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Short communication

Influence of selective VIP receptor agonists in the rat gastric fundus

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Abstract

The receptor subtypes involved in the relaxant effect of vasoactive intestinal polypeptide (VIP) in the rat gastric fundus were investigated in vitro. The selective VIP_2 receptor agonist [Ac-H^1,E^8,K^{12},Nle^{17},A^{19},D^{25},L^{26},$K^{27,28}$,$G^{29,30}$,T^{31}]VIP(cyclo21-25) (RO25-1553) induced a concentration-dependent relaxation (EC_{50} 2.8 nM), while the selective VIP_1 receptor agonist derived from growth hormone-releasing factor (GRF) [K^{15},R^{16},L^{27}]VIP-(1-7)/GRF-(8-27) had no effect up to 1 μM. [R^{16}] chicken secretin, a selective VIP_1 receptor agonist, induced relaxation with a potency of 4.8 nM but its maximal effect was clearly lower than that of VIP, pituitary adenylate cyclase-activating peptide [PACAP-(1-27)] and RO25-1553. This effect was reproduced by porcine secretin (EC_{50} 2.1 nM). It is concluded that the rat gastric fundus contains functional VIP_2 receptors but not VIP_1 receptors, and that specific secretin receptors are also present.

Keywords: Stomach; Smooth muscle; Relaxation; VIP (vasoactive intestinal polypeptide); Secretin

1. Introduction

Two subtypes of G protein-coupled vasoactive intestinal polypeptide (VIP) receptors have been cloned from rat and human tissues and are named the VIP_1/PACAP and VIP_2/PACAP receptors (PACAP: pituitary adenylate cyclase-activating peptide; Ishihara et al., 1992; Lutz et al., 1993; Couvineau et al., 1994; Svoboda et al., 1994). These receptors recognize VIP and both forms of PACAP, PACAP-(1-27) and PACAP-(1-38), with a similar high affinity (Usdin et al., 1994). In contrast, at $PACAP_1$ receptors, PACAP-(1-27) and PACAP-(1-38) have a similar potency while that of VIP is clearly lower (Christophe, 1993). No pharmacological tools were available to clearly distinguish between VIP_1/PACAP and VIP_2/PACAP receptors in functional experiments but, recently, the development of highly selective agonists, such as [R^{16}] chicken secretin and [K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27) (GRF: growth hormone-releasing factor) for the VIP_1/PACAP receptor, and the cyclic VIP analog [Ac-H^1,E^8,K^{12}, Nle^{17},A^{19},D^{25},L^{26},$K^{27,28}$,$G^{29,30}$,T^{31}]VIP(cyclo21-25) (RO25-1553) for the VIP_2/PACAP receptor, was reported (Gourlet et al., 1997b,c). A high-affinity selective antagonist for VIP_1/PACAP receptors has also been developed (Gourlet et al., 1997a). The aim of this study was to investigate the effect of these agents in comparison with that of VIP and PACAP-(1-27) in the longitudinal muscle of the rat gastric fundus in order to determine the subtype of VIP receptor involved in the relaxant effect of VIP in this tissue (Lefebvre et al., 1991; Shirahase et al., 1994), where VIP has been proposed as a non-adrenergic non-cholinergic inhibitory neurotransmitter (Li and Rand, 1990). The experiments were performed according to the Belgian regulations for the protection of experimental animals.

2. Materials and methods

2.1. Tissue preparation and measurement of relaxant effect

Male Wistar rats (290–350 g body weight) were maintained on a 12-h light/dark cycle. After an overnight fast with free access to water, they were killed by a blow to the head and two longitudinal muscle strips (15 mm long × 2.5 mm wide) per gastric fundus were mounted under a load of 1 g in 10-ml organ baths containing Krebs solution (for composition see Lefebvre et al., 1991) at 37°C, gassed with carbogen. The Krebs solution also contained 50 mg

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l^{-1} bovine serum albumin and 30 mg l^{-1} bacitracin. Tension was recorded auxotonically (Grass force-displacement transducer FT03 coupled in series with a 1 g cm^{-1} spring) on Kipp and Zonen recorders (type BD 112). After 1 h of equilibration, contraction was induced by administration of 1 μM prostaglandin $F_{2\alpha}$. Once a stable contraction was obtained, the tissue was relaxed by administration of 30 μM sodium nitroprusside as a control relaxation. After repeated rinsing for at least 60 min, 1 μM prostaglandin $F_{2\alpha}$ was again added and once the plateau was reached, a peptide was added cumulatively. In parallel tissues from the same animals, the relaxant effect of the peptides was studied after incubation with 3 μM tetrodotoxin for 15 min. In additional series, the relaxant effect of VIP, PACAP-(1-27) and RO25-1553 was tested in the absence or, in parallel tissues from the same animals, 15 min after administration of 1 μM [Ac-His^1,D-Phe^2,K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27). The relaxant effect of sodium nitroprusside and the peptides is expressed as the percentage reduction of prostaglandin $F_{2\alpha}$-induced tone. The peptide potency was measured as the concentration required to produce 50% of the maximal relaxant effect of a particular peptide (EC_{50}) by linear interpolation from individual concentration–response curves, and the relative potency vs. VIP was calculated as the ratio EC_{50} VIP/EC_{50} other peptide.

2.2. Drugs and peptides used

Bacitracin, bovine serum albumin, prostaglandin $F_{2\alpha}$, sodium nitroprusside and tetrodotoxin were obtained from Sigma (St. Louis, MO, USA). Bacitracin and bovine serum albumin were added to the Krebs solution; sodium nitroprusside was daily dissolved in deionized water and kept in a vial protected from light; prostaglandin $F_{2\alpha}$ and tetrodotoxin were dissolved in deionized water and kept as 1 mM stock solutions at −20°C.

The following peptides were investigated in this study: VIP, PACAP-(1-27), RO25-1553, [K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), [Ac-His^1,D-Phe^2,K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), porcine secretin and [R^{16}] chicken secretin. All the non-cyclic peptides were synthesized as C-terminal amides by solid-phase methodology, using the Fmoc (9-fluorenylmethoxy-carbonyl) strategy with an Applied Biosystems apparatus 431A (Foster City, CA, USA). The cleavage and the purification of the peptides have already been described (Gourlet et al., 1997b). The cyclic compound RO25-1553 was synthesized as described (O'Donnell et al., 1994) using the N^{α}-Boc derivatives. The purity of the material was at least 95%, as judged by capillary electrophoresis and analytical reverse-phase chromatography, and the peptide conformity was assessed by electrospray mass spectrometry (O'Donnell et al., 1994). The peptides were dissolved in a 20-mM phosphate buffer, 0.15 M NaCl, pH 7.4, as a 0.24 mM stock solution.

2.3. Data analysis

Values are expressed as means ± S.E.M. of *n* experiments with tissues from different animals. The relaxant responses to sodium nitroprusside in the different series were compared by a one-way analysis of variance (ANOVA). The relaxant responses, the EC_{50} and the E_{max} in the absence and presence of tetrodotoxin or the VIP_1/PACAP receptor antagonist were compared by using the Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

Prostaglandin $F_{2\alpha}$ at 1 μM, a submaximal concentration, was used to increase the tone of the rat gastric fundus in order to investigate relaxant stimuli (Lefebvre et al., 1991). The relaxant effect of 30 μM sodium nitroprusside ranged from 71.5 ± 6.6% to 85.1 ± 5.3% in the different series, and was not significantly different. All peptides induced a concentration-dependent relaxation (Fig. 1), except for [K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), which had no relaxant influence up to a concentration of 1 μM. VIP, PACAP-(1-27) and RO25-1553 had a similar maximal effect (Table 1) that was somewhat more pronounced than the reversal of the contraction induced with prostaglandin $F_{2\alpha}$ (= 100%), illustrating that the preparations had some minimal intrinsic tone. Porcine secretin and [R^{16}] chicken secretin were less effective and their E_{max} values were

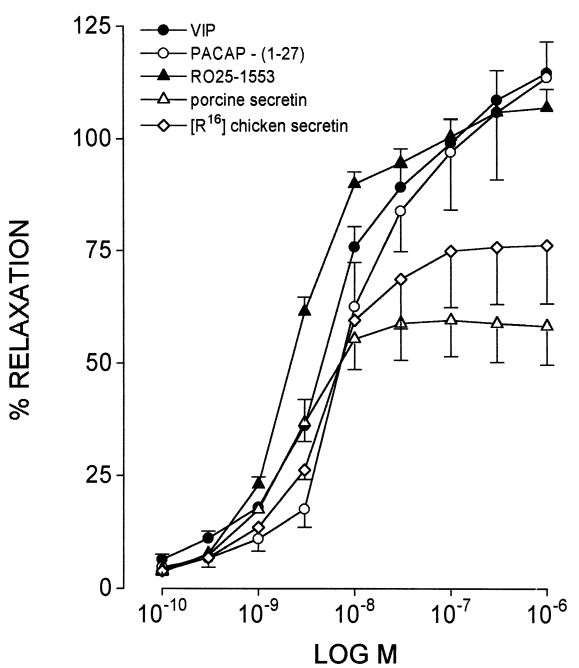

Fig. 1. Mean concentration–response curves for the relaxant effect produced in longitudinal muscle strips of the rat gastric fundus by VIP, PACAP-(1-27), RO25-1553, porcine secretin and [R16] chicken secretin. Mean S.E.M. of $n = 6–7$.

Table 1
Potency and efficacy of VIP receptor agonists in the rat gastric fundus

	EC_{50} (nM)	Relative potency[a]	E_{max} (%)
VIP ($n = 7$)	6.6 ± 1.1	1	114.7 ± 7.1
PACAP-(1-27) ($n = 6$)	12.2 ± 2.6	0.54	113.6 ± 16.5
RO25-1553 ($n = 7$)	2.8 ± 0.4	2.36	107.0 ± 4.0
Porcine secretin ($n = 6$)	2.1 ± 0.3	3.14	58.3 ± 8.6
[R^{16}] chicken secretin ($n = 6$)	4.8 ± 0.8	1.38	76.4 ± 13.1
[K^{15},R^{16},L^{27}] VIP(1-7)/GRF(8-27) ($n = 5$)	Not effective		

[a]Relative potency: EC_{50} VIP/EC_{50} peptide.

58.3 ± 8.6 and 76.4 ± 13.1%, respectively. The overall order of potency was porcine secretin > RO25-1553 > [R^{16}] chicken secretin > VIP > PACAP-(1-27). The administration of 3 μM tetrodotoxin did not influence the basal length of the tissues nor the amplitude of the contraction induced by prostaglandin $F_{2\alpha}$. The relaxant effect of the peptides was not influenced by the presence of tetrodotoxin.

The antagonist of the VIP_1/PACAP receptors [Ac-His[1],D-Phe[2],K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27) had no relaxant effect per se in prostaglandin $F_{2\alpha}$-contracted tissues (10 nM–1 μM, $n = 4$). At a concentration of 1 μM, the antagonist had no influence on the relaxant effect of VIP, PACAP-(1-27) and RO25-1553. The EC_{50} and E_{max} values for VIP were 12.1 ± 4.2 nM and 106.9 ± 4.6% in the absence and 13.8 ± 6.2 nM and 108.1 ± 7.7% in the presence of the antagonist ($n = 6$). For PACAP-(1-27), these values were 4.3 ± 0.9 nM, 87.4 ± 6.2%, 4.3 ± 0.9 nM and 95.2 ± 11.5% ($n = 6$), and for RO25-1553, 3.4 ± 0.7 nM, 104.7 ± 3.1%, 4.9 ± 0.7 nM and 109.8 ± 3.6% ($n = 6$).

4. Discussion

The relaxant effect of the peptides in the rat gastric fundus was not influenced by tetrodotoxin, pointing to a muscular site of action. The potency of VIP was similar to that reported before (Lefebvre et al., 1991; Shirahase et al., 1994), and the results obtained with the selective agonists point to the presence of a functional VIP_2/PACAP receptor and the absence of a functional VIP_1/PACAP receptor in the longitudinal muscle of the rat gastric fundus. Indeed, the VIP_2/PACAP receptor-selective agonist RO25-1553 induced a concentration-dependent relaxation and was 2.4 and 4.4 times more potent than VIP and PACAP-(1-27), respectively. This potency order corresponds to that observed at human recombinant VIP_2/PACAP receptors (Gourlet et al., 1997c). In contrast, the VIP_1/PACAP receptor-selective agonist [K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), up to a concentration of 1 μM, had no effect; its affinity at the rat and human recombinant VIP_1/PACAP receptor is 1–2 nM (Gourlet et al., 1997b). Furthermore, the antagonist [Ac-His[1],D-Phe[2],K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), which has been shown to have an affinity of 15 nM at the rat VIP_1/PACAP receptor but to not affect the rat VIP_2/PACAP receptor in a concentration of 300 nM (Gourlet et al., 1997a), did not influence the relaxant effect of VIP, PACAP-(1-27) and RO25-1553 at 1 μM. The conclusion that there is a functional VIP_2/PACAP receptor is consistent with the results of in situ hybridization studies investigating the presence of the messenger RNA (mRNA) for the two VIP receptor subtypes, where the external muscular layers of the rat stomach showed VIP_2/PACAP receptor mRNA but not VIP_1/PACAP receptor mRNA (Usdin et al., 1994).

[R^{16}] chicken secretin has also been proposed as an agent to discriminate between rat VIP_1/PACAP and VIP_2/PACAP receptors (Gourlet et al., 1997b) because it has a high affinity for the rat VIP_1/PACAP receptor. In view of the absence of a functional VIP_1/PACAP receptor, the relaxant effect of [R^{16}] chicken secretin in the rat gastric fundus might seem surprising. However, in contrast to [K^{15},R^{16},L^{27}]VIP(1-7)/GRF(8-27), which has negligible affinity at secretin receptors, [R^{16}] chicken secretin is a potent agonist at this type of receptor (Gourlet et al., 1997b). The relaxant effect of [R^{16}] chicken secretin in the rat gastric fundus can thus be explained by an interaction with the secretin receptors that are proposed to exist in the rat gastric fundus (Steiner et al., 1993). The potency of porcine secretin also points to an interaction with secretin receptors, because the EC_{50} of porcine secretin corresponds with the concentration of non-radioactive porcine secretin required to provide half-maximal inhibition of saturable binding of [^{125}I]porcine secretin in the rat gastric fundus (Steiner et al., 1993). The maximal relaxant effect obtained with the two secretin peptides was clearly lower than that of VIP and RO25-1553, suggesting that the efficacy of these agonists at the secretin receptors is lower than that of VIP receptor agonists at the VIP_2 receptors. PACAP-(1-27) had a similar maximal effect as VIP and was nearly equipotent with VIP. This has been reported before for the rat gastric fundus (Huang et al., 1993) but contrasts with the results for rat ileal longitudinal muscle, where PACAP-(1-27) was much more potent than VIP (Ekblad and Sundler, 1997). The similar affinity of VIP and PACAP-(1-27) in the rat gastric fundus meets the

criteria of VIP/PACAP non-selective receptors. Still, the presence of a separate PACAP-selective receptor besides the VIP/PACAP receptor cannot be excluded, because the affinity of PACAP-(1-27) at both types of receptors is the same (Christophe, 1993). Furthermore, in the guinea-pig stomach, PACAP-(1-27) is also equipotent with VIP but the presence of a PACAP-specific apamin-sensitive receptor has been proposed in addition to a VIP/PACAP receptor (Katsoulis et al., 1996).

In conclusion, this study suggests the presence of functional VIP_2/PACAP receptors in the longitudinal muscle of the rat gastric fundus.

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References

Christophe, J., 1993. Type I receptors for PACAP (a neuropeptide even more important than VIP?). Biochim. Biophys. Acta 1154, 183–199.

Couvineau, A., Rouyer-Fessard, C., Darmoul, D., Maoret, J.J., Carrero, I., Ogier-Denis, O., Laburthe, M., 1994. Human intestinal VIP receptor: Cloning and functional expression of two cDNA encoding proteins with different N-terminal domains. Biochem. Biophys. Res. Commun. 200, 769–776.

Ekblad, E., Sundler, F., 1997. Distinct receptors mediate pituitary adenylate cyclase-activating peptide- and vasoactive intestinal peptide-induced relaxation of rat ileal longitudinal muscle. Eur. J. Pharmacol. 334, 61–66.

Gourlet, P., De Neef, P., Cnudde, J., Waelbroeck, M., Robberecht, P., 1997a. In vitro properties of a high affinity selective antagonist of the VIP_1 receptor. Peptides 18, 1555–1560.

Gourlet, P., Vandermeers, A., Vertongen, P., Rathé, J., De Neef, P., Cnudde, J., Waelbroeck, M., Robberecht, P., 1997b. Development of high affinity selective VIP_1 receptor agonists. Peptides 18, 1539–1545.

Gourlet, P., Vertongen, P., Vandermeers, A., Vandermeers-Piret, M.C., Rathé, J., De Neef, P., Waelbroeck, M., Robberecht, P., 1997c. The long-acting vasoactive intestinal polypeptide agonist RO25-1553 is highly selective of the VIP_2 receptor subclass. Peptides 18, 403–408.

Huang, M., Shirahase, H., Rorstad, O.P., 1993. Comparative study of vascular relaxation and receptor binding by PACAP and VIP. Peptides 14, 755–762.

Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K., Nagata, S., 1992. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. Neuron 8, 811–819.

Katsoulis, S., Schmidt, W.E., Schwarzhoff, R., Folsch, U.R., Jin, J.-G., Grider, J.R., Makhlouf, G.M., 1996. Inhibitory transmission in guinea pig stomach mediated by distinct receptors for pituitary adenylate cyclase-activating peptide. J. Pharmacol. Exp. Ther. 278, 199–204.

Lefebvre, R.A., Sas, S., Cauvin, A., 1991. Relaxant effect of rat PHI, PHI-Gly and PHV(1-42) in the rat gastric fundus. Peptides 12, 271–274.

Li, C.G., Rand, M.J., 1990. Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. Eur. J. Pharmacol. 191, 303–309.

Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G., Harmar, A.J., 1993. The VIP_2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide. FEBS Lett. 334, 3–8.

O'Donnell, M., Garippa, R.J., Rinaldi, N., Selig, W.M., Simko, B., Renzetti, L., Tannu, S.A., Wasserman, M.A., Welton, A., Bolin, D.R., 1994. RO25-1553: A novel, long-acting vasoactive intestinal peptide agonist: Part I. In vitro and in vivo bronchodilator studies. J. Pharmacol. Exp. Ther. 270, 1282–1288.

Shirahase, H., Coy, D.H., Rorstad, O.P., 1994. Structure–activity relationships for relaxation of smooth muscle by VIP. Peptides 15, 383–385.

Steiner, T.S., Mangel, A.W., McVey, D.C., Vigna, S.R., 1993. Secretin receptors mediating rat forestomach relaxation. Am. J. Physiol. 264, G863–867.

Svoboda, M., Tastenoy, M., Van Rampelbergh, J., Goossens, J.-F., De Neef, P., Waelbroeck, M., Robberecht, P., 1994. Molecular cloning and functional characterization of a human VIP receptor from SUP-T1 lymphoblasts. Biochem. Biophys. Res. Commun. 205, 1617–1624.

Usdin, T.B., Bonner, T.I., Mezey, E., 1994. Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. Endocrinology 135, 2662–2680.