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PYY-preferring receptor in the dorsal vagal complex and its involvement in PYY stimulation of gastric acid secretion in rats

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1 Microinjection of peptide YY (PYY, 7–46 pmol) into the dorsal vagal complex (DVC) stimulated gastric acid secretion in urethane-anaesthetized rats. Using a variety of neuropeptide Y (NPY) and PYY derivatives, we characterized the pharmacological profile of the receptor mediating the acid secretory response to PYY.

2 [Pro^{34}]rat(r)/porcine(p)PYY and [Pro^{34}]human(h)PYY (23–117 pmol), microinjected unilaterally into the DVC resulted in a similar maximal increase in net acid secretion reaching 68 ± 11 and 89 ± 31 μmol 90 min^{-1} respectively.

3 Rat/hNPY and pNPY (47 pmol) microinjected into the DVC induced a similar net gastric acid secretion (27 ± 8 and 23 ± 8 μmol 90 min^{-1} respectively) and a higher dose (116 pmol) tended to reduce the response.

4 Pancreatic polypeptide (PP, 4–46 pmol), [Leu^{31},Pro^{34}]r/hNPY (47 and 117 pmol) and the Y2 selective agonists, hPYY$_{3\text{-}36}$, pNPY$_{5\text{-}36}$ and pNPY$_{13\text{-}36}$ (25–168 pmol) microinjected into the DVC failed to influence basal gastric acid secretion.

5 The rank order of potency of PYY $\geqslant$ [Pro^{34}]r/pPYY = [Pro^{34}]hPYY > r/hNPY = pNPY to stimulate gastric acid secretion upon injection into the DVC and the ineffectiveness of PP, [Leu^{31},Pro^{34}]NPY and C-terminal NPY/PYY fragments suggest that a PYY-preferring receptor subtype may be involved in mediating the stimulating effect.

Keywords: Neuropeptide Y; PYY$_{3\text{-}36}$; [Pro^{34}]PYY; pancreatic polypeptide; Y1 receptor agonists; Y2 receptor agonists

Introduction

Peptide YY (PYY), neuropeptide Y (NPY) and pancreatic polypeptide (PP) are structurally and functionally related endogenous 36-amino acid peptides (Mannon & Taylor, 1994). Recently, PYY$_{3\text{-}36}$ was shown to occur endogenously as a result of cleavage by specific dipeptidyl exopeptidases (Grandt *et al*., 1994). It is now widely accepted that NPY/PYY/PP bind to and activate at least six Y receptor subtypes (Balasubramaniam, 1997). The Y1 subtype exhibits similar affinity for NPY, PYY and [Leu^{31},Pro^{34}]NPY/PYY analogues and poor affinity for the long carboxyl (C)-terminal fragments of NPY/PYY, while the Y2 subtype displays a similar affinity for NPY/PYY and a much higher affinity for the C-terminal fragments than the [Pro^{34}]-substitute analogues of the peptides (Balasubramaniam, 1997). The newest established Y3 subtype does not bind PYY while NPY, [Pro^{34}]NPY, or C-terminal NPY fragments, all displaced [^{125}I]-NPY binding. Therefore the commonly used NPY derivatives [Leu^{31},Pro^{34}]NPY and NPY$_{13\text{-}36}$ have rather poor selectivity for Y1 compared to Y3 and Y2 compared to Y3 receptors respectively (Wahlestedt *et al*., 1992; Gehlert, 1994; Dumont *et al*., 1995; Wan & Lau, 1995). The Y4 subtype (the PP$_1$ receptor) binds PP > PYY $\geqslant$ [Leu^{31},Pro^{34}]NPY > NPY > PP C-terminal fragments (Bard *et al*., 1995; Gehlert *et al*., 1996a; Gregor *et al*., 1996a). The recently cloned Y5 receptor (or atypical Y1) is activated by NPY = PYY $\geqslant$ NPY$_{2\text{-}36}$ = [Leu^{31},Pro^{34}]NPY > NPY$_{3\text{-}36}$ = PYY$_{3\text{-}36}$ > PP (Gerald *et al*., 1996; Hu *et al*., 1996; Weinberg *et al*., 1996). The PYY preferring receptor subtype has a rank order of potency of PYY > NPY > PP and also binds with a low potency to C-terminal fragments or [Leu^{31},Pro^{34}]NPY (Inui *et al*., 1989). Molecular cloning of cDNAs encoding corresponding receptor proteins has been carried out (Bard *et al*., 1995; Nakamura *et al*., 1995; Gehlert *et al*., 1996a,b; Gerald *et al*., 1996; Gregor *et al*., 1996a,b; Hu *et al*., 1996; Weinberg *et al*., 1996) except for the Y3 and PYY-preferring receptor (Kalivas *et al*., 1987; Mannon *et al*., 1993; Gehlert, 1994).

Convergent findings indicate that peptides of the NPY/PYY/PP family act in the medullary dorsal vagal complex (DVC) to influence gastric, pancreatic and gallbladder function (McTigue *et al*., 1993; Chen & Rogers, 1995; Okumura *et al*., 1995; Yang & Taché, 1995; Chen *et al*., 1997; Yoneda *et al*., 1997). However, both gastrointestinal stimulant (McTigue *et al*., 1993; Yang & Taché, 1995; Chen *et al*., 1997; Yoneda *et al*., 1997) and inhibitory (Chen & Rogers, 1995; Okumura *et al*., 1995; Chen *et al*., 1997) responses have been obtained when NPY/PYY/PP peptides were injected into the rat DVC at various doses. The different patterns of gastrointestinal response to NPY/PYY/PP microinjected into the DVC may result from the different affinities of the peptides to the various receptor subtypes (Balasubramaniam, 1997) which are present in the DVC (Leslie *et al*., 1988; Hernandez *et al*., 1994; Dumont *et al*., 1996; Larsen & Kristensen, 1997). One example is somatostatin, which interacts with 5 receptor subtypes (Schlinder *et al*., 1996), showing stimulation or inhibition of gastric acid secretion depending upon the receptor subtypes activated at specific brain sites injected (Martinez *et al*., 1995; 1996; Yoneda & Taché, 1995). Recently, based on the use of [Leu^{31},Pro^{34}]NPY and NPY$_{3\text{-}36}$ microinjected into the DVC, the Y1 and Y2 receptors were proposed to induce a stimulating and inhibitory effect, respectively, on gastric motility in anaesthetized rats (Chen *et al*., 1997).

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We previously showed that PYY stimulated basal gastric acid secretion when microinjected into the DVC at doses ranging from 7 to 46 pmol in urethane-anaesthetized rats (Yang & Taché, 1995). The receptor subtype involved in PYY action is still unknown. In the present study, we used several peptides having different affinities on various receptor subtypes, including NPY (Y3/Y5/Y1/Y2 agonist), PP (Y4), [Pro^{34}]PYY (Y1/PYY-preferring), PYY_{3-36} (Y2 > PYY-preferring), [Leu^{31}, Pro^{34}]NPY (Y1/Y3), NPY_{5-36} and NPY_{13-36} (Y2/Y3) (Balasubramaniam, 1997), to investigate the receptor subtype(s) involved in PYY microinjected into the DVC-induced stimulation of basal gastric acid secretion in urethane-anaesthetized rats. Since there may be differences in the potency of NPY-related peptides obtained from different origins (rat, human and bovine) as has been found for PP (McTigue *et al*., 1995; Gerald *et al*., 1996; Lundell *et al*., 1996), we also compared the potency of PYY/NPY-related peptides derived from rat with those from man or pig.

Methods

Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–330 g were housed 5 animals per cage under conditions of controlled temperature (22 ± 1°C) and illumination (06 h to 18 h). Animals were fed with Purina Laboratory Chow *ad libitum* (Diet No. 5001, Ralston Purina, St. Louis, MO) and tap water. Animals were deprived of food but not water for 24 h before each experiment. All experiments were performed in rats under urethane anaesthesia (1.25 g kg^{-1}, i.p.).

Peptides

The following peptides were used: rat/human (r/h)NPY, porcine (p)NPY, $pNPY_{5-36}$, $pNPY_{13-36}$ and p[Leu^{31}, Pro^{34}]NPY (Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA), [Pro^{34}]r/pPYY, [Pro^{34}]hPYY, $r/pPYY_{3-36}$, and $hPYY_{3-36}$ (Peptides Synthesis Core Facility, UCLA, Los Angeles, CA), and rPP (Peninsula Laboratories, Belmont, CA). Peptides were dissolved either in saline or in 0.1 M phosphate buffer (pH = 7.4). Since both vehicles microinjected into the DVC did not influence basal gastric acid secretion in urethane-anaesthetized rats and peptides dissolved in the both vehicles had the same potency, the results obtained from experiments with saline and 0.1 M phosphate buffer as vehicles were pooled together.

Measurement of gastric acid secretion

In urethane-anaesthetized rats, the oesophagus was ligated at the cervical level and a laparotomy was performed. The pylorus was ligated and a double-lumen gastric cannula was implanted into the forestomach. Gastric acid secretion was measured every 10 min by flushing the gastric lumen through the double-lumen gastric cannula with two 5 ml boluses of saline at room temperature and one 5 ml bolus of air at the end of each 10 min period. Acid output was determined by titration of the flushed perfusate with 0.01 N NaOH with an autotitrator (TTT80 Titrator, Radiometer, Copenhagen).

Microinjections

After implantation of the gastric cannula, urethane-anaesthetized rats were positioned on a Kopf model 900 stereotaxic instrument. The obex region of the dorsal medulla was exposed by resecting the dorsal cervical musculature and removing the occipital skull plate. After brain surgery and a 30 min stabilization period, basal gastric acid secretions were collected for 20 min. Then a glass micropipette (50–70 μm diam) filled with vehicle or test peptides was positioned into the right DVC. Unilateral microinjection of vehicle or peptides were delivered in 50 nl by pressure ejection over 1 min with a 1 μl Hamilton syringe attached to a glass micropipette with a PE-50 polyethylene catheter filled with water. The micropipette was left in place for another 3 min, then withdrawn and the animal was removed from the stereotaxic apparatus. The coordinates used for microinjection into the DVC were as previously specified (Yang *et al*., 1993): V, ventral from the surface of the brainstem: 0.6; A, anterior from the caudal tip of the area postrema: 0.5; L, lateral from the midline: 0.5 (numbers are in mm). At the end of the experiment, rats were killed by decapitation, and brains were removed and fixed in a 10% formalin, 20% sucrose solution for at least 2 days. Frozen sections were sliced at 30 μm, mounted and stained with toluidine blue. Histological sections were examined microscopically. The location of the microinjection sites was identified as the point of termination of the cannula track and marked on plates reproduced from the atlas of Paxinos & Watson (1996).

Statistics

Results are expressed as means ± s.e.mean. The net gastric acid output was calculated by subtracting the average basal value of acid output from each postinjection value. Comparisons between two groups were calculated by Student's *t* test and multiple group comparisons were performed by ANOVA followed by Duncan's contrast. A $P < 0.05$ was considered statistically significant.

Results

Effect of NPY and PP microinjected into the DVC on gastric acid secretion

Microinjection of saline or 0.1 M phosphate buffer (50 nl) into the DVC did not influence basal gastric acid secretion which ranged between 2–4 μmol 10 min^{-1} in urethane-anaesthetized rats. The net increase in the acid output during the 90 min period after microinjection of vehicles was 4 ± 3 μmol 90 min^{-1} ($n = 9$).

Rat/hNPY and pNPY microinjected unilaterally into the DVC (both at 47 and 116 pmol) significantly increased gastric acid secretion with a peak secretion occurring at 20 min (r/hNPY) and 40 min (pNPY) post microinjection (Figure 1). The net acid responses (μmol 90 min^{-1}) observed upon microinjection into the DVC of NPY at 47 and 116 pmol were similar and did not show a dose-response relationship (27 ± 8 and 21 ± 7, respectively, for r/hNPY and 23 ± 8 and 15 ± 3, respectively, for pNPY) (Figure 2). The dose of 23 pmol microinjected into the DVC did not significantly influence gastric acid secretion (Figures 1 and 2). Comparison of gastric acid output over time at the various doses showed no difference in potency between r/hNPY and pNPY (*t* test: 23 pmol, $P = 0.557$; 47 pmol, $P = 0.810$; 116 pmol, $P = 0.356$; Figures 1 and 2).

Rat PP microinjected into the DVC at 4–46 pmol did not influence basal gastric acid secretion in urethane-anaesthetized rats (Table 1).

Effect of [Pro^{34}]-PYY and -NPY derivatives microinjected into the DVC on gastric acid secretion

Unilateral microinjection of [Pro^{34}]r/pPYY (23, 47 and 117 pmol) dose-dependently increased gastric acid secretion to 21 ± 5, 39 ± 7 and 68 ± 11 μmol 90 min^{-1}, respectively, in urethane-anaesthetized rats ($F(3,30)=16.35$, $P<0.001$) (Figures 2 and 3). Time course studies showed that the peak acid response occurred at 30 min post injection of [Pro^{34}] r/pPYY (23, 47 and 117 pmol) with values reaching 9 ± 1, 11 ± 1 and 19 ± 4 μmol 10 min^{-1} respectively (Figure 3a); thereafter gastric acid secretion gradually returned to basal levels but was still significantly higher than the vehicle injected group at 70 min after microinjection of [Pro^{34}] r/pPYY at 47 and 117 pmol (Figure 3a). Likewise, [Pro^{34}]hPYY (23, 47 and 117 pmol) microinjected into the DVC increased gastric acid secretion to 27 ± 10, 52 ± 17 and 89 ± 31 μmol 90 min^{-1}, respectively, ($F(3,25)=4.79$, $P<0.01$) (Figures 2 and 3b). Peak responses reached 10 ± 2, 14 ± 8 and 22 ± 6 μmol 10 min^{-1}, respectively, at 30 to 40 min post injection; gastric acid secretion values were back to basal levels at 70 min post injection of 23 or 47 pmol [Pro^{34}]hPYY, while after the 117 pmol dose, gastric acid secretion was still significantly increased (12 ± 4 μmol 10 min^{-1}, $F(3,25)=6.85$, $P<0.005$) (Figure 3b). The acid responses to [Pro^{34}]r/pPYY and [Pro^{34}]hPYY were significantly more potent than those of r/hNPY and pNPY ($F(3,26)=6.85$, $P<0.001$) (Figure 2).

Porcine [Leu^{31},Pro^{34}]NPY at 47 ($n=17$) or 119 ($n=7$) pmol microinjected into the DVC did not influence basal gastric acid secretion compared with the vehicle group ($F(2,30)=1.59$, $P=0.220$, net increase in gastric acid secretion) (Figure 2).

When [Pro^{34}]r/pPYY, [Pro^{34}]hPYY, r/hNPY or pNPY (46 to 119 pmol) were microinjected into sites nearby but outside the DVC (the nucleus hypoglossal and the ventral medullary reticular nucleus), a weaker and delayed effect in 2 rats and no effect in 7 rats were observed (data not shown).

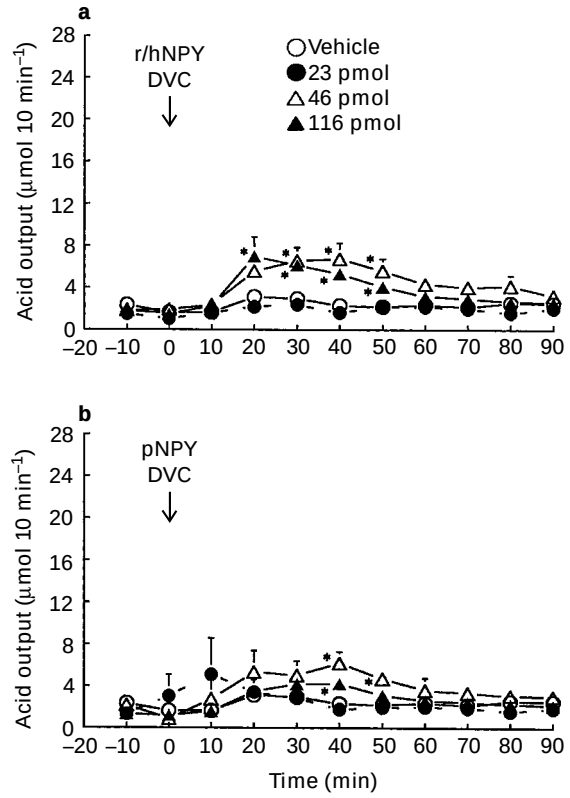

Figure 1 Time course of r/hNPY (a) and pNPY (b) microinjected into the dorsal vagal complex (DVC)-induced stimulation of gastric acid secretion in urethane-anaesthetized rats. Doses injected were 0 (vehicle, $n=9$), 23 ($n=4$ for r/hNPY and 5 for pNPY), 46 ($n=13$ for r/hNPY and 5 for pNPY) and 116 pmol ($n=7$ for r/pNPY and 11 for pNPY). Values are means and vertical lines show s.e.mean; values without s.e. mean are <0.7 μmol 10 min^{-1}. *$P<0.05$ compared with the value of the respective vehicle group.

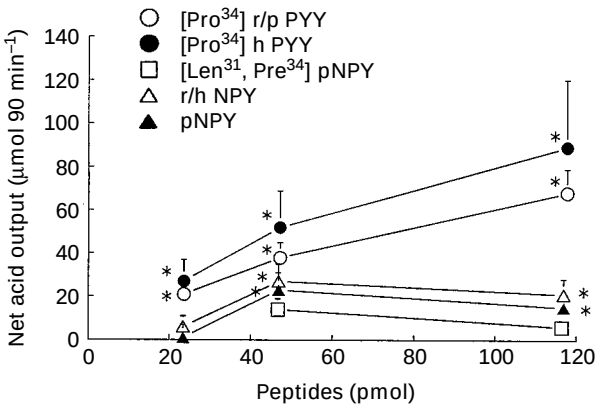

Figure 2 Net increase in gastric acid output for the 90 min after microinjection into the DVC of [Pro^{34}]r/pPYY, [Pro^{34}]hPYY, [Leu^{31},Pro^{34}]pNPY, r/hNPY and pNPY. Each point represents the mean and vertical lines show s.e.mean of 4 to 17 rats. *$P<0.05$ compared with the effect of vehicle microinjected into the DVC.

Effect of C-terminal PYY and NPY fragments microinjected into the DVC on gastric acid secretion

Rat/pPYY$_{3\text{-}36}$ (25, 50 and 125 pmol), hPYY$_{3\text{-}36}$ (25, 49, 124 pmol), pNPY$_{5\text{-}36}$ (53 pmol) and pNPY$_{13\text{-}36}$ (67 or 168 pmol) did not significantly stimulate gastric acid secretion (Table 1).

Table 1 Influence of PYY/NPY-related peptides microinjected into the DVC on gastric acid secretion in urethane-anaesthetized rats

Treatment[a]	*Dose* (pmol)	n	*Net gastic acid output* (μmol 90 min^{-1})[b]
Vehicle	–	9	4 ± 3
r/pPYY$_{3-36}$	25	5	4 ± 2
	50	6	13 ± 7
	124	6	2 ± 4
hPYY$_{3-36}$	25	4	3 ± 5
	50	4	7 ± 5
	124	4	2 ± 7
pNPY$_{5-36}$	53	8	0 ± 3
pNPY$_{13-36}$	67	6	7 ± 3
	168	6	10 ± 2
rPP	4	3	6 ± 4
	7	3	9 ± 5
	11	4	5 ± 5
	46	6	6 ± 4

[a]After basal secretion rats were injected unilaterally into the DVC with vehicle (50 nl) or peptide and gastric acid output was monitored for the following 90 min. [b]Mean$\pm$s.e.mean of number of rats indicated (n).

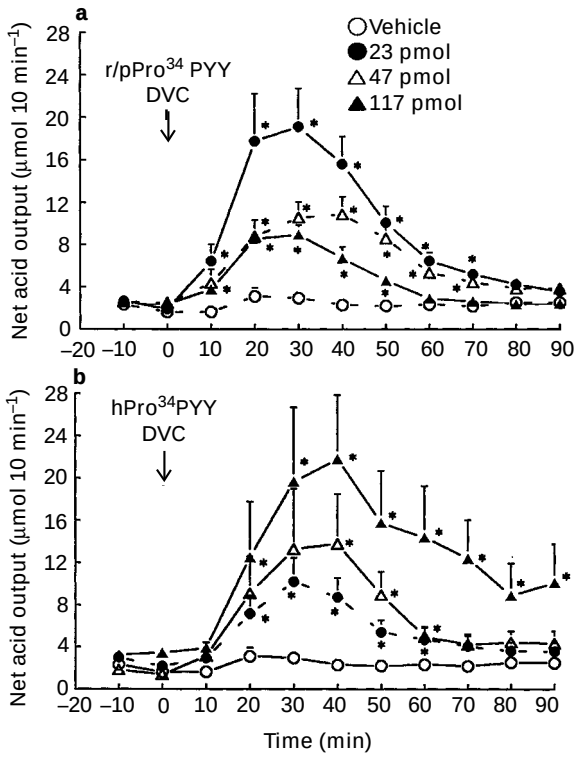

Figure 3 Time course and dose-related stimulation of gastric acid secretion induced by [Pro^{34}]r/pPYY (a) and [Pro^{34}]hPYY (b) microinjected into the dorsal vagal complex (DVC) in urethane-anaesthetized rats. Doses injected were 0 (vehicle, $n=9$), 23 ($n=6$ for both [Pro^{34}]r/pPYY and [Pro^{34}]hPYY), 47 ($n=13$ for [Pro^{34}]r/pPYY and 8 for [Pro^{34}]hPYY), and 117 pmol ($n=6$ for both [Pro^{34}]r/pPYY and [Pro^{34}]hPYY). Values are means and vertical lines show s.e.mean; values without s.e.mean are <0.7 μmol 10 min^{-1}. *$P<0.05$ compared with the values of the respective vehicle group.

Discussion

In the present study we showed that r/hNPY and pNPY microinjected into the DVC at 47 or 116 pmol were equally effective at inducing a similar increase in gastric acid secretion while 23 pmol had no effect in urethane-anaesthetized rats. Recent studies showed that NPY (7–30 pmol) microinjected into the DVC increased bile secretion through vagal pathways (Yoneda *et al*., 1997), as well as basal gastric motility at 4 pmol dose (Chen *et al*., 1997) in anaesthetized rats. Other studies also indicate that intracerebroventricular or intracisternal injection of NPY results in a vagally mediated stimulation of gastric motility, acid and pepsin secretion as well as pancreatic exocrine and bile secretion in urethane-anaesthetized rats and awake dogs, which may represent an integrated NPY-dependent mechanism linking the onset of feeding behaviour and the cephalic phase of digestive control (Matsuda *et al*., 1991; Farouk *et al*., 1992; Geoghegan *et al*., 1993; 1994). Another member of the NPY family, PYY microinjected unilaterally into the DVC at 2–46 pmol stimulated basal gastric acid secretion and motility through vagal pathways in urethane anaesthetized rats (Yang & Taché, 1995; Chen *et al*., 1997).

The definition of the Y receptor subtype(s) involved in NPY action still mainly rests on the order of potency of peptide analogues action at these receptor subtypes (Balasubramaniam, 1997). In the present study, r/hNPY microinjected into the DVC at 23 pmol did not stimulate gastric acid secretion. By contrast, our previous studies, performed under similar conditions, showed that r/pPYY microinjected into the DVC at this dose induces a net increase of 40 ± 6 μmol 90 min^{-1} (Yang & Taché, 1995). Likewise, the present data showed that [Pro^{34}]r/pPYY microinjected into the DVC at 117 pmol induced a three fold higher acid response than r/hNPY at the same dose. Take together these findings indicate that PYY is more potent than NPY. This difference is not related to the heterologous origin of the peptide since PYY and NPY of both rat and human origins were tested for each peptide (Yang & Taché, 1995, present study). In addition, no species difference between the potencies of r/hNPY and pNPY or [Pro^{34}]hPYY and [Pro^{34}]r/pPYY were observed.

Although [Pro^{34}]PYY stimulated gastric acid secretion, PYY and NPY microinjected into the DVC do not seem to be mediated through interaction with the classical Y1 and Y5 subtypes since [Leu^{31},Pro^{34}]NPY, which is a preferential Y1/Y5 agonist (Fuhlendorff *et al*., 1990; Gehlert, 1994; Wan & Lau, 1995), microinjected into the DVC had no effect at similar doses (47–119 pmol). Intracerebroventricular injection of [Leu^{31},Pro^{34}]NPY (2–23 pmol) was shown to inhibit vagally stimulated gastric acid secretion in conscious rats (Penner *et al*., 1993) or to have no effect on basal acid secretion in dogs (Geoghegan *et al*., 1994). By contrast, [Leu^{31},Pro^{34}]NPY microinjected into the DVC at 4 pmol stimulated basal gastric motility in urethane-anaesthetized rats (Chen *et al*., 1997). Therefore the central action of [Leu^{31},Pro^{34}]NPY on gastric function appears to vary with the brain site of injection, species and gastric parameters investigated.

The Y2 receptor also does not seem to be involved in mediating the PYY/NPY stimulating action. This inference is based on the observations that several C-terminal fragments, which either are selective for the Y2 receptor subtype such as r/pPYY$_{3\text{-}36}$ and hPYY$_{3\text{-}36}$, or interact with both Y2 and Y3 receptors such as pNPY$_{5\text{-}36}$ and pNPY$_{13\text{-}36}$ (Grundemar *et al*., 1991; Wahlestedt *et al*., 1992; Gehlert, 1994; Dumont *et al*., 1995), when tested at doses up to 10 fold the minimally effective dose of PYY, did not significantly stimulate gastric acid secretion in urethane-anaesthetized rats. Likewise, the Y2/Y3 receptor agonist, NPY$_{13\text{-}36}$ microinjected into the DVC did not influence basal gastric motility, but inhibited vagally stimulated gastric contractions in urethane-anaesthetized rats (Chen *et al*., 1997). The possible inhibitory action of Y2 agonists cannot be detected in the present study due to the already low basal acid secretion under the condition of urethane anaesthesia (Yang *et al*., 1990). In contrast, in dogs, NPY$_{13\text{-}36}$ injected intracerebroventricularly stimulates basal acid secretion (Geoghegan *et al*., 1994). Such a discrepancy may be related to either brain sites or species differences.

The lower potency of NPY to stimulate acid secretion compared with that of PYY does not support the view that Y3 receptor subtype mediates the stimulating effect, since NPY has a higher affinity on the pharmacologically defined Y3 receptor (NPY preferring subtype) compared to PYY (Wahlestedt & Reis, 1993). It is also unlikely that the stimulation of acid secretion induced by PYY/NPY is mediated by Y4 subtype, to which PP has a higher affinity (Bard *et al*., 1995; Gregor *et al*., 1996a,b). In the present study, rPP (4–46 pmol) microinjected into the DVC did not influence basal gastric acid secretion in urethane-anaesthetized rats. In addition, [Leu^{31},Pro^{34}]pNPY which displays a similar potency as rPP on the rat Y4 (PP$_1$) receptors (Gregor *et al*., 1996b; Gehlert *et al*., 1997) did not influence basal acid secretion. Also, *in vitro* studies showed that r/pPYY and pNPY, unlike rPP, do not bind to the rPP receptors (Lundell *et al*., 1996), while in our study pNPY microinjected into the DVC stimulated gastric acid secretion. However, the lack of stimulation of acid secretion in response to DVC microinjec-

tion of PP contrasts with previous results indicating that rPP microinjected into the DVC at low doses (0.4–4 pmol) increased basal and stimulated gastric acid secretion in urethane-anaesthetized rats (McTigue *et al*., 1993). Such a discrepancy may be related to differences in rat strains (Long Evans versus Sprague Dawley), experimental conditions (such as the use of dexamethasone) or concentration of peptide injected.

The rank order of potency of PYY $\geqslant$ [Pro^{34}]PYY > NPY at stimuling basal gastric acid secretion in urethane-anaesthetized rats upon DVC microinjection at doses of 7–119 pmol and the lack of the effect of [Leu^{31},Pro^{34}]NPY, $PYY_{3\text{-}36}$, $NPY_{5\text{-}36}$, $NPY_{13\text{-}36}$ and PP under similar conditions may be indicative of the peptides action at the putative PYY-preferring receptor subtype. This is supported by binding studies showing the presence of a PYY-preferring receptor subtype in the DVC (Leslie *et al*., 1988; Whitcomb *et al*., 1993; Hernandez *et al*., 1994). Although this receptor subtype has not yet been cloned, pharmacological characterization showed a rank order of potency of PYY > NPY > C-terminal fragments > > [Leu^{31},Pro^{34}]NPY for this receptor binding (Inui *et al*., 1989; 1992; Castan *et al*., 1992; Mannon *et al*., 1993; Balasubramaniam, 1997). However, it seems that the PYY-preferring receptor mediating an acid stimulating effect in the present study differs from the one defined in peripheral tissues. In the intestine and the kidney, the PYY-preferring receptor resembles more the conventional Y2 receptor subtype in its ability to bind to C-terminal fragments of PYY (Voisin *et al*., 1996; Balasubramaniam, 1997).

Specific binding for Y1, Y2 and Y4 receptors have also been shown to be present in the DVC by binding or *in situ* hybridization studies (Dumont *et al*., 1996; Gustafson *et al*., 1997; Larsen & Kristensen, 1997). It is likely that the difference in gastric responses observed on microinjection of the members of the NPY/PYY/PP family depend upon their affinity at receptor subtypes and possible interactions at multiple receptors depending upon the dose used. In particular, recent functional and electrophysiological evidence indicates that the Y2 subtype exerts an inhibitory effect on DVC neurones (Chen & Rogers, 1997). In the present study, the lack of a dose-response relationship obtained with r/hNPY or pNPY at 46 and 116 pmol and the ineffectiveness of $PYY_{3\text{-}36}$ may reflect an additional interaction of the peptides with the Y2 receptor or other subtypes, which reduces their stimulant action. Based on this complex situation, it is difficult to make a firm identification of receptor types by only using rank order of potency of agonists. The use of more selective agonists and specific antagonists for these receptor subtypes is needed to provide definite conclusions as to which subtypes contribute to the stimulation versus inhibition of gastric acid secretion, upon microinjection into the DVC.

In summary, microinjection of PYY/NPY analogues into the DVC showed a rank order of potency PYY $\geqslant$ [Pro^{34}]-PYY > NPY for stimuling gastric acid secretion, while [Leu^{31},Pro^{34}]NPY, $PYY_{3\text{-}36}$, $NPY_{5\text{-}36}$ and $NPY_{13\text{-}36}$ had no effect in urethane-anaesthetized rats. No difference in potency was observed between rat, porcine and human origins of NPY or [Pro^{34}]PYY. These results suggest that the PYY stimulating effect on gastric acid secretion, when microinjected into the DVC, may involve a PYY-preferring receptor subtype in rats. Further proof will require the development of receptor agonists and antagonists that are selective for the various NPY/PYY/PP receptor subtypes.

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