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Reversal by NPY, PYY and 3-36 molecular forms of NPY and PYY of intracisternal CRF-induced inhibition of gastric acid secretion in rats

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1 The Y receptor subtype involved in the antagonism by neuropeptide Y (NPY) of intracisternal corticotropin-releasing factor (CRF)-induced inhibition of gastric acid secretion was studied in urethane-anaesthetized rats by use of peptides with various selectivity for Y_1, Y_2 and Y_3 subtypes: NPY, a Y_1, Y_2 and Y_3 agonist, peptide YY (PYY), a Y_1 and Y_2 agonist, [Leu^{31}, Pro^{34}]-NPY, a Y_1 and Y_3 agonist, NPY(3-36) and PYY(3-36), highly selective Y_2 agonists and NPY(13-36) a weak Y_2 and Y_3 agonist. Peptides were injected intracisternally 10 min before intracisternal injection of CRF (10 μg) and gastric acid secretion was measured by the flushed technique for 1 h before and 2 h after pentagastrin- (10 μg kg^{-1} h^{-1}, i.v.) infusion which started 10 min after CRF injection.

2 Intracisternal injection of CRF (10 μg) inhibited by 56% gastric acid secretion stimulated by pentagastrin. Intracisternal injection of NPY and PYY (0.1-0.5 μg) did not influence the acid response to pentagastrin but blocked CRF-induced inhibition of pentagastrin-stimulated acid secretion. NPY(3-36) (0.5 μg) and PYY(3-36) (0.25 and 0.5 μg) also completely blocked the inhibitory action of CRF on pentagastrin-stimulated acid secretion.

3 [Leu^{31}, Pro^{34}]-NPY (0.5-5 μg) and NPY(13-36) (0.5-5 μg) injected intracisternally did not modify gastric acid secretion induced by pentagastrin or CRF inhibitory action.

4 The sigma antagonist, BMY 14802 (1 mg kg^{-1}, s.c.) did not influence the acid response to pentagastrin but prevented the antagonism by PYY(3-36) (0.5 μg) of the CRF antisecretory effect.

5 These results show that both PYY and NPY and the 3-36 forms of PYY and NPY are equipotent in blocking central CRF-induced inhibition of pentagastrin-stimulated gastric acid secretion. The structure-activity profile suggests a mediation through Y_2 receptor subtype and the involvement of sigma binding sites.

Keywords: Neuropeptide Y (NPY); peptide YY (PYY); sigma receptor; corticotropin-releasing factor (CRF); gastric acid secretion; central nervous system; pentagastrin

Introduction

Functional studies have provided evidence that NPY acts in the brain to modulate the central action of exogenous or endogenous corticotropin-releasing factor (CRF). Neuropeptide Y (NPY) injected into the lateral ventricle inhibited central CRF- and conditioned fear-induced stimulation of colonic motility (Jiménez & Buéno, 1990; Junien *et al.*, 1991; Gué *et al.*, 1992a). Likewise, NPY injected intracisternally or into the paraventricular nucleus of the hypothalamus also prevented intracisternal or intrahypothalamic CRF-induced decrease in gastric acid secretion (Gue *et al.*, 1992b).

The term sigma receptor was first established in 1987 to describe naloxone-insensitive receptors with a higher affinity for (+)- than (−)-benzomorphan, such as SKF-10047 (+)-N-allylnormetazocine) (Walker *et al.*, 1990). There is *in vivo* evidence for an interaction of NPY and sigma receptors (Wan & Lau, 1995). In particular, sigma agonists mimicked the inhibitory effect of NPY on central CRF-induced alterations of gastrointestinal function (Junien *et al.*, 1991; Gué *et al.*, 1992a, b; Yoneda *et al.*, 1992). In addition, the putative sigma antagonist, BMY 14802 (Su *et al.*, 1988; Taylor *et al.*, 1991), blocked the antagonist action of NPY on CRF- and/or stress-induced stimulation of colonic motor function and inhibition of gastric acid secretion (Gué *et al.*, 1992a,b). In an *in vivo* binding study, NPY and PYY displaced in a concentration-dependent manner the specific binding of the prototypical sigma radioligand, [^{3}H]-(+)-SKF-10047 in the mouse hippocampal formation (Bouchard *et al.*, 1993).

It is now widely accepted on the basis of functional and ligand binding studies that NPY binds to and activates at least three different receptor subtypes designated Y_1, Y_2 and Y_3 (Wahlestedt *et al.*, 1991; Gehlert, 1994; Wan & Lau, 1995). The Y_1 receptor exhibits similar affinity for NPY, PYY and [Leu^{31}, Pro^{34}]-NPY analogues and poor affinity for the C-terminal fragments of NPY/PYY while the Y_2 receptor displays affinity for NPY/PYY and C-terminal fragments of the peptides but not to the Y_1-selective agonist, [Leu^{31}, Pro^{34}]-NPY (Gehlert, 1994). By contrast, the newest established Y_3 subtype has weak efficacy for PYY while NPY, [Leu^{31}, Pro^{34}]-NPY, or C-terminal NPY fragments all displaced [^{125}I]-NPY (Wahlestedt *et al.*, 1991; Gehlert, 1994; Wan & Lau, 1995). Autoradiographic binding assays demonstrated that Y_2 binding sites are more broadly and abundantly represented than Y_1 receptors in the rat brain (Aicher *et al.*, 1991; Dumont *et al.*, 1993; Larsen *et al.*, 1993). The identity of the receptor subtype for NPY in the brain that mediates the antagonism of CRF action remains to be established. The originally developed selective agonists for the Y_1, [Leu^{31}, Pro^{34}]-NPY, and Y_2, NPY(13-36), receptor subtypes are now known to display similar and rather high potency for the Y_3 receptor subtype as well (Sheikh *et al.*, 1989; Fuhlendorff *et al.*, 1990; Wahlestedt *et al.*, 1991; Dumont *et al.*, 1994; Gehlert, 1994; Wan & Lau, 1995). By contrast, PYY, which shares approximately 70% homology with NPY, acti-

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vates Y_1 and Y_2 receptors while being devoid of affinity for the Y_3 subtype (Sheikh *et al.*, 1989; Grundemar *et al.*, 1991; Wahlestedt *et al.*, 1991; Gehlert, 1994; Dumont *et al.*, 1995). Moreover, the 3-36 truncated forms of PYY and NPY bind selectively and with high affinity to the Y_2 receptor subtype and are devoid of affinity for the Y_1 or Y_3 receptors (Grandt *et al.*, 1992a,c; Dumont *et al.*, 1994, 1995). Biochemical studies indicate that the 3-36 long C-terminal fragments of PYY and NPY are produced in the brain or peripheral tissues of various species through enzymatic cleavage suggesting that these peptides are the selective endogenous agonists for the Y_2 receptor (Grandt *et al.*, 1992a,b,c; 1994a,b).

In the present study, we determined the selectivity of various NPY/PYY agonists for the previously reported blockade by intracisternal NPY of intracisternal CRF-induced inhibition of gastric acid secretion in urethane-anaesthetized rats (Gué *et al.*, 1992b). We compared the capacity of [Leu^{31}, Pro^{34}]-NPY, PYY and NPY/PYY truncated C-terminal fragments to block the inhibition of gastric acid secretion induced by intracisternal injection of CRF. In addition, the role of sigma receptors in mediating selective NPY subtype agonist-induced inhibition of CRF action was investigated with a sigma antagonist, BMY 14802 (Su *et al.*, 1988; Taylor *et al.*, 1991).

Methods

Animals

Male Sprague-Dawley albino rats (Simonsen Laboratories, Gilroy, CA, U.S.A.) weighing 250-350 g were housed 8-10 animals per cage under conditions of controlled temperature (22±1°C) and illumination (06 h 00 min to 18 h 00 min). Animals were maintained on Purina Laboratory Chow *ad libitum* (diet no 5001; Ralston-Purina, St Louis, MO, U.S.A.) and tap water. Animals were deprived of food for 18 h but given free access to water up to the beginning of the study. All experiments were performed in rats under urethane anaesthesia (1.25 g kg^{-1}, i.p.)

Drugs and treatments

The following peptides were used: porcine NPY, porcine PYY, porcine [Leu^{31}, Pro^{34}]-NPY, porcine NPY(3-36), porcine PYY(3-36), porcine NPY(13-36), and rat CRF. Peptides were synthesized as previously described (Reymond *et al.*, 1992; Grandt *et al.*, 1994a). Peptides in powder form were dissolved in saline before administration. Intracisternal injection (5 µl) was performed by direct puncture of the occipital membrane with a 50 µl Hamilton syringe in rats maintained in stereotaxic apparatus. BMY 14802 [α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidyl)-1-piperazine butanol hydrochloride: Bristol-Meyers Squib Co., Wallingford, CT, U.S.A.] was dissolved in 0.9% saline and injected subcutaneously in 0.2 ml. Pentagastrin (Peptavlon, Ayerst Lab, New York, NY, U.S.A.) was diluted in saline and infused through the femoral vein at a rate of 1.1 ml h^{-1}.

Measurement of acid secretion

A cannula was inserted into the trachea of urethane-anaesthetized rats and a laparotomy was performed. The pylorus was ligated and double-lumen cannula was acutely inserted through a small incision into the forestomach. Care was taken not to occlude or damage major vessels. The abdominal musculature and the skin were closed with silk sutures. Thirty minutes after surgery, gastric acid output was monitored for 40 min before and 140 min after intracisternal injection of peptides or vehicle. Acid output was determined by flushing the gastric lumen every 10 min with two 5 ml boluses of saline and one bolus of air at the end of each 10 min period. The flushed perfusate was titrated with 0.1N NaOH to pH 7.0 on an automatic titrator (Radiometer, Copenhagen).

Experimental protocol

In a first series of experiments, after basal measurement of gastric acid secretion, saline (5 µl), NPY (0.01, 0.1 or 0.5 µg 5 µl^{-1}), PYY (0.01, 0.1 or 0.5 µg 5 µl^{-1}), NPY(3-36) (0.5 µg 5 µl^{-1}) or PYY(3-36) (0.1, 0,25 or 0.5 µg 5 µl^{-1}) was injected intracisternally, and 10 min later, a second intracisternal injection of either saline (5 µl) or CRF (10 µg 5 µl^{-1}) was made. In a second series of experiments, saline (5 µl), [Leu^{31}, Pro^{34}]-NPY or NPY(13-36) (each peptide at 0.5 or 5 µg 5 µl^{-1}) was injected intracisternally 10 min before injection of saline (5 µl) or CRF (10 µg 5 µl^{-1}). In the last study, BMY 14802 (1 mg kg^{-1}) was injected subcutaneously 20 min before the first intracisternal injection of either saline or PYY(3-36) (0.5 µg 5 µl^{-1}), and 10 min later, CRF (10 µg 5 µl^{-1}) was injected intracisternally. The choice of BMY 14802 dose and route of administration was based on previous studies showing antagonism of centrally administered sigma agonists and NPY (Gué *et al.*, 1992a,b). In all experiments, 10 min after the second intracisternal injection, pentagastrin (10 µg kg^{-1} h^{-1}) was infused through the femoral vein for 2 h to provide a maximal stimulation of gastric acid secretion.

Statistics

Gastric acid output, expressed in µmol 90 min^{-1}, represents the integrated acid response during the plateau from 30 min to 120 min period following pentagastrin infusion. Data were analysed by Denied's procedure for multiple comparisons after a two-way ANOVA. $P<0.05$ was considered statistically significant.

Results

Effects of intracisternal injection of NPY/PYY and related peptides on CRF action

In urethane anaesthetized rats, basal gastric acid secretion is low (3.1±0.7 µmol 10 min^{-1}). Intravenous infusion of pentagastrin (10 µg kg^{-1} h^{-1}) in saline-injected rats stimulated acid secretion. A plateau was reached 30 min after the beginning of the infusion (20.8±2.4 µmol 10 min^{-1}) (Figure 1). Intracisternal injection of CRF (10 µg), 10 min before pentagastrin infusion, resulted in a significant ($P<0.05$) inhibition

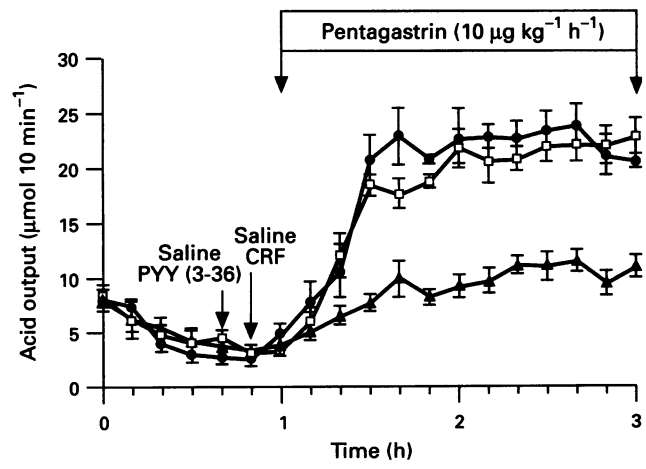

Figure 1 Time course of the action of PYY(3-36) on intracisternal CRF-induced inhibition of pentagastrin-stimulated acid secretion in urethane-anaesthetized rats. Rats were injected intracisternally at 10 min apart with: saline+saline (●), saline+CRF. 10 µg, (▲), PYY(3-36), 0.5 µg, +CRF, 10 µg, (□). Ten minutes later, pentagastrin was infused and this continued throughout the experiment. Each point represents mean±s.e.mean of 6 rats per group.

which was maintained throughout the experimental period (Figure 1). In CRF-treated rats, the mean gastric acid output (μmol 90 min^{-1} for the 30 to 120 min period after the beginning of pentagastrin infusion) was 100.2±6.8 (n=6) compared with 226.2±12.2 (n=6) in saline-treated group.

Intracisternal injection of NPY (0.01, 0.1 and 0.5 μg) and PYY (0.01, 0.1 and 0.5 μg) followed within 10 min by another intracisternal injection of saline did not modify pentagastrin-stimulated gastric acid secretion (Figure 2a and b). Gastric acid values (μmol 90 min^{-1}) were 197.9±12.3 for saline, and 208.9±3.7, 205.8±3.1, and 189.5±4.1 for NPY at 0.01, 0.1 and 0.5 μg respectively (Figure 2a) and 218.5±4.3 for saline and 209.1±3.6, 208.4±5.3, and 201.5±4.8 for PYY at 0.01, 0.1 and 0.5 μg respectively (Figure 2b). Both NPY and PYY injected intracisternally at 0.1 and 0.5 μg completely prevented intracisternal CRF-induced inhibition of gastric acid secretion while having no effect at 0.01 μg (Figure 2).

Gastric acid response to pentagastrin infusion (μmol 90 min^{-1} for the 30–120 min period after the start of pentagastrin infusion) was not influenced by intracisternal injection of NPY(3–36) (0.5 μg, n=5): 201.4±3.7 or PYY(3–36) (0.1, 0.25 and 0.5 μg): 199.4±15.4 (n=5), 190.0±6.2 (n=5) and 194.9±3.1 (n=6) respectively (Figure 3 and data not shown).

However, at 0.5 μg dose, the 3–36 fragments of PYY and NPY abolished the inhibitory effect of intracisternal CRF (Figures 1,3). PYY(3–36) at 0.25 μg also prevented the antisecretory action of CRF while 0.1 μg had no effect (Figure 3). By contrast, intracisternal injection of [Leu^{31}, Pro^{34}]-NPY (0.5 and 5 μg) and NPY(13–36) (0.5 and 5 μg) did not influence pentagastrin-stimulated gastric acid secretion or the antisecretory effect of intracisternal CRF (Table 1).

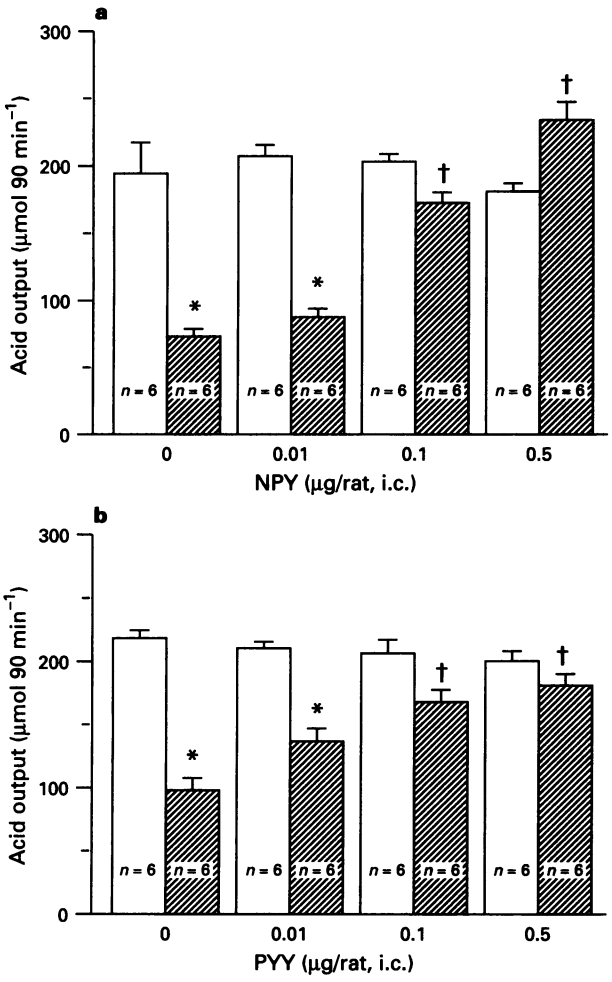

Figure 2 Dose-related antagonism of intracisternal CRF-induced inhibition of gastric acid secretion by NPY (a) and PYY (b) in urethane-anaesthetized rats. Intracisternal injection of saline, or various doses of NPY or PYY were followed 10 min later by intracisternal injection of saline (open columns) or CRF, 10 μg, (hatched columns). All animals were infused for 2 h with pentagastrin (10 μg kg^{-1} h^{-1}) 10 min after the second intracisternal injection. Each column represents the mean with s.e.mean of the number of rats indicated at the bottom of each column. Gastric acid output was calculated during the 30 to 120 min collection period after the beginning of pentagastrin perfusion. *P<0.05 compared to respective saline-treated groups; †P<0.05 compared with CRF alone.

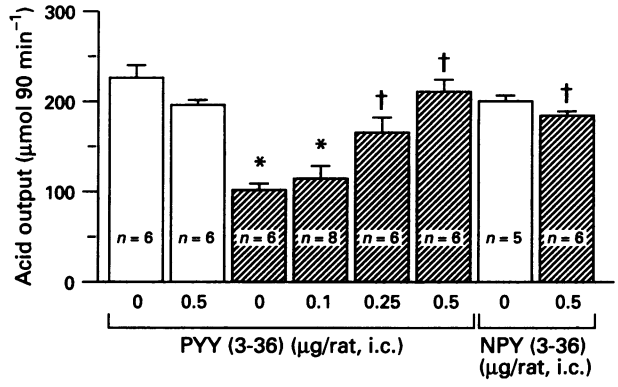

Figure 3 Dose-related antagonism of CRF-induced inhibition of gastric acid secretion by PYY(3–36) and NPY(3–36) in urethane-anaesthetized rats. Intracisternal injection of saline, various doses of PYY(3–36) or NPY(3–36) (0.5 μg) were followed, 10 min later, by intracisternal injection of saline, (open columns) or CRF, 10 μg, (hatched columns). All animals were infused for 2 h with pentagastrin (10 μg kg^{-1} h^{-1}) 10 min after the second intracisternal injection. For other details, see legend to Figure 2. *P<0.05 compared to saline+saline-treated group; †P<0.05 compared with saline+CRF-treated group.

Table 1 Effects of intracisternal injection of NPY analogues on intracisternal CRF-induced inhibition of gastric acid secretion in urethane-anaesthetized rats

Treatment[a]	*Dose* (μg, i.c.)	*Gastric acid output* (μmol 90 min^{-1})[b] *Saline* (i.c.)	*CRF* (10 μg, i.c.)
Saline		226.2±12.2	100.1±6.8*
[Leu^{31}, Pro^{34}]-NPY	0.5	200.4±12.8	122.3±8.6*
	5	193.8±5.5	101.5±13.3*
NPY (13–36)	0.5	198.0±3.4	93.3±9.8*
	5	209.1±5.6	122.6±11.3*

[a]Fasted rats under urethane anaesthesia were injected intracisternally with saline, NPY analogue or C-terminal fragment and, 10 min later, with either saline or CRF (10 μg). Ten minutes later, pentagastrin (10 μg kg^{-1} h^{-1}) was infused through the femoral vein. [b]Mean±s.e. mean of the 90 min period starting 30 min after pentagastrin infusion. *P<0.05 compared with corresponding saline-treated control group.

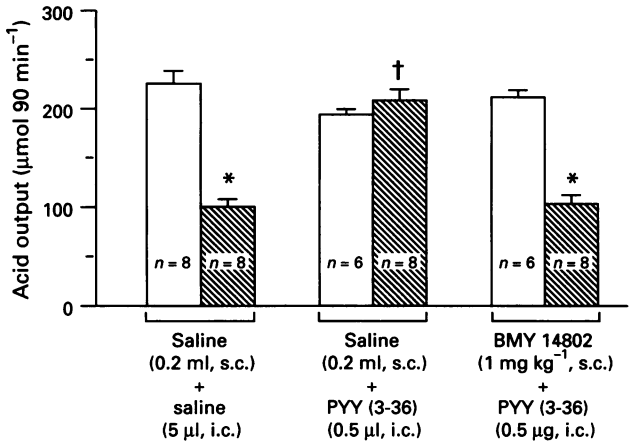

Figure 4 Influence of BMY 14802, a sigma antagonist, on PYY(3–36)-induced blockade of CRF action on gastric acid secretion in urethane-anaesthetized rats. BMY 14802 (1 mg kg^{-1}) or saline was injected subcutaneously 20 min before intracisternal injection of PYY(3–36). Ten minutes after the PYY(3–36) injection, saline (open columns) or CRF, 10 μg, (hatched columns) was injected intracisternally. All animals were infused for 2 h with pentagastrin (10 μg kg^{-1} h^{-1}) 10 min after the second intracisternal injection. For other details, see legend to Figure 2. *$P<0.05$ compared with corresponding non CRF-treated group; †$P<0.05$ compared with saline + saline + CRF-treated group.

Effects of BMY 14802 on PYY(3–36)-induced blocking of CRF antisecretory effect

We previously showed that BMY 14802 (1 mg kg^{-1}, s.c.) tested under the same conditions did not modify the acid response to pentagastrin or CRF-induced inhibition of gastric acid secretion (Gué *et al.*, 1992b). However, BMY 14802 (at the same single dose s.c.) abolished intracisternal PYY(3–36) (0.5 μg)-induced blockade of CRF action (Figure 4). In BMY 14802 (1 mg kg^{-1}) and PYY(3–36)-pretreated group, gastric acid output (μmol 90 min^{-1}) was 102.0 ± 9.1, a value which was not different from CRF-treatment alone (100.1 ± 6.8) (Figure 4).

Discussion

In the present study, we sought to characterize the Y receptor subtype that mediates intracisternal NPY-induced blockade of CRF action on gastric function. As previously reported (Taché *et al.*, 1983; Gué *et al.*, 1992b), intracisternal injection of CRF, 10 min before pentagastrin injection, inhibited gastric acid secretion stimulated by pentagastrin in urethane-anaesthetized rats. The antisecretory effect of CRF injected intracisternally represents a central nervous system action of the peptide mediated by alterations of the autonomic outflow to the stomach (Taché *et al.*, 1983; Gunion & Taché, 1987; Gué *et al.*, 1992b). In agreement with previous observations (Gué *et al.*, 1992b), NPY injected intracisternally 10 min before CRF, antagonized CRF-induced inhibition of pentagastrin-stimulated acid secretion. The present study also showed that NPY action can be reproduced by intracisternal injection of PYY. Dose-response studies indicate that PYY and NPY are equipotent in antagonizing CRF action. The finding that PYY had a similar effect to NPY is of interest with respect to the classification of the Y receptor mediating NPY action. This would exclude an interaction with the Y_3 receptor subtype since PYY is inactive in this receptor class (Grundemar *et al.*, 1991; Wahlestedt *et al.*, 1991; Gehlert, 1994; Wan & Lau, 1995). Y_1 and Y_2 receptors displayed a similar affinity for NPY and PYY (Wahlestedt *et al.*, 1991; Gehlert, 1994; Wan & Lau, 1995) suggesting that NPY acts through either of these two classes of receptors.

The Y_1 receptor subtype does not seem to be involved in mediating an NPY antagonist effect on CRF action. This inference is based on the observation that [Leu^{31}, Pro^{34}]-NPY, a preferential Y_1/Y_3 agonist (Fuhlendorff *et al.*, 1990; Gehlert, 1994; Wan & Lau, 1995) did not influence CRF action even when injected intracisternally at a dose 50 fold higher than the maximal effective dose of NPY or PYY. In addition, the mapping of the Y_1 NPY subtype receptor distribution in the rat brain using quantitative *in vitro* autoradiography or *in situ* hybridization histochemistry shows high concentrations of Y_1 subtype in the cortical areas rather than in hypothalamic or medullary sites (Aicher *et al.*, 1991; Dumont *et al.*, 1993) where NPY and CRF are known to act to influence gastric function (Gunion & Taché, 1987; Heymann-Mönnikes *et al.*, 1991; Gué *et al.*, 1992b).

The selectivity profile observed for NPY/PYY is most consistent with an action at the Y_2 receptor subtype. Intracisternal injection of the 3–36 molecular form of either NPY or PYY exhibited similar potency to NPY/PYY in preventing CRF-induced inhibition of gastric acid secretion. Recent evidence indicates that PYY(3–36) and NPY(3–36), which do not bind to Y_1 or Y_3 receptors (Grandt *et al.*, 1992a, b, c; Dumont *et al.*, 1994), are selective and high affinity Y_2 agonists (Grandt *et al.*, 1992a,c; Dumont *et al.*, 1994; 1995; Wan & Lau, 1995). These studies provide the first evidence of a biological action of PYY(3–36)/NPY(3–36) injected into the cerebrospinal fluid. Upon peripheral administration, PYY(3–36) has been reported to be as potent as PYY in inhibiting pancreatic secretion in dogs (Grandt *et al.*, 1992c). NPY(3–36) and PYY(3–36) occur endogenously in the brain or peripheral tissues as a result of cleavage by specific dipeptidyl exopeptidase (Grandt *et al.*, 1992a,b,c; 1994a). By contrast, we found that NPY(13–36), the C-terminal fragment most often used to differentiate Y_2 receptors (Gehlert, 1994), did not mimic the effect of NPY or PYY or the 3–36 forms of NPY and PYY even when tested at a 10 fold higher dose. The lack of biological action of NPY(13–36) compared with NPY(3–36) and PYY(3–36) may be related to differences in affinity and selectivity of the two fragments for the Y_2 receptors (Gehlert, 1994). In porcine splenic membrane, PYY(3–36) and NPY(3–36) displayed a similar affinity for Y_2 receptor subtypes as NPY or PYY while NPY(13–36) was found to have a 10 fold (or more) lower affinity than NPY(3–36) (Grandt, unpublished observation). Alternatively, these functional studies could bring additional support to the concept of NPY receptor heterogeneity. Other observations indicate that [^{125}I]-PYY-labelled sites in the rat brain are resistant to inhibition by both [Pro^{34}]-NPY and NPY(13–36), but not to the long carboxyl-terminal fragment of NPY(2–36) (Dumont *et al.*, 1993).

The antagonist effect of NPY, PYY and the 3–36 forms of NPY and PYY injected intracisternally occurred at doses (0.1–0.5 μg) that did not influence basal and pentagastrin-stimulated gastric acid secretion. Previous studies also showed that intracisternal injection of PYY (0.2 μg) or NPY (0.01–2 μg) did not influence gastric acid secretion under basal or pentagastrin-stimulated conditions in urethane anaesthetized rats (Matsuda *et al.*, 1991; Gué *et al.*, 1992b; Yang & Taché, 1995). By contrast, higher intracisternal doses or direct microinjection of the peptides into the dorsal motor nucleus of the vagus induced a vagal-dependent stimulatory effect on gastric secretion in urethane-anaesthetized rats (Matsuda *et al.*, 1991; Yang & Taché, 1995).

The central NPY-induced antagonistic effect of CRF action on gastrointestinal function was previously reported to be reversed by the sigma antagonist, BMY 14802 (Gué *et al.*, 1992a, b). In the present study, pretreatment with the sigma receptor antagonist, BMY-14802, (Su *et al.*, 1988; Taylor *et al.*, 1991) resulted in the blockade of intracisternal PYY(3–36)-induced prevention of CRF antisecretory effect. Likewise, in other studies, NPY-induced potentiation of N-methyl-D-aspartate (NMDA) activation of rat CA3 dorsal hippocampus pyramidal neurones was abolished by BMY-14802 (Monnet *et al.*, 1992a,b). In addition, *in vivo* binding studies indicate that NPY

and PYY inhibited labelling by the prototypical sigma ligand, [^{3}H]-(+)SKF-10047 in the mouse hippocampal formation suggesting an interaction between NPY and sigma receptors (Bouchard *et al.*, 1993). These observations have raised the possibility that NPY action may be mediated through an atypical NPY/sigma receptor for which both PYY and NPY and the 3-36 truncated form demonstrate high affinities in the rat brain (Monnet *et al.*, 1992a; Bouchard *et al.*, 1993; Dumont *et al.*, 1993). Alternatively, the activation of the Y_2 receptor subtype by NPY/PYY and the highly selective and high affinity Y_2 agonists NPY(3-36)/PYY(3-36) can induce the release of a yet to be characterized endogenous sigma ligand mediating the action of NPY.

The brain sites at which NPY injected intracisternally acts to influence the action of CRF are still to be established. We previously showed that CRF and PYY act in the dorsal motor nucleus of the vagus to influence gastric function (Heymann-Mönnikes *et al.*, 1991; Yang & Taché, 1995). Autoradiographic studies indicate that NPY and PYY receptors are localized in the area postrema and dorsal vagal complex (Nakajima *et al.*, 1986; Leslie *et al.*, 1988; Hernandez *et al.*, 1994) where the Y_2 receptor represents the major subtype (Aicher *et al.*, 1991; Wan & Lau, 1995). Recent studies also showed that [^{125}I]-PYY(3-36) binding is identified almost exclusively in the nucleus tractus solitarius (Whitcomb, 1995). In agreement with these binding studies, several actions of NPY in the nucleus tractus solitarius to influence cardiorespiratory and visceroendocrine controls have been assigned to an action on a Y_2 receptor subtype (Ergene *et al.*, 1993).

In summary, intracisternal injection of NPY-evoked blockade of central CRF-induced inhibition of pentagastrin-stimulated gastric acid secretion is mediated by activation of the Y_2 receptor. This is supported by the equipotency of PYY and NPY and the Y_2 selective agonists, NPY(3-36) and PYY(3-36) while the selective Y_1/Y_3 agonist, [Leu^{31}, Pro^{34}]-NPY is inactive. In addition, the blockade of the biological action of PYY(3-36) by the putative sigma antagonist, BMY 14802, suggests an interaction between Y_2 and sigma receptors through sites and mechanisms that remain to be elucidated.

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