Comparison of the inhibitory effects of PYY(3-36) and PYY(1-36) on gastric emptying in rats

Prasanth K. Chelikani, Alvin C. Haver, and Roger D. Reidelberger

Veterans Affairs–Nebraska Western Iowa Health Care System, Omaha, NE 68105; and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska 68178

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Comparison of the inhibitory effects of PYY(3-36) and PYY(1-36) on gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol 287: R1064–R1070, 2004. First published July 8, 2004; doi:10.1152/ajpregu.00376.2004.—We compared the effects of the two molecular forms of the brain-gut peptide YY (PYY), PYY(1-36) and PYY(3-36), on gastric emptying. Unanesthetized rats received 20-min intravenous infusions of rat PYY(1-36) (0, 1.7, 5, 17, 50, 100, 170 pmol·kg⁻¹·min⁻¹) and rat PYY(3-36) (0, 0.5, 1.7, 5, 17, 50, 100, 170 pmol·kg⁻¹·min⁻¹), either alone or combined, and gastric emptying of saline was measured during the last 10 min of infusion. For comparison, human PYY(3-36) was administered at 0, 17, and 50 pmol·kg⁻¹·min⁻¹. Gastric emptying was decreased by 11, 24, 26, and 38% in response to 17, 50, 100, and 170 pmol·kg⁻¹·min⁻¹ of rat PYY(1-36); by 10, 26, 41, 53, and 57% in response to 5, 17, 50, 100, and 170 pmol·kg⁻¹·min⁻¹ of rat PYY(3-36); and by 35 and 53% in response to 17 and 50 pmol·kg⁻¹·min⁻¹ of human PYY(3-36), respectively. Estimated ED₅₀ s were 470 and 37 pmol·kg⁻¹·min⁻¹ for rat PYY(1-36) and PYY(3-36), respectively. In general, within an experiment, coadministration of PYY(1-36) and PYY(3-36) inhibited gastric emptying by an amount that was comparable to that produced when either peptide was given alone. We conclude that 1 intravenous infusion of PYY(1-36) and PYY(3-6) each produces a dose-dependent inhibition of gastric emptying in rats, 2) PYY(3-36) is an order of magnitude more potent than PYY(1-36) in inhibiting gastric emptying, 3) human PYY(3-36) and rat PYY(3-36) inhibit gastric emptying similarly, and 4) PYY(1-36) and PYY(3-36) do not appear to interact in an additive or synergistic manner to inhibit gastric emptying.

intravenous infusion; dose response; interaction

Address for correspondence: R. D. Reidelberger, Nebraska Western Iowa Health Care System (151), 4101 Woolworth Ave., Omaha, NE 68105 (E-mail: roger.reidelberger@med.va.gov).

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MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Charles River, Kingston, NY) weighing 300–485 g were housed individually in hanging wire-mesh cages in a room with controlled temperature (19–21°C) and a 12:12-h light-dark cycle (lights off at 1700 h). The animals were provided pelleted rat chow (Lambiet, 5001 Rodent diet; PMI Nutrition International, Brentwood, MO) and water ad libitum. The Animal Studies Subcommittee of the Omaha Veterans Affairs Medical Center approved the experimental protocol. Animal experimentation was conducted in conformity with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society (1).

Peptides

Rat PYY(1-36) [rPYY(1-36)] was purchased from Phoenix Pharmaceuticals (Belmont, CA), and human PYY(3-36) [hPYY(3-36)] was purchased from Bachem (Torrance, CA). Rat PYY(3-36) [rPYY(3-36)] was synthesized and purified as follows.

Synthesis and Purification of PYY(3-36)

5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxycarboxylic acid linker attached to a polyethylene-graft polystyrene support (PAL-PEG-PS) resin for solid-phase peptide synthesis was purchased from Applied Biosystems (Foster City, CA). O-(benzotriazol-1-yl)-N,N,N',N'-tetramethylurea (HBTU), 1-hydroxybenzotriazole (HOBr), and protected amino acid derivatives were purchased from Chem-Impex International (Wood Dale, IL). Dimethylformamide (DMF), piperidine, trifluoroacetic acid (TFA), acetonitrile, and diethyl ether were purchased from Fisher Scientific (Pittsburgh, PA). Thiouanisole, anisole, and ethanedithiol were purchased from Aldrich (St. Louis, MO).

rPYY(3-36) was assembled by continuous flow solid-phase methodology on the PAL-PEG-PS support at a 0.1 mmol scale using a Pioneer Peptide Synthesizer (Foster City, CA). α-Amino groups were protected with the fluorenylmethoxycarbonyl (Fmoc) group and side chains were protected with the trityl group for Gln, His, and Asn; tert-butyloxycarbonyl (Boc) group for Ser, Asp, Glu, Thr and Tyr; tert-butyloxycarbonyl group for Lys; and pentamethyldihydrobenzofuransulfonyl group for Arg. After removal of the Fmoc group from the resin with 20% piperidine in DMF (vol/vol), HBTU- and HOBr-activated Fmoc-Tyr(Bu)-OH in DMF were coupled to the resin. This process was repeated with each amino acid derivative. After assembly, the peptide-resin was washed with diethyl ether and dried under vacuum. Cleavage of peptide from the resin and removal of side chain protecting groups were accomplished in a 10-ml mixture of TFA, thioanisole, ethanedithiol, and anisole (9.0:0.5:0.3:0.2, vol/vol). After being stirred for 2 h at room temperature, the peptide was precipitated by the addition of cold diethyl ether. The peptide/resin was filtered. The peptide was dissolved in TFA, reprecipitated in cold diethyl ether, and isolated by filtration and lyophilized.

Purification of rPYY(3-36) was accomplished by reverse-phase high-performance liquid chromatography on a Waters (Milford, MA) model 600 HPLC system. The crude peptide was dissolved in 78% solvent A (0.1% TFA/water) and 22% solvent B (0.095% TFA/acetonic acid) and subjected to a gradient of 22% B to 34% B over 50 min on a semipreparative VyDAC (Hesperia, CA) C18 column (10 × 250 mm). Flow rate was 4 ml/min, and the peptide was detected by UV absorbance at 230 nm. Fractions containing the pure peptide were collected and lyophilized. Proof of structure was provided by electrospray mass spectrometry.

Surgical Procedures

The procedures for implantation of jugular vein catheters for peptide infusions have been described previously (48). The catheters were kept patent by flushing with 0.2 ml of 50% dextrose on alternate days and plugged with stainless steel wire. The rats were also implanted with gastric cannulas using established procedures (43). The gastric cannulas were used for instilling saline containing the nonabsorbable marker phenol red and for collecting the gastric contents. The animals were allowed at least 1 wk to recover from surgery and adapted to restraint in Bollman cages before being subjected to experimentation. All gastric emptying studies were conducted on rats that were food deprived for at least 18 h but with free access to water. At the end of each experiment, the patency of the jugular vein catheters was determined by intravenous infusion of 0.2 ml of the short-acting anesthetic Propofol (Abbott Laboratories). The catheters were considered patent if the rats lost consciousness immediately on injection of the anesthetic; only data from such propofo-positive rats were included in statistical analyses.

Experiments

Effects of intravenous infusion of rPYY(1-36) on gastric emptying. On experimental days, the fasted rats were restrained in Bollman cages, gastric cannulas were opened, and residual gastric contents were flushed with warm water. The rats were then infused intravenously for 20 min with a single dose of rPYY(1-36) (0, 1.7, 5, 17, 50, 100, or 170 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA). Ten minutes after the onset of infusion, 5 ml of 0.9% saline containing 60 mg/l phenol red was instilled into the stomach. Ten minutes later, gastric contents were collected, the stomach was flushed with 5 ml of 0.9% saline, the flushings were combined with the gastric contents, and the total volume collected was measured gravimetrically. The concentration of phenol red in the instilled and recovered fluid was determined spectrophotometrically, and the volume of saline emptied during the 10-min period was calculated. Each rat (n = 12) received each dose of rPYY(1-36) in random order at ~48-h intervals. During the intervening days between experiments, the rats had free access to rat chow and a liquid diet (1.5 kcal/ml Ensure Plus, Abbott Laboratories).

Effects of intravenous infusions of rPYY(3-36) and hPYY(3-36) on gastric emptying. With the use of identical procedures as described above for rPYY(1-36), gastric emptying was measured in rats (n = 12) receiving intravenous doses of rPYY(3-36) (0, 0.5, 1.7, 5, 17, 50, 100, and 170 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA) in random order at ~48-h intervals. For determining the effects of the human homologue of rPYY(3-36), gastric emptying was determined in o-positive rats (n = 12) receiving intravenous doses of hPYY(3-36) (0, 17, and 50 pmol·kg⁻¹·min⁻¹ in 0.15 M NaCl, 0.1% BSA) in random order at ~48-h intervals.

Effects of coadministration of rPYY(1-36) and rPYY(3-36) on gastric emptying. The experimental procedures were essentially the same as described above for rPYY(1-36) and rPYY(3-36). Five experiments were performed to determine the effects of coadministration of different dose combinations of rPYY(1-36) and rPYY(3-36) on volume of saline emptied from the stomach. In one such experiment, rats (n = 12) received in a randomized order, at ~48-h intervals, intravenous infusions of vehicle (0.15 M NaCl, 0.1% BSA) and (in pmol·kg⁻¹·min⁻¹) 1.7 of rPYY(1-36), 1.7 of rPYY(3-36), and 1.7 of rPYY(1-36) combined with 1.7 of rPYY(3-36). In the other experiments of identical design, rats received dose combinations (in pmol·kg⁻¹·min⁻¹) of 17 of rPYY(1-36) and 5 of rPYY(3-36), 50 of rPYY(1-36) and 17 of rPYY(3-6), 170 of rPYY(1-36) and 5 of rPYY(3-36), and 170 of rPYY(1-36) and 17 of rPYY(3-36).

Statistical Analysis

Values are presented as group means ± SE. Data from each of the experiments determining the dose-response effects of rPYY(1-36), rPYY(3-36), and hPYY(3-36) on gastric emptying were analyzed separately by one-way repeated-measures ANOVA. Data from each of the experiments determining the effects of coadministration of
rPYY(1-36) and rPYY(3-36) on gastric emptying were analyzed by a two-way repeated-measures ANOVA. Planned comparisons of treatment means were evaluated by paired t-tests; differences were considered significant if \( P < 0.05 \). A general nonlinear, least-squares curve fitting method was used to fit the dose-response data for the effects of rPYY(1-36) and rPYY(3-36) on gastric emptying to the following sigmoidal dose-response equation: \( Y = a + (d - a)/(1 + 10^{(X - X_0)/s}) \); where \( Y \) is the response, \( X \) is the logarithm of the administered dose, \( a \) is the response for 0 dose, \( d \) is the response for infinite dose, \( c \) is the logarithm of the dose producing a response halfway between \( a \) and \( d \) (ED\(_{50}\)), and \( b \) denotes the steepness of the dose-response curve. The ED\(_{50}\) for rPYY(1-36) and rPYY(3-36) were compared as described previously (33).

**RESULTS**

**Effects of Intravenous Infusion of rPYY(1-36) on Gastric Emptying**

Intravenous infusion of rPYY(1-36) dose dependently reduced the volume of saline emptied from the stomach (Fig. 1; \( F_{5,60} = 6.15, P < 0.0001 \)). Compared with the vehicle-infused control group, the volume of saline emptied from the stomach was significantly decreased by 11.24, 26, and 38% in response to 17, 50, 100, and 170 pmol kg\(^{-1}\)min\(^{-1}\) of rPYY(1-36), respectively. Gastric emptying of saline was not significantly (\( P > 0.05 \)) affected by rPYY(1-36) at 1.7 (3.58 ± 0.15 ml) or 5 (3.43 ± 0.18 ml) pmol kg\(^{-1}\)min\(^{-1}\) compared with the control group (3.64 ± 0.20 ml). Nonlinear regression fitting of the dose-response data to the sigmoidal equation gave the following relationship between rPYY(1-36) and gastric emptying: volume emptied (ml) = \( 3.9/[1 + 10^{(2.67 - X - 0.60)/0.26}] \) and volume emptied (ml) with rPYY(3-36) = \( 2.54/[1 + 10^{(2.35 - X - 0.92)/0.96}] + 0.96 \), where \( X \) denotes logarithm of peptide dose. The estimated ED\(_{50}\) were 470 pmol kg\(^{-1}\)min\(^{-1}\), and their inhibition of gastric emptying, were 17 (11%) and 170 (38%), respectively.

**Effects of Intravenous Infusions of rPYY(3-36) and hPYY(3-36) on Gastric Emptying**

Intravenous infusion of rPYY(3-36) dose dependently reduced the volume of saline emptied from the stomach (Fig. 1; \( F_{6,70} = 19.2, P < 0.0001 \)). Compared with the vehicle-infused control group, the volume of saline emptied from the stomach was significantly decreased by 10, 26, 41, 53, and 57% in response to 5, 17, 50, 100, and 170 pmol kg\(^{-1}\)min\(^{-1}\) of rPYY(3-36), respectively. Gastric emptying of saline was not significantly (\( P > 0.05 \)) affected by rPYY(3-36) at 0.5 (3.38 ± 0.22 ml) or 1.7 (3.27 ± 0.16 ml) pmol kg\(^{-1}\)min\(^{-1}\) compared with the vehicle-infused control group (3.48 ± 0.14 ml).

Nonlinear regression fitting of the dose-response data to the sigmoidal equation gave the following relationship between rPYY(3-36) and gastric emptying: volume emptied (ml) = \( 3.7/[1 + 10^{(1.57 - X - 0.92)/0.76}] + 0.96 \), \( r^2 = 0.76 \) (Fig. 1). The estimated ED\(_{50}\) was 37 pmol kg\(^{-1}\)min\(^{-1}\), which was an order of magnitude lower than that for rPYY(1-36) (\( F_{1,158} = 42.9, P < 0.0001 \)). The minimal and maximal effective doses of rPYY(3-36), and their inhibition of gastric emptying, were 5 pmol kg\(^{-1}\)min\(^{-1}\) (10%) and 170 pmol kg\(^{-1}\)min\(^{-1}\) (57%), respectively.

Intravenous infusion of rPYY(3-36) dose dependently reduced the volume of saline emptied from the stomach (Fig. 1; \( F_{2,30} = 30.7, P < 0.0001 \)). Compared with the vehicle-infused control group, the volume of saline emptied from the stomach was significantly decreased by 35 and 53% in response to 17 and 50 pmol kg\(^{-1}\)min\(^{-1}\) of hPYY(3-36), respectively. The same doses of rPYY(3-36) produced comparable reductions in gastric emptying.

**Effects of Coadministration of rPYY(1-36) and rPYY(3-36) on Gastric Emptying**

Intravenous infusion of subthreshold doses of rPYY(1-36) and rPYY(3-36), at 1.7 pmol kg\(^{-1}\)min\(^{-1}\) each, had no significant effect on gastric emptying of saline when given alone or combined (Fig. 2A). The main effects for rPYY(1-36) (\( F_{1,9} = 2.25, P > 0.05 \)) and rPYY(3-36) (\( F_{1,9} = 0.07, P > 0.05 \)), and the interaction between them (\( F_{1,9} = 0.05, P > 0.05 \)), were not significant.

Intravenous infusion of near-threshold doses of rPYY(1-36) at 17 pmol kg\(^{-1}\)min\(^{-1}\) and rPYY(3-36) at 5 pmol kg\(^{-1}\)min\(^{-1}\), alone and in combination, inhibited gastric emptying (Fig. 2B). The main effects for rPYY(1-36) (\( F_{1,11} = 4.66, P < 0.05 \)) and rPYY(3-36) (\( F_{1,11} = 8.47, P < 0.05 \)) were significant, but the interaction between them was not significant (\( F_{1,11} = 0.53, P > 0.05 \)). Relative to the vehicle-treated group, gastric emptying was significantly inhibited by 11% in response to rPYY(3-36) alone (\( P < 0.05 \)) and 26% in response to coadministration of rPYY(1-36) and rPYY(3-36) (\( P < 0.05 \)). The response to rPYY(1-36) alone was not quite significant (\( P = 0.06 \)). The response to

Fig. 1. Gastric emptying responses to intravenous infusions of rat peptide YY(1-36) [rPYY(1-36)], rat PYY(3-36) [rPYY(3-36)], and human PYY(3-36) [h PYY(3-36)] in 11 rats each. Different doses of each peptide or vehicle (0.1% BSA/saline) were infused intravenously for 20 min in rats food deprived for 18 h. At 10 min after the onset of infusion, 5 ml of saline containing phenol red was instilled intragastrically, followed by collection of gastric contents 10 min later. Individual curves for rPYY(1-36) and rPYY(3-36) represent nonlinear regression fitting of data to a sigmoidal dose-response equation. Resulting best-fit equations were volume emptied (ml) with rPYY(1-36) = \( 3.9/[1 + 10^{(2.67 - X - 0.60)/0.26}] \) and volume emptied (ml) with rPYY(3-36) = \( 2.54/[1 + 10^{(2.35 - X - 0.92)/0.96}] + 0.96 \), where \( X \) denotes logarithm of peptide dose. The estimated ED\(_{50}\) were 470 and 37 pmol kg\(^{-1}\)min\(^{-1}\) for rPYY(1-36) and rPYY(3-36), respectively. \( *P < 0.05, \hat{P} < 0.01, \ddot{P} < 0.001 \), compared with the vehicle-treated group for each peptide.
coadministration of rPYY(1-36) and rPYY(3-36) was not different from the response to rPYY(3-36) alone.

Intravenous infusion of suprathreshold doses of rPYY(1-36) at 50 pmol·kg⁻¹·min⁻¹ and rPYY(3-36) at 17 pmol·kg⁻¹·min⁻¹, alone and in combination, significantly inhibited gastric emptying (Fig. 2C). The main effects for rPYY(1-36) ($F_{1,11} = 5.93, P < 0.05$) and rPYY(3-36) ($F_{1,11} = 26.67, P < 0.001$) as well as the interaction between them ($F_{1,11} = 11.24, P < 0.01$) were significant. Compared with the vehicle-treated group, gastric emptying was inhibited by 25% in response to rPYY(1-36) alone.
**DISCUSSION**

We demonstrate here that intravenous infusion of rPYY(1-36) and rPYY(3-36) each produces a dose-dependent inhibition of gastric emptying in rats. Minimal effective doses were 17 and 5 pmol·kg⁻¹·min⁻¹, ED₅₀ were 470 and 37 pmol·kg⁻¹·min⁻¹, and maximal effective doses were 170 pmol·kg⁻¹·min⁻¹ for each inhibiting gastric emptying by 38 and 57%, respectively. Thus rPYY(3-36) is an order of magnitude more potent than rPYY(1-36) in reducing gastric emptying in rats. Our results also suggest that hPYY(3-36) and hPYY(3-36), which differ by two amino acids at positions 3 and 18, inhibit gastric emptying in rats with a similar potency and efficacy. We have used the same experimental model to determine the dose-dependent effects of CCK-8, amylin, calcitonin (CT), salmon CT (sCT), CT-gene-related peptide (CGRP), and adrenomedullin (ADM) on gastric emptying (41, 42). Our results suggest an order of potency of sCT > amylin > CCK-8 = PYY(3-36) > ADM = hPYY(1-36) > CT (estimated ED₅₀ are 1, 3, 35, 37, 130, 160, 470, and 730 pmol·kg⁻¹·min⁻¹, respectively).

In rats, intravenous PYY(1-36) infusion at doses from 0.83 to 2 pmol·kg⁻¹·min⁻¹ appear to increase plasma PYY by an amount comparable to that produced by a mixed-nutrient liquid meal (26, 46). In the present study, intravenous infusion of PYY(1-36) at 1.7 and 5 pmol·kg⁻¹·min⁻¹ had no effect on gastric emptying; the minimal effective dose was 17 pmol·kg⁻¹·min⁻¹. Together, these results suggest that postprandial levels of PYY(1-36) are not sufficient to inhibit gastric emptying in rats. The intravenous doses of PYY(3-36) that reproduce meal-induced increases in plasma PYY(3-36) have not been determined for rats. Thus it is unknown whether postprandial plasma levels of PYY(3-36) are sufficient to inhibit gastric emptying. It also remains to be determined whether circulating PYY(1-36) or PYY(3-36) interact with other factors to inhibit gastric emptying, or whether these peptides act by neuromodulation or paracrine mechanisms, rather than endocrine mechanisms, to inhibit gastric emptying. Raybould et al. (40) provided evidence suggesting that PYY is not an essential mediator of the inhibitory effect of intestinal lipid on gastric emptying in rats. Intraperitoneal injection of PYY antiserum blocked the inhibitory effect of intraperitoneal injection of PYY(1-36) on gastric emptying but had no effect on gastric emptying responses to lipid administration into the proximal or distal small intestine. However, the extent to which the PYY antiserum bound PYY(3-36) was not determined.

Our results indicate that PYY(3-36) is an order of magnitude more potent than PYY(1-36) in reducing gastric emptying in rats. Possible reasons for this difference in potency include differences in plasma clearance rates, access to target tissues (e.g., brain uptake), and receptor binding properties of the two PYY analogs. PYY(1-36) and PYY(3-36) appear to have similar plasma half-lives of 8 to 12 min in dogs (21, 31, 39). Plasma half-lives of PYY(1-36) and PYY(3-36) in rats, and the relative blood-brain barrier permeabilities of each peptide, remain to be determined. The enzyme dipeptidyl-peptidase IV (DPP IV) cleaves the NH₂-terminal Tyr-Pro of PYY(1-36) to yield PYY(3-36) (35). DPP IV is located on the endothelial cells of blood vessels as a soluble enzyme in plasma and in a number of other tissues (34). The extent to which PYY(1-36) is converted to PYY(3-36) on entering the circulation has not been determined. Our results suggest that the effects of PYY(1-36) on gastric emptying are not solely mediated by its conversion to PYY(3-36); otherwise, coadministration of PYY(1-36) and PYY(3-36) should have produced an additive effect on gastric emptying in the present study, which it did not (further discussed below).

The difference in potency of PYY(1-36) and PYY(3-36) in reducing gastric emptying may be due to differences in receptor binding properties of the two peptides. PYY(1-36) and PYY(3-36) bind with various degrees of affinity to at least six receptor subtypes, Y₁ to Y₅, all of which have been cloned except for the Y₃ receptor (5, 36). Ligand binding studies reveal that PYY(1-36) binds to Y₁, Y₂, Y₄, and Y₅ receptors, whereas PYY(3-36) selectively binds to Y₂ receptors with an affinity similar to that exhibited by PYY(1-36) (5, 28, 29). It was postulated that differences in the tertiary structure of both forms likely explain the differences in their ligand binding affinities (28, 29). The juxtaposition of both the amino and COOH termini of the molecule is required for Y₁-receptor binding, whereas only the COOH-terminal helix confers selectivity for Y₂-receptor binding (29). PYY(1-36) contains both structural features, and it can bind both Y₁ and Y₂ receptors. The cleavage of the amino terminal dipeptide from PYY(1-36) to form PYY(3-36) eliminates the juxtaposition of the amino and COOH termini, resulting in reduced binding of PYY(3-36) to the Y₁-receptor (29). It is likely that this tertiary structure is conserved in both rat and hPYY(3-36), which might explain their similar potencies in inhibiting gastric emptying in rats.
Several lines of evidence suggest that circulating PYY gains access to the dorsal vagal complex (DVC) to activate Y2 receptors on vagal efferents to inhibit gastric emptying. Findings include 1) peripherally administered PYY (1-36) binds in a saturable manner to specific areas in the DVC (23) and induces c-Fos immunoreactivity in these areas (6); 2) PYY(1-36) and NPY(3-36) (a Y2 agonist) dose dependently inhibit excitatory postsynaptic currents in cholinergic neurons of the dorsal motor nucleus of the vagus (10); 3) injection of PYY(1-36) or Y2 agonists [NPY(3-36) or PYY(3-36)] into the DVC inhibits neurons of the dorsal motor nucleus of the vagus, as well as TRH-induced gastric motility (11, 12, 14); 4) intracisternal injections of PYY(1-36) inhibit gastric emptying, and this inhibition is attenuated by vagotomy (13); and 5) intracerebroventricular injection of NPY(3-36) (Y2 agonist) dose dependently delays gastric emptying of solids, as well as impairs antral contractions and antropyloric coordination in the emptying period (25). In contrast to these Y2 receptor-dependant effects, Y1 receptor-mediated effects on gastric emptying appear to be stimulatory. Central administration of the Y1 agonist (Leu3, Pro4) NPY was shown to stimulate gastric motility under basal conditions without further augmenting TRH-stimulated gastric motility in rats (14). These results together with our finding that PYY(3-36) is more potent than PYY(1-36) in inhibiting gastric emptying, suggest that PYY(1-36) and PYY(3-36) may each stimulate a Y2 receptor-mediated inhibition of gastric emptying, whereas PYY(1-36) might also activate a Y1 receptor-mediated stimulation of gastric emptying, which partially counters its action at Y2 receptors to inhibit gastric emptying.

The studies just cited provide strong evidence that PYY(3-36), acting by a hormonal mechanism, gains access to the DVC to activate Y2 receptors on vagal efferents to inhibit gastric emptying. In addition to this central mechanism, it is also possible that PYY(3-36) interacts directly with Y2 receptors along the gastrointestinal tract to decrease gastric emptying. The mRNA for Y2 receptors has been detected in the intestine of rats (18), and activation of colonic Y2 receptors has been reported to increase longitudinal smooth muscle tone in human and mouse colon (24). In addition to these possible endocrine mechanisms, the presence of PYY immunoreactivity in the gastric mucosa and enteric nervous system (15) suggests that PYY(3-36) might also act by paracrine or neurocrine mechanisms to inhibit gastric emptying.

In contrast to our results, Batterham et al. (4) reported that PYY(3-36) had no effect on gastric emptying in rats or humans. However, PYY(3-36) dosing and methods of measuring gastric emptying were not adequately described in that article to permit speculation on why this discrepancy has occurred. Because PYY(1-36) and PYY(3-36) coexist in the circulation, our interest was in determining whether these peptides interact in an additive or synergistic manner to inhibit gastric emptying. Five different doses of PYY(1-36) were paired with three different doses of PYY(3-36). Five combinations of PYY(1-36) and PYY(3-36) were 1) subthreshold doses of each (1.7 pmol·kg⁻¹·min⁻¹ each); 2) near-threshold effective doses of each (17 and 5 pmol·kg⁻¹·min⁻¹, respectively); 3) a suprathreshold dose of PYY(1-36) (170 pmol·kg⁻¹·min⁻¹) with a near-threshold dose of PYY(3-36) (5 pmol·kg⁻¹·min⁻¹); and 4) two different suprathreshold doses of PYY(1-36) (50 and 170 pmol·kg⁻¹·min⁻¹) each with a suprathreshold dose of PYY(3-36) (17 pmol·kg⁻¹·min⁻¹). In general, coadministration of PYY(1-36) and PYY(3-36) inhibited gastric emptying by an amount that was comparable to the inhibition produced when either peptide was given alone. The apparent lack of additivity or synergy between PYY(1-36) and PYY(3-36) in reducing gastric emptying is similar to that observed between Y1 and Y2 agonists in reducing Ca²⁺ influx and glutamate release in rat hippocampal synaptosomes (45). Coadministration of Y1 and Y2 agonists or Y2 and Y5 agonists inhibited glutamate release and Ca²⁺ influx by an amount that was comparable to that produced by each agonist independently. Furthermore, the inhibitory response to coadministration of Y2 agonist with either Y1 or Y5 agonist was reversed by a Y2 antagonist but not by either a Y1 or Y5 antagonist (45). Thus Silva et al. (45) proposed the existence of a physiological cross-talk between Y1, Y2, and Y5 receptors, such that when Y2 receptors are activated, Y1 and Y5 receptors remain "silent."

In summary, the present study demonstrates that 1) intravenous infusion of PYY(1-36) and PYY(3-36) each produces a dose-dependent inhibition of gastric emptying in rats, 2) PYY(3-36) is an order of magnitude more potent than PYY(1-36) in inhibiting gastric emptying, 3) hPYY(3-36) and rat PYY(3-36) inhibit gastric emptying similarly, and 4) PYY(1-36) and PYY(3-36) do not appear to interact in an additive or synergistic manner to inhibit gastric emptying. It remains to be determined whether endogenous molecular forms of PYY act by endocrine, paracrine, and/or neurocrine mechanisms to decrease gastric emptying; whether PYY inhibits gastric emptying of solid food; and whether PYY inhibits gastric emptying during as well as after gastric filling.

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