Intracisternal PYY increases gastric mucosal resistance: role of cholinergic, CGRP, and NO pathways

HONG YANG, KEISHI KAWAKUBO, AND YVETTE TACHE
CURE: Digestive Diseases Research Center, West Los Angeles Veterans Affairs Medical Center, and Department of Medicine, Digestive Diseases Division and Brain Research Institute, University of California, Los Angeles, 90073

ALTHOUGH THE VAGUS NERVE has long been established to play a permissive role in the development of gastric lesions, there is also evidence suggesting its physiological relevance in the enhancement of the gastric mucosal resistance to pending injury (33). Ablation of the dorsal motor nucleus of the vagus (DMN) or vagotomy in rats worsens gastric lesion formation induced by ethanol and suppresses adaptive gastroprotection (5, 9, 13, 17, 36). Recent studies showed that stimulation of thyrotropin-releasing hormone (TRH) receptor (TRHR) in the dorsal vagal complex (DVC) plays a physiological role in the vagal-dependent increase of the gastric mucosa resistance to injury (17, 19, 34). Low doses of the stable TRH analog RX-77368 injected into the cisterna magna or into the DVC, as well as TRH endogenously released into the DVC by a weak activation of the raphe pallidus cell bodies, reduce gastric mucosal lesions induced by 60% ethanol in rats (8, 19, 20). Vagal cholinergic-dependent pathways involving calcitonin gene-related peptide (CGRP) and L-arginine/nitric oxide (NO)-induced gastric vasodilatation mediate the central action of exogenous and endogenous TRH (18, 20, 31). Gastric prostaglandins also play a role in the central TRH protective action, which is not related to changes in gastric mucosal blood flow (18, 20, 31).

Whether the activation of medullary TRHr and the associated peripheral CGRP/NO pathways represent a final common mechanism mediating the central vagal modulation of the gastric mucosal resistance to injury is still to be investigated.

Besides TRH, only a few peptides have been consistently reported to act in the brain to induce a vagal-dependent stimulation of gastric secretory and motor function (32). In particular, a growing number of reports in rats and dogs indicate that members of the neuropeptide Y (NPY)/peptide YY (PY) family may have physiological relevance in coordinating the vagal-dependent visceral responses to feeding (12). Microinjection of PYY or NPY into the DVC induced vagal-dependent stimulation of gastric acid and hepatic bile secretion and gastric motor function through Y1 receptors./"PYY-prefering receptors binding sites" in urethan-anesthetized rats (7, 39–41). Our previous studies showed that rat PYY and [Pro34]PYY microinjected into the DVC in doses ranging from 20 to 124 pmol are more potent than rat NPY or rat pancreatic polypeptide in stimulating gastric acid secretion in urethan-anesthetized rats (39, 40). In the present study, we examined the influence of PYY, injected intracisternally at doses below the threshold that stimulates gastric acid secretion, on the development of gastric lesions induced by diluted ethanol in urethan-anesthetized rats. The central and peripheral mechanisms mediating the PYY action were assessed with the use of an antisense oligodeoxynucleotide strategy to interfere with medullary TRHr receptors (24, 30), with a pharmacological approach to block peripheral muscarinic and CGRP receptors, and with prostaglandins and NO synthases.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 250–310 g were maintained under conditions of con-
trolled temperature (22–24°C) and illumination (12:12 h light-dark cycle starting at 6 AM). Rats had ad libitum access to Purina Laboratory Chow (Ralston Purina, 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 experiments were performed in rats anesthetized by intraperitoneal injection of urethan (1.5 g/kg body wt, Sigma, St. Louis, MO).

Drugs and Treatments
Porcine rat PYY (kindly provided by Dr. J. Rivier, Salk Institute, La Jolla, CA) was dissolved in saline before intracisternal injection. The stable TRH analog RX-77368 (pGlu-His-[3,3'-dimethyl]-Pro-NH2; Ferring Pharmaceuticals, Feltham, Middlesex, UK) was diluted in saline before intracisternal injection from aliquots of a stock solution (30 µg/ml in 0.1% BSA-saline, kept at –70°C). Ethanol (Fisher Scientific, Fair Lawn, NJ) was diluted in saline and given intragastrically by oral gavage with the use of a stainless steel cannula. Atropine sulfate (Sigma) was dissolved in saline and injected subcutaneously (1 mg/kg). Human CGRP receptor subtype 1 (R1) antagonist CGRP-8–37 (1) in powder form (kindly provided by Dr. S. St. Pierre, University of Québec, Montreal, PQ, Canada) was dissolved in 0.1% BSA-saline and injected intravenously (100 µg/kg). Indomethacin (Sigma) was dissolved in 1% NaHCO3 solution and intraperitoneally injected (5 ml/kg). The NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 3 mg/kg; Sigma) and L- or D-arginine (300 mg/kg; Sigma) were dissolved in saline and intravenously injected. Intravenous injections were performed through the jugular vein in 1 ml/kg body wt, and the volume was reduced to 0.5 ml/kg when consecutive intravenous injections were performed. Control groups were injected with the corresponding vehicles.

Antisense oligodeoxynucleotides complementary to the first 18 bases downstream from the initiation codon of the rat TRH receptor mRNA (10) were synthesized with phosphorothioate derivatives and were used as one of the control treatments. The mismatch sequence has neither significant complementarity to any part of the TRH receptor mRNA nor significant complementarity to any other gene sequences in the GenBank database. The oligodeoxynucleotides were purified by polyacrylamide gel electrophoresis and were diluted in sterile saline to a final concentration of 10 µg/ml, and aliquots were maintained at –70°C until use.

For intracisternal injection, rats were placed in ear bars of a stereotactic equipment, and the atlanto-occipital membrane was punctured with a 50-µl Hamilton syringe (Hamilton, Reno, NV). The correctness of needle placement into the cisterna magna was ensured by the presence of cerebrospinal fluid in the Hamilton syringe on aspiration before injection of vehicle or peptides in 10 µl volume.

Gastric Acid Secretion
In urethan-anesthetized rats, the esophagus 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 stomach through the double lumen 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 the use of an autotitrator (TTT titrator, Radiometer, Copenhagen, Denmark).

Gastric Lesion Formation and Assessment
Gastric lesions were induced by intragastric administration of 45% ethanol (5 ml/kg) by oral intubation with a stainless steel cannula in urethan-anesthetized rats. One hour after ethanol administration, rats were killed by decapitation. Stomachs were isolated, opened along the greater curvature, and rinsed with saline, except in the last experiment, in which, before opening the stomach, the pylorus and cardia were ligated and 8 ml of 1% formalin was injected and left in the stomach for a 30-min fixation period. Then stomachs were pinned flat to a hard paperback and photographed. The images were transferred to a computer to measure gastric lesions in the glandular part with NIH Image 1.54. Gastric lesions were expressed as percent coverage of the corpus as previously described (19).

Experimental Protocols
Effect of intracisternal PYY on gastric acid secretion and ethanol-induced gastric lesions. In urethan-anesthetized rats, basal gastric acid secretion was monitored every 10 min for 80 min before and 80 min after the intracisternal injection of PYY (23, 47, or 117 pmol) or saline (10 µl). In separate studies, PYY (23, 47, or 117 pmol) or saline was injected intracisternally, and, 30 min later, 45% ethanol was administered intragastrically. Gastric lesions were monitored 1 h later.

Effect of intracisternal TRHr antisense oligodeoxynucleotides on intracisternal PYY and TRH analog-induced gastro-protection. Rats were injected intracisternally twice with either TRHr antisense or mismatch oligodeoxynucleotides (100 µg/injection) under short enflurane anesthesia (2–3 min, 5.5% vapor in O2; Ethrane-Anaquest, Madison, WI) at 24-h intervals. Twenty-four hours after the second injection, rats were anesthetized with urethan, and the stable TRH analog RX-77368 (4 pmol), PYY (47 pmol), or saline (10 µl) was injected intracisternally. Thirty minutes after the peptide or saline intracisternal injection, 45% ethanol was given intragastrically, and gastric lesions were monitored 1 h later.

Effect of low doses of RX-77368 and PYY given singly or simultaneously on ethanol-induced gastric lesions. Rats were injected intracisternally with saline, RX-77368 (2.6 pmol/10 µl), PYY (6 pmol/10 µl), or RX-77368 (2.6 pmol) plus PYY (6 pmol, mixed in 10 µl of vehicle). Thirty minutes after the intracisternal injection, 45% ethanol was administered intragastrically, and rats were killed 1 h later.

Effect of intracisternal PYY on ethanol-induced gastric lesions: influence of various pretreatments. The following pretreatments were performed before intracisternal injection of either saline or PYY (47 pmol): atropine (1 mg/kg) or saline injected subcutaneously 30 min before; CGRP-8–37 (100 µg/kg) or its vehicle (0.1% BSA-saline) injected intravenously 15 min before; indomethacin (5 mg/kg) or its vehicle (1.0% NaHCO3 in saline) injected intraperitoneally 15 min before; L-NAME (3 mg/kg) or saline injected intravenously 15 min before; and L-arginine or its enantiomer D-arginine (300 mg/kg) injected intravenously immediately before the intravenous injection of L-NAME (3 mg/kg). In all experiments, 45% ethanol was given intragastrically 30 min after the intracisternal injection of saline or PYY, and gastric lesions were assessed 1 h later.

The regimen of drug pretreatment was based on previous studies, which showed the inhibition of vagally stimulated
gastric prostaglandin release by indomethacin (43) and the blockade of vagally mediated gastric protection against 60% ethanol by the CGRP receptor antagonist and the NO synthase inhibitor (18, 19).

Statistical analysis. All results are expressed as means ± SE. Comparisons among multiple groups were performed by one-way ANOVA followed by Duncan’s contrast. P values of <0.05 were considered statistically significant.

RESULTS

Effect of Intracisternal PYY on Acid Secretion and Ethanol-Induced Gastric Lesions

Gastric acid outputs per 80-min period were not significantly different between intracisternal saline- or PYY (23, 47, or 117 pmol)-injected groups in urethan-anesthetized rats (Table 1). Time course studies showed similar peak values at 30–40 min after intracisternal injection of either saline or PYY at 23, 47, and 117 pmol (3.7 ± 0.5, 4.0 ± 0.3, 3.8 ± 0.8, and 3.8 ± 0.9 µmol/10 min, respectively).

One hour after intragastric administration of 45% ethanol, macroscopic gastric lesions were visualized as long dark-red vertical lines covering 18.4 ± 2.3% (n = 15) of the corpus mucosa in urethan-anesthetized rats injected intracisternally with saline. PYY injected intracisternally at 47 and 117 pmol significantly decreased gastric lesion areas to 6.8 ± 1.5% and 7.5 ± 1.6% of the corpus mucosa, respectively, whereas at 23 pmol, the reduction of lesions (lesion area 13.5 ± 2.8%) did not reach statistical significance (Fig. 1).

Influence of Intracisternal TRHr Antisense Oligodeoxynucleotides on Intracisternal PYY and TRH Analog-Induced Gastric Protection

Pretreatment with TRHr antisense or mismatch oligodeoxynucleotides did not influence the formation of gastric lesions induced by 45% ethanol in rats injected intracisternally with saline 30 min before ethanol administration (19.0 ± 2.6% and 24.9 ± 5.2%, respectively; Fig. 2). RX-77368 (4 pmol) injected intracisternally significantly reduced ethanol-induced gastric lesions in mismatch oligodeoxynucleotide-pretreated rats (10.3 ± 1.3%). Pretreatment with TRHr antisense oligodeoxynucleotides prevented the gastroprotective effect of intracisternal RX-77368 (19.8 ± 2.1%). PYY (47 pmol) injected intracisternally also significantly reduced ethanol-induced gastric lesions in mismatch oligodeoxynucleotide-pretreated rats (8.1 ± 1.9%). However, pretreatment with TRHr antisense oligodeoxynucleotides did not influence the gastroprotective effect of intracisternal PYY (8.0 ± 1.0%; Fig. 2).

Effect of Low Doses of RX-77368 and PYY Given Singly or Simultaneously on Ethanol-Induced Gastric Lesions

RX-77368 (2.6 pmol) or PYY (6 pmol) injected intracisternally induced a small but nonsignificant reduction (−23% and −13%, respectively) of gastric lesions induced by diluted ethanol (Fig. 3). When the two peptides were injected intracisternally together (in 10 µl of vehicle), the gastric lesion formation was significantly reduced (−44%; Fig. 3).

Table 1. Effect of intracisternal injection of PYY on gastric acid secretion in urethan-anesthetized rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose, pmol/rat ic</th>
<th>n</th>
<th>Basal, µmol/80 min</th>
<th>Post ic injection, µmol/80 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
<td>6</td>
<td>20.5 ± 2.3</td>
<td>25.8 ± 2.6</td>
</tr>
<tr>
<td>PYY 23</td>
<td>2</td>
<td>5</td>
<td>22.3 ± 1.3</td>
<td>27.2 ± 1.2</td>
</tr>
<tr>
<td>PYY 47</td>
<td>5</td>
<td>11</td>
<td>18.2 ± 2.0</td>
<td>27.0 ± 3.8</td>
</tr>
<tr>
<td>PYY 117</td>
<td>7</td>
<td>11</td>
<td>17.9 ± 3.3</td>
<td>26.3 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. Urethan-anesthetized rats were injected intracisternally (ic) with saline or peptide YY (PYY; 23, 47, or 117 pmol), and gastric acid secretion was monitored 80 min before and 80 min after the intracisternal injection.
Effect of Various Pretreatments on Intracisternal PYY-Induced Reduction of Gastric Lesions Induced by Ethanol

In vehicle-pretreated rats, intracisternal injection of PYY (47 pmol) resulted in a significant decrease in the formation of gastric erosions (Figs. 4 and 5 and Table 2). Atropine (1 mg/kg sc), CGRP-(8—37) (100 µg/kg iv), or L-NAME (3 mg/kg iv) pretreatment completely prevented the gastroprotective effect of intracisternal PYY (47 pmol). The areas of gastric lesions in these groups reached 24.9 ± 2.0%, 35.0 ± 3.9%, and 20.3 ± 1.7% of the corpus mucosa, respectively, and were not significantly different from intracisternal saline-injected groups with the same respective pretreatments (Figs. 4 and 5). L-Arginine (300 mg/kg iv) injected intravenously immediately before L-NAME administration completely prevented L-NAME-induced blockade of the gastroprotective effect of intracisternal PYY (47 pmol; Fig. 5). D-Arginine injected under the same conditions did not reverse the L-NAME action (Fig. 5). Pretreatment with indomethacin (5 mg/kg ip) did not influence the protective effect of intracisternal PYY at 47 pmol (Table 2). In intracisternal saline-injected groups, pre-treatment with subcutaneous atropine, intravenous CGRP-(8—37), intravenous L-NAME, or intraperitoneal indomethacin did not significantly alter the gastric lesions induced by 45% ethanol compared with respective vehicle-pretreated control groups (Figs. 4 and 5 and Table 2). There was a tendency for enhanced ethanol lesions with intravenous CGRP-(8—37) pre-treatment alone, but the difference did not reach statistical significance (Fig. 4).

DISCUSSION

The present data show that PYY injected intracisternally (23–47 pmol) dose-dependently decreased 45% ethanol-induced gastric lesions by 27–63% in urethan-anesthetized rats. At the highest dose tested (117 pmol), the PYY protective effect was not further enhanced, showing that intracisternal PYY did not completely prevent gastric damage induced by diluted ethanol. Cholinergic receptor blockade by atropine did not influence gastric injury induced by ethanol in intracisternal vehicle-injected rats, in agreement with previous findings (11, 19, 42). However, atropine completely prevented intracisternal PYY-induced gastric protection, suggesting that cholinergic mechanisms are involved in the peptide action. We previously reported that microinjection of PYY (23–47 pmol) into the DMN induced a vagally mediated stimulation of gastric acid secretion in urethan-anesthetized rats (40). By contrast, the same dose of peptide injected intracisternally did not modify basal acid secretion (40). Likewise, in the present study, there was only a small transient and non-dose-related increase in peak values after intracisternal injection of PYY (23, 47, or 117 pmol), and the total gastric outputs in 80 min were not significantly different from the acid secretion in the intracisternal...
saline group. Several reports indicate that low activation of medullary TRH increases the resistance of the gastric mucosa to ethanol injury through vagal atropine-sensitive pathways in rats (8, 17–19, 34). Under these conditions, TRH increases gastric mucosal blood flow without enhancing gastric acid secretion because of the prevalent prostaglandins and CGRP inhibitory mechanisms, thereby displaying a gastroprotective action (18, 21, 27, 42, 43). These results indicate similarities between intracisternal PYY and the TRH analog actions on gastric function. Both peptides injected intracisternally exhibit a gastroprotective effect against diluted ethanol-induced lesions through atropine-sensitive mechanisms at doses below threshold that stimulate gastric acid secretion (18, 19, 42).

Evidence showing interactions between TRH and PYY in the medullary regulation of gastric function has been reported. PYY microinjected into the DVC in a 12-pmol dose potentiated exogenous and endogenous TRH in DVC-induced gastric acid secretion (40). By contrast, femtomolar doses of PYY microinjected into the DVC inhibited gastric motility stimulated by TRH (6). However, the possible role of medullary TRH pathways in the gastroprotective action of intracisternal PYY was not supported by the present observations. PYY injected intracisternally similarly reduced ethanol-induced gastric lesions by 67% and 68%, respectively, in intracisternal TRHr antisense- or mismatched oligodeoxynucleotide-pretreated rats. The efficacy of the TRHr antisense oligodeoxynucleotides was assessed by their blocking action on intracisternal TRHr analog-induced gastric protection against ethanol lesions. RX-77368 injected intracisternally at 4 pmol reduced 45% ethanol-induced gastric lesions by 59% in rats pretreated with the mismatch oligodeoxynucleotides. These results confirm previous observations showing reduction of 60% ethanol-induced gastric lesions by RX-77368 at similar low doses (8, 34). The prevention of the gastroprotective action of intracisternal RX-77368 by TRHr antisense oligodeoxynucleotides pretreatment is consistent with the abolishment of TRHr-induced stimulation of gastric emptying and contractility by a similar antisense strategy (24, 30). These functional findings support that TRHr antisense oligodeoxynucleotide pretreatments selectively block TRH receptor-mediated actions. In vitro binding studies also showed that TRHr antisense oligodeoxynucleotides reduced medullary TRH receptor binding (16). The present results indicate that intracisternal PYY-induced gastric protection is not secondary to the induction of medullary TRH release or activation of TRH receptors. This is further supported by the additive effect of simultaneous intracisternal injection of PYY and RX-77368. Low doses of RX-77368 and PYY, which did not significantly influence ethanol-induced gastric lesion formation when injected intracisternally alone, showed a gastroprotective effect when injected intracisternally together. These findings indicate that the activation of TRH receptors in the DVC do

### Table 2. Effect of indomethacin on intracisternal PYY-induced gastric protection against ethanol in urethan-anesthetized rats

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Injection (ic)</th>
<th>n</th>
<th>Gastric Lesions, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Saline</td>
<td>7</td>
<td>14.9 ± 2.6</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Saline</td>
<td>8</td>
<td>16.1 ± 2.8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>PYY</td>
<td>8</td>
<td>7.3 ± 1.0*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>PYY</td>
<td>7</td>
<td>8.2 ± 2.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Vehicle (1% NaHCO₃) or indomethacin (5 mg/kg) was injected intraperitoneally 60 min before intracisternal injection of saline or PYY (47 pmol), and 30 min later 45% ethanol (5 ml/kg) was administrated intragastrically. The gastric lesions were monitored 1 h later. *P < 0.05 compared with vehicle/saline group. †P < 0.05 compared with indomethacin/saline group.
not provide the final common pathway for the vagal cholinergic-dependent action of other peptides, such as PYY. The Y receptor subtypes through which PYY mediates its gastroprotective action need to be further assessed as PYY binds to the cloned Y1, Y2, Y4, and Y5 receptors and a PYY-preferring binding site yet to be cloned (2, 25). Recent reports showed that PYY and related analogs microinjected into the DVC stimulated gastric acid secretion and motility with a rank order of potency suggesting activation of PYY-preferring Y1 receptor subtype (7, 39). Dense representation of [125I]-PYY binding sites in the medullary area, especially in those nuclei regulating gastric function through vagal pathways such as the DVC (14, 23), supports the functional observations.

Pharmacological approaches used to investigate the peripheral mediators involved in intracisternal PYY-induced gastroprotection showed both differences and similarities with those previously established for medullary TRH. Although consistent reports indicate a role of gastric prostaglandins in the gastric protective effect of intracisternally or DVC-injected TRH analog or endogenous medullary TRH (4, 19, 20, 42), indomethacin did not alter the gastric protection induced by intracisternal PYY in the present study. Indomethacin was used at a dose that did not influence ethanol-induced gastric lesions (28, 38, 42) but blocked vagal-mediated gastric prostaglandin release (43). The results indicate that gastric prostaglandins are not major mediators for the intracisternal PYY gastroprotective action.

There is conclusive evidence that exogenous administration or endogenous release of CGRP from capsaicin-sensitive afferent nerves induces gastric hyperemia and counteracts gastric mucosal damage caused by ethanol and other damaging agents via activation of CGRP-R1 (15). We recently reported that peripheral muscarinic stimulation induced either by central vagal activation or by peripheral administration of the muscarinic receptor agonist bethanechol increases gastric mucosal blood flow through a CGRP-dependent mechanism (21). In the present study, intravenous injection of the CGRP-R1 antagonist CGRP-(8—37) completely prevented the gastroprotective effect of intracisternal PYY, indicating a mediation through activation of peripheral CGRP-R1 receptors. The intravenous injection of CGRP-(8—37) showed a tendency to worsen 45% ethanol-induced gastric lesions as previously reported (15), suggesting the importance of CGRP in the maintenance of gastric mucosa integrity against damaging agents (15).

NO is an essential mediator of the gastric hyperemia and protective effects of peripheral CGRP, whereas prostaglandins do not play a role (15). Pretreatment with L-NAME, unlike indomethacin, completely prevented the gastroprotective effect of intracisternal PYY. At the dose used in the present study (3 mg/kg), L-NAME did not significantly influence the ethanol lesions, in agreement with our previous observations (22). The action of L-NAME was reversed in an enantioselectively specific manner by the coadministration of the natural substrate of NO synthase (26) L-arginine, whereas d-arginine, which is not a substrate for NO synthase, had no effect. These data indicate a critical role of L-arginine/NO as a second messenger mediating peripheral CGRP action in intracisternal PYY-induced gastric protection. The mechanisms through which CGRP/NO pathways enhance gastric mucosa resistance to ethanol injury have been recently reviewed (15). Both gastric vascular-dependent and -independent mechanisms as well as a direct protective action on gastric mucosal cells are involved (15, 37). These observations, along with our previous reports regarding the gastroprotective action of medullary TRH (18, 20), may support the notion that CGRP and NO are common underlying mechanisms conferring protection against ethanol injury when central vagal-cholinergic pathways are recruited by selective peptides injected intracisternally at subeffective doses for stimulating acid secretion.

The physiological significance of the present observations needs to be further established. However, considering the saturable binding of PYY in the DVC at physiological circulating concentrations (14), it is tempting to speculate that postprandially released PYY (35) may act in the DVC and contribute to the maintenance of gastric mucosal integrity. Besides the peripheral source, PYY may originate from the central nervous system. The highest concentration of PYY immunoreactivity is located in the brain stem (3). A restricted population of neurons in the midline of the rostral medulla expresses PYY mRNA (29). Also, PYY nerve terminals are present in the DVC (29). Whether the activation of these brain stem PYY immunoreactive neurons could influence gastric function through an action in the DVC is still unknown.

In conclusion, the present study demonstrates that PYY injected intracisternally at doses that do not influence gastric acid secretion reduced 45% ethanol-induced gastric lesions in urethan-anesthetized rats. The PYY action is mediated by peripheral cholinergic activation of CGRP/L-arginine/NO pathways but not through medullary TRH receptors and peripheral prostaglandins. These results indicate that activation of vagal-cholinergic pathways by central peptides may constitute an important regulatory mechanism to enhance the resistance of gastric mucosa against deleterious agents through a common CGRP/NO-dependent action.

We thank Dr. J. Rivier (Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, CA) for the supply of rat PYY, Dr. Serge St. Pierre (Department of Biochemistry, University of Quebec, Montreal, PQ, Canada) for the supply of CGRP-(8—37), Dr. Vincent Wu for preparing the TRHr antisense oligodeoxynucleotides, and Paul Kirsch for his assistance in the preparation of the manuscript. This work was supported by National Institutes of Health Grants DK-30110 (Y. Taché), DK-50255 (H. Yang), and DK-41301 (Animal Core, Y. Taché).
REFERENCES


