Peripheral PYY inhibits intracisternal TRH-induced gastric acid secretion by acting in the brain

HONG YANG, KEISHI KAWAKUBO, HELEN WONG, GORDON OHNING, JOHN WALSH†, AND YVETTE TACHE´
CURE: Digestive Diseases Research Center, Veterans Affairs Greater Los Angeles Healthcare System, and Digestive Diseases Division, Department of Medicine and Brain Research Institute, School of Medicine, University of California, Los Angeles, California 90073

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Peripheral PYY inhibits intracisternal TRH-induced gastric acid secretion by acting in the brain. Am J Physiol Gastrointest Liver Physiol 279: G575–G581, 2000.—The site of action of peripheral peptide YY (PYY)–induced inhibition of vagally stimulated gastric acid secretion was studied using immunoneutralization with PYY antibody in urethan-anesthetized rats. Gastric acid secretion (59 ± 7 μmol/90 min) stimulated by intracisternal injection of the stable thyrotropin-releasing hormone (TRH) analog RX-77368 (14 pmol/rat) was dose-dependently inhibited by 52%, 69%, and 85% by intravenous infusion of 0.25, 0.5, and 1.0 nmol·kg⁻¹·h⁻¹ PYY, respectively. PYY or PYY₃₋₃₆ (2.4 pmol/rat) injected intracisternally also inhibited the acid response to intracisternal RX-77368 by 73% and 80%, respectively. Intravenous pretreatment with PYY antibody (4.5 mg/rat), which shows a 35% cross-reaction with PYY₃₋₃₆ by RIA, completely prevented the inhibitory effect of intravenously infused PYY (1 nmol·kg⁻¹·h⁻¹). When injected intracisternally, the PYY antibody (280 μg/rat) reversed intracisternal PYY (2.4 pmol)- and intravenous PYY (1 nmol·kg⁻¹·h⁻¹)-induced inhibition of acid response to intracisternal RX-77368 by 64% and 93.5%, respectively. These results provide supporting evidence that peripheral PYY inhibits central vagal stimulation of gastric acid secretion through an action in the brain.

PEPTIDE YY (PYY) is released by endocrine cells in the ileum in response to the presence of fatty acids in the intestinal lumen (30). PYY₁–₃₆ and PYY₃–₃₆ are the two molecular forms of PYY abundant in the blood (9). Peripheral administration of PYY produces a potent antisecretory effect that is selective for central vagally mediated stimulation of gastric acid and pancreatic exocrine secretions (24, 25), while having little, if any, effect on secretions elicited by peripherally acting exogenous secretagogues (24). Convergent studies indicate that the inhibition of gastric and pancreatic secretory and motor functions elicited by peripheral PYY involves a central vagal dependent mechanism. Circulating PYY may enter the brain through the area postrema and portions of the nucleus of the solitary tract (NTS), where the blood-brain barrier is incomplete and can be the portal of entry for circulating peptide hormones (10). PYY binding sites are presented in the area postrema and the dorsal vagal complex (DVC) (7, 18), which includes the NTS and the dorsal motor nucleus of the vagus (DMN) (16). The PYY derivatives [Leu⁻¹⁷,Pro³⁴]PYY (or Pro³⁴ PYY) and PYY₃–₃₆ (or PYY₁₃–₃₆) show selective affinity to Y₁ and Y₂ receptors, respectively (1). Specific [¹²⁵I][Leu⁻¹⁷,Pro³⁴] PYY (Y₁) and [¹²⁵I]PYY₃–₃₆ (Y₂) binding sites have been detected in the NTS and the area postrema (7). PYY infused into peripheral circulation at physiological concentrations gains access to PYY binding sites located in specific portions of the DVC (12). In addition, peripheral injection of PYY activates neurons in the area postrema and dorsalmedial NTS, as shown by Fos induction in these areas (4). Microinjection of PYY or PYY₁₃–₃₆ into the DVC inhibits gastric motility through Y₂ receptors (6), whereas PYY or Pro³⁴ PYY stimulates gastric motility and acid secretion through Y₁/PYY-prefering receptors (6, 39). Both PYY and PYY₁₃–₃₆ administered in vivo, or in vitro to brain stem slice preparation, inhibit the activity of cholinergic vagal efferent neurons in the DMN, suggesting mediation through Y₂ receptors (5). However, despite these convergent findings supporting a possible action of circulating PYY in the brain, direct evidence showing that the antisecretory effect of peripheral PYY is initiated at a central site is still lacking.

In vivo immunoneutralization has been extensively used to inhibit the biological actions of gastrointestinal peptides or neuropeptides and to assess their physiological relevance in the brain or periphery (32, 40, 41). Antibodies do not necessarily require access to intracellular compartments to inhibit peptide action and are extremely stable within biological tissues (36, 37). Antibodies administered into the lateral ventricles have been shown to diffuse through brain tissue (34, 35) and

†Deceased 14 June 2000.

Address for reprint requests and other correspondence: H. Yang, CURE: Digestive Diseases Research Center, Veterans Affairs Greater Los Angeles Healthcare System, Bldg 115, Rm. 203, 11301 Wilshire Blvd., Los Angeles, CA 90073 (E-mail: hoyang@ucla.edu).
to become concentrated at sites expressing the immuno
genetic epitopes (31). In the present study, we first
established that the specific PYY polyclonal antibody
[Center for Ulcer Research and Education (CURE) no.
9153] injected intravenously or intracisternally pre
vents intravenous or intracisternal PYY-induced inhibi
tion, respectively, of the acid response to central thyrotropin-releasing hormone (TRH) analog, and sec
ond, we administered the antibody intracisternally to
assess whether peripherally infused PYY inhibits gas
tric acid secretion at a central site.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Harlan Labora
tory, San Diego, CA) weighing 250–310 g were maintained under
conditions of controlled temperature (22–24°C) and illumi
nation (12:12 h light-dark cycle starting at 6 A.M.). Rats had
ad libitum access to Purina laboratory chow (St. Louis, MO)
and tap water. Animals were deprived of food for 24 h but had
free access to water until 2 h before the beginning of the
experiments. All studies were performed in rats anesthetized
by intraperitoneal injection of urethan (1.5 g/kg, Sigma
Chemical, St. Louis, MO).

Drugs and treatments. Porcine/rat PYY (p/r PYY) (kindly
provided by Dr. J. Rivier, Salk Institute, La Jolla, CA) was
dissolved in 0.1% BSA (Sigma Chemical)/saline before intra
venous infusion. P/r PYY and p/r PYY3–36 (Peptides Synthe
sis Core Facility, University of California, Los Angeles, CA)
were dissolved in saline before intracisternal injection. The
stable TRH analog RX-77368 [pGlu-His-(3,3’-dimethyl)-Pro
NH2], Ferring Pharmaceuticals, Feltham, UK) was diluted in
saline before intracisternal injection from aliquots of a stock
solution (30 μg/ml in 0.1% BSA and saline, kept at −70°C).
The control groups were infused intravenously or injected
intracisternally with the corresponding vehicles.

The protein A-Sepharose column-purified IgG from a poly
clonal PYY antisera (CURE 9153) was provided by the Antibody
Core Laboratory of CURE: Digestive Diseases Re
search Center (Los Angeles, CA). This antibody has been
used previously for in vivo immunoneutralization to assess
endogenous PYY actions (3, 43). By RIA, the PYY antibody
has no cross-reactivity with rat or human pancreatic
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nal injection of the TRH analog. PYY (0.25, 0.5, or 1 nmol·kg⁻¹·h⁻¹) infused intravenously starting at 30 min before intracisternal RX-77368 (14 pmol) dose-dependently inhibited the acid response to intracisternal RX-77368 by 52%, 69%, and 83%, respectively, compared with vehicle-infused control, whereas PYY at 0.125 nmol·kg⁻¹·h⁻¹ had no significant inhibitory effect (61.7 ± 13.9 μmol/90 min) (Fig. 2). Intravenous PYY infusion (0.125, 0.25, 0.5, or 1 nmol·kg⁻¹·h⁻¹ for 30 min) did not influence basal gastric acid secretion in urethan-anesthetized rats (data not shown).

**Effect of intravenous PYY antibody on intravenous PYY antisecretory action.** The PYY antibody injected intravenously (2.25 or 4.5 mg/rat, −10 min) dose-dependently blocked peripheral PYY (1 nmol·kg⁻¹·h⁻¹ iv)-induced inhibition of the acid response to intracisternal RX-77368 (14 pmol) in urethan-anesthetized rats. PYY antibody at 2.25 or 4.5 mg/rat returned gastric acid output values to 29.5 ± 16.3 and 79.3 ± 5.4 μmol/90 min, respectively (3- and 8-fold increases, respectively, compared with 10.1 ± 5.2 μmol/90 min in rats without antibody pretreatment). PYY antibody at a lower dose (1 mg/rat iv) did not influence the PYY (1 nmol·kg⁻¹·h⁻¹ iv) inhibitory effect on intracisternal RX-77368-induced gastric acid secretion (14.7 ± 2.0 μmol/90 min) (Figs. 2 and 3). PYY antibody alone injected intravenously at 4.5 mg/rat did not influence basal acid secretion in urethan-anesthetized rats (net acid output, 2.7 ± 5.8 μmol/90 min; n = 4).

**Effect of intracisternal PYY and PYY₃₋₃₆ on gastric acid secretion**-induced by intracisternal RX-77368. In the saline-pretreated group (5 μl saline ic, administered at −10 min), the acid response to RX-77368 (14 pmol/5 μl) was 112 ± 28 μmol/90 min (Fig. 4). Pretreatment with intracisternal PYY (2.4 pmol/5 μl) or PYY₃₋₃₆ (2.4 pmol/5 μl) significantly inhibited the acid response to intracisternal RX-77368 by 80% and 73%, respectively, compared with the intracisternal saline-pretreated group (Fig. 4). PYY (2.4 pmol) injected intracisternally did not influence basal gastric acid secretion (see Fig. 7).

**Effect of intracisternal PYY antibody on intracisternal PYY antisecretory action.** The control antibody (280 μg/20 μl ic) injected 10 min before intracisternal injection of a mixture of PYY (2.4 pmol) and RX-77368 (1.4 pmol) in a volume of 5 μl did not influence the inhibitory effect of intracisternal PYY on RX-77368-induced acid secretion. The net acid output (25.5 ± 15.4 μmol/90 min, Fig. 5) was similar to that obtained when the two peptides were injected intracisternally separately (Fig. 4). In contrast, intracisternal PYY antibody pretreatment (280 μg/20 μl, −10 min) reversed the PYY
inhibitory effect by 64%, and the net acid output returned to 77.0 ± 13.2 μmol/90 min (Fig. 5).

Effect of intracisternal PYY antibody on intravenous PYY antisecretory action. The PYY antibody (280 μg/20 μl) injected intracisternally 10 min before the intravenous infusion of PYY (1 nmol·kg⁻¹·h⁻¹) reversed the intravenous PYY-induced inhibition of gastric acid secretion stimulated by intracisternal RX-77368 (14 pmol) (Fig. 6). The net acid responses to intracisternal RX-77368 in intracisternal control and PYY antibody-pretreated rats infused intravenously with PYY (1 nmol·kg⁻¹·h⁻¹) were 4.5 ± 3.5 and 55.3 ± 10.0 μmol/90 min, respectively. This represents a 93.5% reversal of the inhibitory effect of intravenous PYY on the acid response to intracisternal RX-77368 (Fig. 6). PYY antibody (280 μg) or the control antibody (280 μg) injected intracisternally did not significantly influence either the basal gastric acid secretion or the acid response to intracisternal RX-77368 (14 pmol) in urethan-anesthetized rats (Fig. 7).

DISCUSSION

TRH is an endogenous neurotransmitter in the medulla that plays an important physiological role in the vagal regulation of gastric functions (28). Medullary TRH-synthesizing neurons are mainly located in the caudal raphe nuclei and project to innervate the DMN (17, 19, 27). TRH microinjected into the DMN stimulates gastric acid secretion through activating the vagus (13, 21). The intracisternal injection of the stable TRH analog RX-77368 is a well established and widely used tool to induce central vagally mediated stimulation of gastric acid secretion and motility in conscious or anesthetized rats (28). In the present study, PYY infused intravenously dose-dependently inhibited gastric acid secretion induced by intracisternal RX-77368 in urethan-anesthetized rats. This finding provides additional evidence that peripheral PYY is a potent inhibitor of central vagally stimulated gastric acid secretion, as previously reported in other models, such as acid responses to sham feeding (24) and 2-deoxy-glucose (25). The minimal effective dose at which intravenous PYY exerts an antisecretory effect (250 pmol·kg⁻¹·h⁻¹) is in the range similar to that previously reported (14) to induce physiological concentrations in the circulation. The acid response to intracisternal RX-77368 (14 pmol) mimics the levels of acid secretion stimulated by cold stress (42), which has been shown to be mediated through medullary TRH (11, 42). Based on the well-established demonstration that medullary TRH stimulates vagal efferent activity (22) and plays a physiological role in the cephalic phase of acid secretion (2, 29), whereas PYY is one of the enterogastrones (30), the present findings suggest a possible physiological relevance of the interaction between peripheral PYY and central TRH to regulate gastric acid secretion during digestion.
Although the physiological significance of endogenous PYY and its interactions with other gastrointestinal peptides to regulate gastric secretion after a meal is important to investigate, the present study was focused on the mechanism of peripheral PYY inhibitory action. Growing evidence (8, 26) has revealed the central vagal components that respond to a meal. In particular, Fos immunoreactivity was induced in neurons of the NTS and area postrema after feeding and intraduodenal loading (8, 26, 38). Peripheral injection of PYY has a similar effect (4). Several lines of morphological and functional evidence (7, 12, 18, 23–25) also support the view that the medulla is the action site for peripheral PYY to inhibit gastric and pancreatic functions. To be consistent with the hypothesis that intravenously administered PYY inhibits central TRH-induced gastric acid secretion by acting in the medulla, PYY injected directly into the cisterna magna at lower doses should mimic the inhibitory effect of peripherally infused PYY. Indeed, PYY or PYY3–36 injected intracisternally at 2.4 pmol inhibits intracisternal RX-77368-stimulated acid secretion similarly to intravenous infusion of PYY at 0.5–1 nmol·kg⁻¹·h⁻¹. PYY1–36 and PYY3–36 are the two molecular forms that are abundant in the blood (9). Both PYY and PYY3–36 (or PYY13–36) exhibit similar affinity to the Y₂ receptors (1), and when applied to the DMN in femtomole levels, inhibited DMN neuronal activity (5), as well as TRH-stimulated gastric motility (6). These findings, together with present results showing that PYY and PYY3–36 injected intracisternally are equipotent to inhibit vagally stimulated acid secretion by intracisternal TRH analog, support the view that the medulla is an action site for a low dose of PYY to inhibit vagally stimulated gastric functions through Y₂ receptors.

In the absence of available specific Y₂ receptor antagonists, to obtain direct evidence that peripheral PYY acts in the medulla to inhibit gastric acid secretion, we used the immunoneutralization method of approach. The PYY antibody CURE 9153 proved to be valuable in evaluating the role of circulatory PYY in intestinal nutrition-induced absorption (3) and inhibiting vagally stimulated acid secretion by intracisternal RX-77368-stimulated gastric acid secretion (43). This provides biological evidence of its immunoneutralizing potency in rats. All three tested antisera injected intracisternally in portions of the DVC (12). The possibility that the intracisternal-injected PYY antibody interacts with intracisternal RX-77368 to increase acid secretion can be excluded, because intracisternal injection of PYY antibody or control antibody did not influence basal and RX-77368-induced gastric acid secretion.

Intracerebroventricular or intracisternal passive immunization has been used extensively to inhibit central actions of peptide (20, 32). It has been proved by immunohistochemistry that antibody injected intracerebroventricularly penetrates into brain tissues (34) and specifically accumulated by neurons with the antigen protein (31). The vast majority of investigators using this technique made the assumption that the antibodies can reach the systemic circulation in substantial quantities after intracisternal or intracerebroventricular administration. However, recent studies (33) revealed that small quantities of antisera or purified antibodies administered into the ventricles inhibit the action of a neuropeptide specifically within the brain and do not gain access to peripheral tissues in substantial quantities. This assumption is based on an understanding of limited transport of large-molecular-weight proteins across the blood-brain barrier (15) and the unlikely that antibodies can reach the systemic circulation in significant amounts after intracisternal or intracerebroventricular administration.

In the present study was not incompatible with the findings of Zhao et al. (43). Our observations are consistent with other previous studies (24, 25) showing that PYY is more potent in inhibiting the cephalic phase of acid secretion, whereas in the study of Zhao et al. (43) there was no cephalic phase involved, as the acid was stimulated by intragastric peptone, which mimics a gastric phase of acid secretion.
In summary, PYY was more potent in inhibiting intracisternal TRH analog-induced stimulation of gastric acid secretion in urethan-anesthetized rats when administered intracisternally rather than intravenously. PYY antibody was more potent in reversing the antisercretory action of intravenously infused PYY when injected intracisternally rather than intravenously. In addition, intracisternal injection of PYY₃₋₃₆ mimics the antisercretory effect of intracisternal PYY. These findings, together with previous observations (4, 5, 7, 12, 18, 23–25), strongly support the view that peripheral PYY-induced inhibition of vagally stimulated gastric functions involves a Y₂-mediated central mechanism, possibly at a medullary site.

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