Intracisternal TRH analog increases gastrin release and corpus histidine decarboxylase activity in rats

MIN SONG, HONG YANG, JOHN H. WALSH, GORDON OHNING, HELEN WONG, AND YVETTE TACHE
CURE: Digestive Diseases Research Center, West Los Angeles Veterans Affairs Medical Center, and Digestive Diseases Division, Department of Medicine and Brain Research Institute, University of California, Los Angeles, California 90073

Song, Min, Hong Yang, John H. Walsh, Gordon Ohning, Helen Wong, and Yvette Tache. Intracisternal TRH analog increases gastrin release and corpus histidine decarboxylase activity in rats. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G901–G908, 1999.—Thyrotropin-releasing hormone (TRH) acts in brain stem nuclei to induce vagally mediated stimulation of gastric secretion. The effects of intracisternal injection of the TRH analog RX-77368 on plasma gastrin levels and corpus histidine decarboxylase (HDC) activity were studied in 48-h fasted conscious rats. RX-77368 (25–100 ng) increased plasma gastrin levels by threefold at 30 min, which remained significantly higher than control at 2 and 4 h postinjection. Corpus HDC activity began to increase at 2 h and reached a peak at 4 h postinjection with a 21-fold maximum response observed at 50 ng. Morphological changes in the appearance of corpus HDC-immunoreactive cells correlated well with HDC activity. Pretreatment with gastrin monovalent antibody completely prevented RX-77368 stimulatory effects on HDC activity. Atropine significantly attenuated gastrin increase at 30 min by 26%. These results indicated that in conscious fasted rats, TRH analog acts in the brain to increase corpus HDC activity in the enterochromaffin-like cells, which involves gastrin release stimulated by central TRH analog.

enterochromaffin-like cell; gastrin monoclonal antibody; vagus; thyrotropin-releasing hormone.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 220–350 g (Harlan, Indianapolis, IN) were given free access to Purina Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water and housed under controlled light (light on 0600–1800) and temperature (22 ± 2°C) conditions. All experiments, except for one normally fed group, were performed in rats deprived of food, but not water, for 48 h. Animal protocols were approved by the Veterans Affairs Medical Center, West Los Angeles Research Service Animal Committee.

Drugs and treatments. The stable TRH analog RX-77368 (pGlu-His,3'-dimethyl-Pro-NH2, Ferring Pharmaceutical, Feltham, Middlesex, UK) was dissolved in 0.1% BSA and 0.9% saline at 100 ng/10 µl and kept frozen at −70°C. Before each experiment, the peptide was diluted in saline and injected intracranially in 10 µl volume.

Atropine (Sigma, St. Louis, MO) in powder form was dissolved in 0.9% saline before use and injected intraperitoneally (1 mg/kg). Gastrin MAb E5 [Center for Ulcer Research and Education (CURE) no. 109.21] was used as control antibody. The antibodies were characterized and purified for in vivo use as previously described and were diluted separately in 0.9% saline before intravenous administration at a concentration previously established to abolish gastrin-17-induced gastric acid secretion (22, 46).

Experimental design. In 48-h fasted rats, RX-77368 (25, 50, or 100 ng) or saline was injected intracranially under a brief anesthesia induced by inhalation of methoxyflurane (Pitman-Moore, Mundelein, IL). Rats were awakened within 2 min after removal from methoxyflurane exposure and were killed at 4 h after the intracranial injection. Time-course studies were performed using intracisternal injections of vehicle or RX-77368 (50 ng) and rats were killed at 30 min, 2 h, or 4 h after the injection. In one experiment, gastrin MAb or Gly-extended gastrin MAb (3 mg·0.5 ml−1·rat−1) was injected through the tail vein under methoxyflurane anesthesia at 30 min before intracisternal RX-77368 (50 ng) injection. In other groups, atropine (1 mg/kg ip) or vehicle (1 ml/kg ip) was injected 30 min before intracisternal injection of saline or RX-77368 (50 ng). At the end of each experiment, rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Blood samples (2 ml) were collected from the left cardiac ventricle for measurement of plasma gastrin levels. The stomach was opened along the great curvature and rinsed with ice-cold 0.9% saline, and corpus samples were taken for detection of HDC-containing ECL cells by immunohistochemistry and/or for measurement of HDC activity, respectively. Then rats were killed by cervical dislocation. Rats with food and water ad libitum and one group of 48-h fasted rats used for the morphological study were killed without intracranial injection. All experiments were performed between 8:30 AM and 3:00 PM.

Gastrin measurements. Blood was collected into ice-chilled tubes containing 0.04 ml of 7.5% EDTA solution. After centrifugation at 2,000 g for 10 min, plasma was separated and stored at −20°C until assay. Gastrin concentrations were determined as previously described (48) by a competitive RIA with gastrin antiserum (CURE no. 1160). Human gastrin-17 (Research Plus, Denville, NJ) was used as standard. Values are expressed as picomoles per liter plasma.

HDC activity determination. Oxyntic mucosa was weighed and homogenized (100 mg/ml) in 0.05 mol/l phosphate buffer (pH 7.0). The homogenate was then centrifuged at 10,000 g for 15 min. Aliquots of 80 µl supernatants were incubated at 37°C for 60 min with a cocktail containing L-[14C]histidine (50 nCi, New England Nuclear, Boston, MA), 5 × 10−2 M histidine, 10 × 10−3 M pyridoxal phosphate, and 10 × 10−2 M dithiothreitol in a total volume of 160 µl as described by Larsson et al. (19). The [14C]CO2 formed was trapped in 50 µl of Solvable (Packard Instrument, Downers Grove, IL) in filter paper during incubation and a reincubation for 50 min at room temperature after addition of 2 M citric acid. The radioactivity was measured by liquid scintillation counting. The HDC activity was expressed as picomoles of CO2 formed per milligram of protein per hour. Tissue protein contents were quantitated using Coomassie protein assay reagent (Pierce Chemical, Rockford, IL). In each series of assays, blanks containing all reagents, except that the tissue homogenate was replaced by 0.05 M phosphate buffer, were included and the blank values were subtracted from sample values.

Duplicate tests were run on all samples.

HDC immunohistochemistry. Tissue specimens taken from the oxyntic gland area were cut in strips and fixed by immersion in fixative containing 4% paraformaldehyde and 14% picric acid in 0.1 M PBS (pH 7.4) for 4 h. After immersion in 20% sucrose overnight at 4°C, tissues were frozen on dry ice and sectioned with a cryostat. Transverse sections (8 µm), including the full thickness of mucosa, were processed for the immunohistochemistry.

HDC immunostaining was performed using a rabbit polyclonal antibody (CURE no. 94211) raised against the COOH-terminal fragment (amino acids 603–615) of rat HDC (HDC-CT), which was prepared using a COOH-terminal peptide conjugated to keyhole limpet hemocyanin as previously described (7, 17). The working dilution of the antibody was 1:1,000. Tissue sections were primarily incubated with HDC antibody for 18 h at 4°C immediately followed by a 30-min incubation at 37°C. After washing in PBS, FITC-conjugated goat anti-rabbit antibody (Vector Laboratory, Burlingame, CA) was applied at room temperature for 60 min. In control sections, the HDC-CT antibody was inactivated by adding excess amounts of corresponding antigen of HDC-CT (100 µg/ml, Peptides Synthesis Core Facility, UCLA, Los Angeles, CA). Corpus HDC-CT immunoreactive cells were visualized and photographed under a Nikon labophot-2 fluorescent microscope (Nikon Instrument, Garden City, NY) with dark field. Immunofluorescent cells were counted in three randomly selected 2.4-mm2 fields in each section and expressed as the number of immunoreactive cells per squared millimeter.

Statistics. Data are expressed as means ± SE. Differences between means of HDC immunoreactive cells with different treatments were determined by Kruskal-Wallis nonparametric ANOVA followed by Dunn’s multiple comparisons test. All other comparisons between two groups were analyzed by Student’s t-test, whereas those among multiple groups by one-way ANOVA followed by Duncan’s contrast. P < 0.05 was considered statistically significant.

RESULTS

Effect of intracranial injection of RX-77368 on plasma gastrin levels and corpus HDC activity. Plasma gastrin levels in normally fed rats were about 110–120 pmol/l (31). After 48-h fast, gastrin levels were low in rats injected intracranially with saline (20–40 pmol/l). RX-77368 injected intracranially at 25, 50,
and 100 ng caused similar increases in plasma gastrin levels measured at 4 h after the injection (Fig. 1B). Time-course studies showed that RX-77368 injected intracisternally at 50 ng stimulates gastrin release to reach a peak at 30 min, which was threefold higher than the intracisternal saline-injected controls (137 ± 13 vs. 33 ± 5 pmol/l). Then plasma gastrin levels gradually decreased but remained significantly elevated at 2 and 4 h after the RX-77368 intracisternal injection (Figs. 1B and 2B).

The corpus HDC activity was barely detectable in 48-h fasted, intracisternal saline-injected rats. Intracisternal injection of RX-77368 at 50 ng significantly increased HDC activity in gastric corpus mucosa compared with saline injection in 48-h fasted conscious rats (Fig. 1A). A higher dose (100 ng) did not further increase the HDC activity response, whereas at a lower dose (25 ng) the change in HDC activity did not reach significance (Fig. 1A). At 50 ng the HDC activity increased significantly at 2 and 4 h after the intracisternal injection with 6- and 20-fold higher values than the saline-injected rats, respectively (20.8 ± 3.7 and 66.5 ± 15.0 vs. 3.2 ± 1.9 pmol CO₂·h⁻¹·mg protein⁻¹; Fig. 2A).

Effect of intracisternal injection of RX-77368 on HDC immunoreactivity in the gastric corpus mucosa. In normally fed rats, the ECL cells could be visualized immunocytochemically using the antibody against rat HDC-CT (Fig. 3A). Most HDC-CT immunoreactive cells were located at the basal third of the oxyntic glands, and a few cells were observed in the neck portion of the oxyntic gland. The number of HDC-CT immunoreactive cells in normally fed rats was 50.6 ± 2.5/mm² (n = 4).

Effect of intracisternal injection of RX-77368 on HDC immunoreactivity in the gastric corpus mucosa. In normally fed rats, the ECL cells could be visualized immunocytochemically using the antibody against rat HDC-CT (Fig. 3A). Most HDC-CT immunoreactive cells were located at the basal third of the oxyntic glands, and a few cells were observed in the neck portion of the oxyntic gland. The number of HDC-CT immunoreactive cells in normally fed rats was 50.6 ± 2.5/mm² (n = 4).

No HDC-CT immunoreactivity was detected when sections were incubated with antibody in the presence of excess amount of HDC-CT antigen (data not shown).

After a 48-h fast, HDC-CT immunoreactive cells had almost disappeared (Fig. 3B). The number of HDC-CT immunoreactive cells was reduced to 5.4 ± 1.4/mm² (P < 0.05, n = 4) in untreated rats. RX-77368 (25, 50, or 100 ng) injected intracisternally in 48-h fasted rats induced the reappearance of HDC-CT immunoreactive cells, which was notable at 2 h postinjection at doses of 50 and 100 ng (data not shown). The number of positive cells was significantly increased from 10.5 ± 3.7/mm² in saline-injected control group (n = 4) to 37.7 ± 3.0/mm² (n = 4), 49.1 ± 3.5/mm² (P < 0.05, n = 4), and 49.9 ± 3.9/mm² (P < 0.05, n = 3) at 4 h after intracisternal injection of RX-77368 at 25, 50, and 100 ng, respectively. The number of corpus HDC-CT immunoreactive cells at 4 h after 50 ng of RX-77368 intracisternal injection in 48-h fasted rats (49.1 ± 3.5/mm²) was similar to that observed in fed rats without intracisternal injection (50.6 ± 2.5/mm²; Fig. 3, A and C). Thereafter, the number of HDC-CT immunoreactive cells diminished but remained higher than the 48-h fasted basal levels even at 8 h after injection (data not shown).

Effect of gastrin MAb on increases of corpus HDC immunoreactivity and HDC activity induced by intracisternal RX-77368. Immunoneutralization of endogenous gastrin by gastrin MAb pretreatment (3 mg·0.5
ml⁻¹·rat⁻¹·iv, −30 min) completely prevented the intracisternal RX-77368 (50 ng)-induced appearance of HDC-CT immunoreactive cells (Fig. 3D) and the increase of HDC activity (Fig. 4) in the gastric corpus mucosa at 4 h after the intracisternal injection, compared with either Gly-extended gastrin MAb- or saline-pretreated groups (Fig. 4). Gly-extended gastrin MAb pretreatment under the same condition did not alter the rise in corpus HDC activity induced by intracisternal injection of RX-77368 (Fig. 4).

Effect of atropine on intracisternal RX-77368-induced increase in plasma gastrin levels. Atropine pretreatment (1 mg/kg ip, −30 min) in 48-h fasted rats did not influence plasma gastrin levels at 30 min after saline intracisternal injection (47 ± 8 vs. 52 ± 4 pmol/l; Fig. 5). Intracisternal injection of RX-77368 (50 ng) in atropine-pretreated rats still induced an increase in gastrin levels which was doubled (102 ± 11 pmol/l) compared with that of intracisternal saline group (47 ± 8 pmol/l, P < 0.05) at 30 min after intracisternal injection. However, the gastrin response in the atropine group was significantly lower (~26%) than in vehicle-pretreated rats receiving intracisternal RX-77368 (137 ± 13 pmol/l; Fig. 5). Atropine pretreatment (1 mg/kg ip, −30 min) significantly increased gastrin levels as well as corpus HDC activities at 4 h after intracisternal saline injection. The increases were similar to those induced by intracisternal RX-77368 (50 ng) in vehicle-pretreated rats (data not shown, n = 4).

DISCUSSION

Results obtained in the present study demonstrate that intracisternal injection of RX-77368 increases plasma gastrin levels, the number of corpus HDC-CT immunoreactive cells, and HDC activity in conscious 48-h fasted rats. These observations add new information on the regulation of gastric endocrine cells by...
Central TRH increases gastrin and corpus HDC activity

Fig. 4. Effect of gastrin MAb pretreatment on intracisternal RX-77368-induced increase in corpus mucosa HDC activity in conscious rats. Animals fasted 48 h were intravenously injected with either gastrin MAb or control antibody Gly-extended gastrin MAb (3 mg·0.5 ml⁻¹·rat⁻¹), and 30 min later saline or RX-77368 (50 ng) was injected intracisternally. HDC activity was measured 4 h after intracisternal injection. Each column represents means ± SE of number of rats indicated beside each column. *P < 0.05 compared with intracisternal saline-injected group. #P < 0.05 compared with intracisternal RX-77368 group and intravenous saline-intracisternal RX-77368 group.

Central TRH. Intracisternal TRH analog injected at 50 ng increased plasma gastrin levels by threefold at 30 min after the injection, and the levels were maintained significantly higher than saline intracisternally injected controls for the 4-h period. By contrast, in previous studies (40, 45, 46) performed in urethan-anesthetized or pylorus-ligated rats, plasma levels of gastrin increased <40% for only 5–10 min after intracisternal TRH or RX-77368, which did not result in a significant difference compared with the intracisternal saline group. In addition, the gastric histamine release in response to RX-77368 was also minimally reduced (0 to 35%) by gastrin MAb pretreatment (45, 46), suggesting that gastrin did not play a major role in central TRH-induced gastric histamine release, in agreement with the absence of rise in circulating gastrin (40). The lack of gastrin influence in mediating central TRH effect on histamine release in these previous studies may be related to the conditions of urethan anesthesia (40, 45, 46) and pylorus ligation (45). Urethan stimulates antral somatostatin release and synthesis (48), which may suppress gastrin response to intracisternal TRH analog as well as the action of gastrin on ECL cells (33), therefore emphasizing the direct cholinergic influence induced by central TRH. On the other hand, pylorus ligation reduces the relative contribution of gastrin due to the activation of cholinergic tone and the negative feedback regulation on gastrin release induced by constant low intraluminal gastric pH in this animal model (27). The present observations in intact conscious rats that intracisternal TRH analog increases plasma gastrin levels, added to the morphological evidence that the vagus nerve synapses with gastric cholinergic and peptidergic neurons innervating gastrin cells (2, 38), strengthen the role of central TRH in the regulation of antral gastrin release.

Because the elevation of plasma gastrin levels in response to intracisternal RX-77368 was only partially (26%) attenuated by atropine pretreatment, noncholinergic postganglionic component(s) of the vagus, such as gastrin-releasing peptide (GRP) (2, 38), may be primarily involved. Such a possibility is supported by the report that in isolated rat stomach model, gastrin secretion from G cells is induced by two routes: an ACh-mediated and a GRP-mediated route (23). In addition, electrical stimulation of the vagus (44) or omeprazole treatment (42) significantly increased plasma gastrin levels and the responses were blocked 50–70% by specific bombesin receptor antagonists. Other mechanisms mediating intracisternal RX-77368-induced gastrin release may involve an indirect regulation through reducing antral somatostatin inhibitory influence on G cells. It has been reported that fasting increases antral somatostatin synthesis and release due to unrestrained stimulation of antral D cell function by luminal acid, which leads to depression of G cell function (35) and results in decreases of plasma gastrin levels, corpus HDC immunoreactive cells, and HDC activity (30). Electrical vagal stimulation has been shown to reduce somatostatin release (15, 25), and therefore an inhibition in somatostatin release may be taken into account as mediating the gastrin response to the vagal activation induced by intracisternal TRH analog. The sympathetic nervous system may also play a role, because central injection of TRH, besides stimulating the vagal efferent activity (31), also increases the sympathetic nervous system outflows (28), and gastrin release is enhanced by activation of the sympathoadrenal system (4, 43). However, the neural-hormonal pathways through which central TRH induces gastrin release need to be further studied.
TRH analog injected intracisternally induced parallel increases in the appearance of corpus HDC immunoreactive cells and HDC activity, suggesting an increased enzyme protein and enzyme activity in the ECL cells after intracisternal TRH analog injection. The strong correlation between immunohistochemical detection of HDC using HDC-CT MAb and the measurement of mucosal HDC enzyme activity in the rat corpus have been observed in a previous study, showing a parallel decrease of the number of immunoreactive HDC-containing cells and HDC enzymatic activity during fasting and a parallel increase during subsequent refeeding (30). We had also established that the HDC-CT immunoreactivity colocalized with histamine immunoreactivity, indicating that the HDC detected by this antibody is located in the histamine-synthesizing ECL cells (30). These data, together with previous observations that intracisternal RX-77368 induced vagal-cholinergic dependent histamine release (45, 46), strongly support that the ECL cell function is influenced by central TRH.

The stimulation of HDC-CT immunoreactive ECL cells and HDC activity reached peak responses at 4 h after TRH analog intracisternal injection, which is consistent with previous findings showing that HDC activity is maximal at 3–4 h after refeeding in 48-h fasted rats (16). However, the kinetics of HDC response to RX-77368 was different from that of histamine release, which became significant at 20 min and reached a peak at 30 min after the intracisternal TRH analog injection at similar doses (45, 46). The differences in the time courses of histamine release and corpus HDC responses to intracisternal TRH analog suggest that there may be two phases of central TRH action on the ECL cells. First, an acute release of histamine from ECL cells, which occurs within 30 min and participates in the stimulation of gastric acid secretion (46), and a subsequent increase in histamine synthesizing enzyme in ECL cells, which reaches its peak at 4 h after the injection when the acid secretion already returned to basal levels.

Convergent evidence indicates that gastrin is involved in the mechanisms mediating the effect of central TRH analog on corpus HDC-containing ECL cells. Gastrin is well established to be the principal stimulator of ECL cell function (9). In the present study, gastrin MAb completely prevented the increase in corpus HDC immunoreactivity and activity induced by intracisternal injection of RX-77368. In addition, the rise in plasma gastrin levels began within 30 min in conscious rats, whereas HDC activity significantly increased at 2–4 h after intracisternal RX-77368. This is consistent with the time course studies showing maximal response of HDC activity at 2–4 h after the start of pentagastrin infusion (10, 16). Gastrin may initially stimulate histamine release, and the subsequent increase in HDC activity may be a result of the feedback regulation on HDC activity by reduced histamine contents in the ECL cells (1, 16). In addition, recent studies indicate that gastrin also directly regulates HDC promoter to enhance HDC gene expression (13, 14).

Atropine pretreatment (4.5 h before taking samples) and bilateral subdiaphragmatic vagotomy (28 h before) significantly increased plasma gastrin levels and corpus HDC activity at 4 h after intracisternal saline injection (data not shown), which impaired the assessment of vagocholinergic involvement in mediating the intracisternal RX-77368 action on the peak response of HDC activity. However, evidence from previous studies suggested that the early phase of ECL cell responses to intracisternal injection of RX-77368 is at least partly vagal-cholinergic dependent. This is supported by the observation that in urethan-anesthetized rats, M1 receptor antagonist and vagotomy completely blocked the histamine release as well as the acid response induced by intracisternal TRH analog, which was 62% mediated by histamine release (46). Second, gastrin antibody pretreatment did not prevent intracisternal TRH analog-induced rise in hepatic portal levels of histamine, whereas vagotomy and atropine did (46). Third, in a short-term 90–95% pure ECL cell culture system, ACh significantly stimulated basal and gastrin-driven histamine secretion via M1 receptor (34). Fourth, administration of gastrin antibody inhibited the increase in corpus HDC mRNA levels after refeeding for 6 h but not for 30 min (8). Likewise we recently obtained preliminary data showing that intracisternal RX-77368 increased corpus HDC mRNA levels at 30 min after the injection, which was prevented by atropine pretreatment but not by gastrin immunoneutralization (47). Recent findings that corpus ECL cells express PACAP receptors and receive PACAP innervation gave new insights on the possibility of direct neural regulation of ECL cells (12, 20, 21, 29, 49).

In summary, studies performed in conscious fasted rats showed that intracisternal TRH analog increased plasma gastrin levels, the number of corpus HDC-CT immunoreactive cells, and HDC activity. The responses of HDC to intracisternal TRH analog is gastrin dependent as shown by the abolition of the HDC responses by gastrin MAb. The increase of gastrin release was largely mediated by noncholinergic mechanisms, suggesting a role of vagal noncholinergic and/or sympathetic influence in the gastrin response to central TRH and associated regulation of HDC activity. Because medullary TRH, with its synthesizing neurons located in the caudal raphe nuclei and projecting to the dorsal motor nucleus of the vagus, plays a physiological role in the central autonomic regulation of gastrointestinal function, especially the cephalic phase of gastric acid secretion (41), the present observations provide a new model to assess the integrated neural and hormonal components regulating ECL cell function.

Paul Kirsch is acknowledged for assistance in the preparation of the manuscript and Debbie Avedian for technical assistance in gastrin radioimmunoassay.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-50255 (H. Yang), DK-17294 (J. H. Walsh), DK-30110 (Y. Taché), and DK-41301 (Antibody-Radioimmunoassay and Animal Cores), the National Institute of Mental Health Grant MH-00663 (Y. Taché), and Veterans Affairs Research Service Funding (J. H. Walsh).
REFERENCES


