shown in the portal plasma in the dog during vagal stimulation (Yasui et al 1987), of VIP and peptide histidine isoleucine (PHI), a homologue of VIP derived from the same precursor (Itoh et al 1983), would point to PHI as possible co-transmitter. PHI has indeed been shown to induce a relaxation in various gastrointestinal smooth muscle preparations (Makhlouf 1985). On the other hand, we have shown that the peptidases trypsin and α-chymotrypsin also did not completely antagonize the relaxation induced by NANC neuron stimulation (De Beurme & Lefebvre 1987); this would suggest a non-peptide as co-transmitter.

The most convincing pharmacological evidence for a role of VIP as inhibitory NANC neurotransmitter should come from studies using selective VIP antagonists. Recently, two molecules were shown to antagonize the effect of VIP: (Ac-Tyr^1 hGRF) which inhibited the VIP-stimulated adenylate cyclase activity in a dose-dependent manner in human and rat intestinal epithelial membranes (Laburthe et al 1986) and (4Cl-D-Phe^6, Leu^{17})VIP which competitively antagonized the VIP-stimulated amylase release in the guinea-pig pancreas (Pandol et al 1986). We studied the effect of the substituted VIP molecule, (4Cl-D-Phe^6, Leu^{17})VIP, on the relaxations induced by exogenous administration of VIP and by electrical stimulation of the NANC neurons. Although the number of experiments is limited, due to the small amount of substance available, it is clear that (4Cl-D-Phe^6, Leu^{17})VIP does not inhibit relaxations induced by either VIP or NANC neuron stimulation. The lack of effect of (4Cl-D-Phe^6, leu^{17})VIP on the smooth muscle effect of VIP in the rat gastric fundus might be due to the known differences in VIP receptors between species and/or tissues (O'Dorisio 1987). Thompson et al (1987) recently reported that (4Cl-D-Phe^6, Leu^{17})VIP, in the concentration used in this study, did not

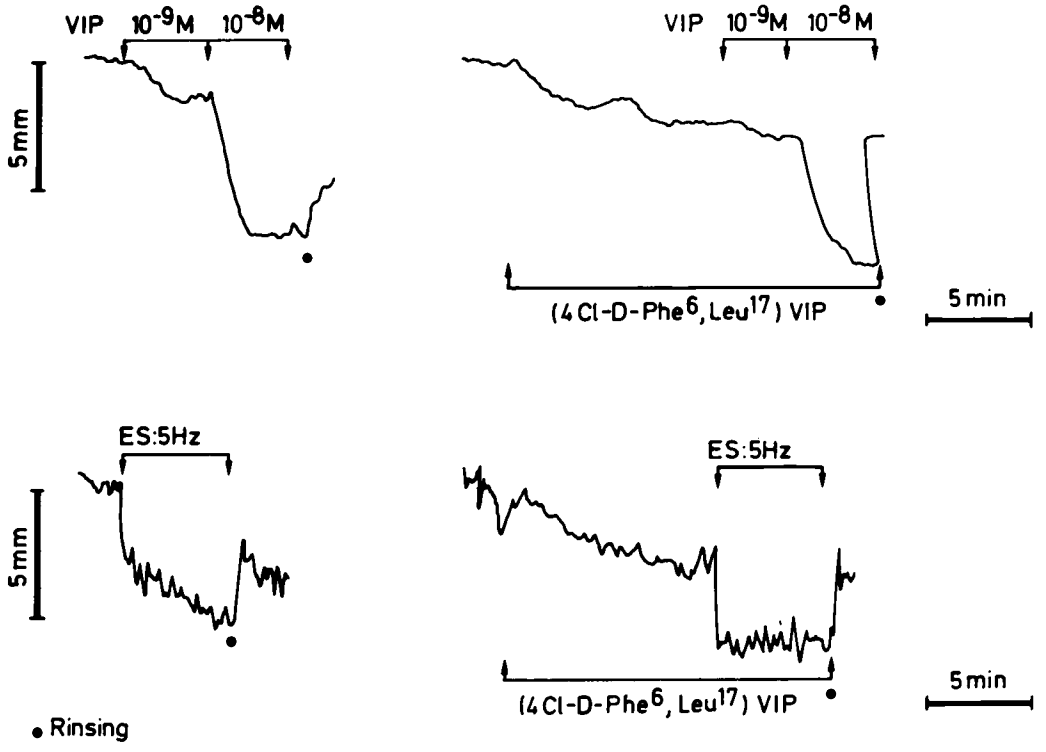

FIG. 3. Longitudinal fundus strips of the rat. Relaxation in response to vasoactive intestinal polypeptide (VIP, 10^{-9} and 10^{-8} M in a cumulative way) and electrical stimulation of the NANC neurons (ES, supramaximal voltage, 5 Hz, 1 ms) before and after administration of (4Cl-D-Phe^6, Leu^{17})VIP ($3{\cdot}10^{-5}$ M, incubation time 10 min).

influence the tissue sensitivity to NANC nerve stimulation and to exogenous VIP in the isolated feline intrapulmonary bronchus. In the feline bronchus, (4Cl-D-Phe^6, Leu^{17})VIP had no influence on tissue tone, while in the rat gastric fundus it has a relaxatory effect itself. This might indicate that (4Cl-D-Phe^6, Leu^{17})VIP has some agonist effect in the rat gastric fundus, but is either less potent that VIP, or acts as a partial agonist. In the latter case, one could, however, expect some antagonism of the full agonist VIP.

In conclusion, our results with VIP antiserum support the hypothesis that VIP is involved in NANC relaxation in the rat gastric fundus. (4Cl-D-Phe^6, Leu^{17})VIP can however not be used to elucidate this further, as it does not block the effect of exogenous VIP.

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