Evaluation of early gastric mucosal permeability induced by central thyrotropin-releasing hormone administration

Takashi Joh,† Tadayuki Oshima,† Nobuo Takahashi, Hiroshi Kaneko, Makoto Sasaki, Hiromi Kataoka, Katsushi Watanabe, Masashi Sobue, Hideo Suzuki, Tomoyuki Nomura, Hirota Ohara, and Makoto Itoh†

†Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Sciences, Nagoya; and ‡Department of Internal Medicine, Division of General Medicine, Aichi Medical University School of Medicine, Aichi, Japan

Submitted 8 March 2004; accepted in final form 8 October 2004

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.

THE ROLE OF THE CENTRAL NERVOUS SYSTEM in the regulation of gastric function has long been recognized. Thyrotropin-releasing hormone (TRH), a stress-related neuropeptide originally isolated from the hypothalamus (1, 10), or its stable analog pGlu-His-(3,3′-dimethyl)-ProNH₂ (RX-77368) (RX-77368), we measured the blood-to-lumen ⁵¹Cr-labeled EDTA clearance and examined the effects of vagotomy, atropine, omeprazole, and hydrochloric acid (HCl) on RX-77368-induced mucosal permeability. A cytoprotective dose of RX-77368 (1.5 ng) did not increase mucosal permeability. However, higher doses significantly increased mucosal permeability. Permeability peaked within 20 min and gradually returned to control levels in response to a 15-ng dose (submaximal dose). Increased mucosal permeability was not recovered after a 150-ng dose (ulcerogenic dose). This increase in permeability was inhibited by vagotomy or atropine. Intragastric perfusion with HCl did not change the RX-77368 (15-ng)-induced increase in permeability, but completely inhibited the recovery of permeability after the peak. Pretreatment with omeprazole did not change the RX-77368 (15-ng)-induced increase in permeability, but quickened the recovery of permeability after the peak. These data indicate that the RX-77368-induced increase in permeability is mediated via the vagal-cholinergic pathway and is not a secondary change in RX-77368-induced acid secretion. Inhibited recovery of permeability on exposure to an ulcerogenic RX-77368 dose or on exposure to HCl plus a submaximal dose of RX-77368 may be crucial for the induction of gastric mucosal lesions by central RX-77368 administration.

intracisternal injection; ⁵¹Cr-labeled EDTA clearance

THE ROLE OF THE CENTRAL NERVOUS SYSTEM in the regulation of gastric function has long been recognized. Thyrotropin-releasing hormone (TRH), a stress-related neuropeptide originally isolated from the hypothalamus (1, 10), or its stable analog pGlu-His-(3,3′-dimethyl)-ProNH₂ (RX-77368) (RX-77368) has been reported to act in the brain to stimulate gastrointestinal secretion, motility, transit, and ulcer formation in conscious or anesthetized rats, rabbits, and cats (14, 15). Many studies (14, 15) indicate that TRH actions on gut function are mediated through activation of the parasympathetic outflow and peripheral muscarinic receptors. TRH or stable TRH analogs injected into the cisterna magna show cytoprotective (19, 25) and ulcerogenic effects (3, 14) on gastric mucosa (17, 18). These stress-related effects on gastric mucosa depend on the balance between vagally activated increases in ulcerogenic agents including acid secretion (16) and cytoprotective factors such as prostaglandins (25) and heat shock proteins (8).

Mucosal permeability plays a key role in regulation of gastric integrity. Although the vagal cholinergic-mediated effects of gastric acid secretion or mucosal blood flow on gut function are well characterized in response to intracisternal injection of TRH (16, 20), little is known about the effects of central RX-77368, a stable TRH analog, on gastric mucosal permeability at cytoprotective (1.5 ng) and ulcerogenic doses (15–150 ng), especially during the first 60 min after intracisternal injection of RX-77368. In the present study, we examined effects of intracisternal injection of RX-77368 on gastric mucosal permeability in vivo by measuring the blood-to-lumen ⁵¹Cr-labeled EDTA clearance, which has been developed to assess the mucosal permeability and has been shown to be an extremely sensitive index of mucosal damage (4–7, 11, 22). We also examined effects of vagotomy, atropine, luminal perfusion with hydrochloric acid (HCl), or omeprazole on RX-77368-induced changes in mucosal permeability.

MATERIALS AND METHODS

Drugs

The following substances were used. The stable TRH analog RX-77368 (Reckitt & Colman, Kingston-upon-Hill, UK) in powder form was dissolved in 0.5% bisinchoninic acid (BSA) and 0.1% acetic acid at an initial concentration of 4 mg/ml and kept at −20°C. The required doses were made by dilution of the stock solution with PBS immediately before experiments. Omeprazole, a selective H⁺-K⁺-ATPase inhibitor in parietal cells, was kindly provided by AstraZeneca (Molndal, Sweden). Omeprazole was dissolved in 100% DMSO (Sigma, St. Louis, MO), and the stock solution (30 mg/ml) was diluted in bicarbonate buffer (0.56 mg/ml) before experiments. Atropine sulfate was obtained from Sigma.

Animal Preparation

Male Sprague-Dawley rats weighing 250–350 g (Japan SLC, Hamamatsu, Japan) were fasted overnight and anesthetized with intraperitoneal urethane (0.6 g/kg, Tokyo Kasei Kogyo, Tokyo, Japan) and α-chloralose (0.12 g/kg; Wako Pure Chemical Industries, Tokyo,

Address for reprint requests and other correspondence: T. Joh, Dept. of Internal Medicine and Bioregulation, Nagoya City Univ. Graduate School of Medical Sciences, 1 Kawasumi, Mizuho, Nagoya, 467-8601, Japan (E-mail: tjiang@med.nagoya-cu.ac.jp).
Fig. 1. Effect of intracisternal injection of thyrotropin-releasing hormone (TRH) on gastric mucosal permeability. The TRH-induced changes in mucosal permeability were assessed by measurement of $^{51}$Cr-labeled EDTA clearance 60 min after intracisternal injection of TRH. At a 1.5-ng TRH dose ($\oplus$), the mucosal permeability was not significantly increased. However, mucosal permeability consistently increased after intracisternal injection of 15 ($\bullet$) and 150 ng ($\ominus$) TRH. Permeability was still significantly elevated 60 min after intracisternal injection of TRH (90 min on the figure) at the highest dose (150 ng). Each value represents the mean ± SE of each group (n = 5). *P < 0.01, *P < 0.05 vs. baseline (the stabilization period before injection).

Assessment of Mucosal Integrity (Measurement of $^{51}$Cr-Labeled EDTA Clearance)

After an abdominal incision was made, the renal blood vessels were ligated to prevent loss of the low-molecular-weight tracer used to assess gastric mucosal permeability. The stomach was exteriorized, and a ligature was placed around the pylorus. An incision was made in the forestomach, and a double-lumen cannula (outer cannula, 3.25 mm in diameter; inner cannula, 1 mm in diameter) was inserted into the stomach and secured to the forestomach with a ligature. The stomach was perfused with PBS or a solution through the inner cannula at a rate of 1.0 ml/min, and the effluent was collected via the outer cannula. Samples of gastric perfusate were collected at 5-min intervals to monitor $^{51}$Cr-labeled EDTA clearance (6). In a series of experiments, the pH of gastric perfusate was measured by using a pH meter (Horiba, Kyoto, Japan). The head was placed on an ear bar of a stereotaxic apparatus. The dura mater covering the foramen magnum was exposed. A small pin hole was made into the membrane with a 25-gauge needle 1–1.5 mm distal to the caudal edge of the occipital bone. A polyethylene tube (PE-10) was carefully inserted through the pin hole into the cisterna magna. The tube (10 cm; 7 µl dead space) was filled with saline. Successful cannulation into the cisterna magna was verified by the leakage of cerebrospinal fluid, and the open end of the catheter was then connected to a 50-µl Hamilton syringe. A tracheotomy was performed, and PE-240 tubing was inserted into the trachea to maintain an open airway. The right femoral artery and vein were cannulated (PE-50) for monitoring systemic arterial pressure and for administration of $^{51}$Cr-labeled EDTA (PerkinElmer Life & Analytical Sciences), respectively. Body temperature was monitored by a rectal thermometer and maintained at 37°C with a heating pad and lamp throughout the experiment. The studies were approved by the Animal Research Committee of the Nagoya City University Graduate School.

Experimental Protocols

In all experiments, gastric mucosal permeability was continuously monitored by measuring $^{51}$Cr-labeled EDTA clearance. Once steady-state clearances were obtained (20–30 min), an additional 30-min perfusion served as a control. Changes in mucosal permeability were assessed by measurement of $^{51}$Cr-labeled EDTA clearance 60–90 min after intracisternal injection of RX-77368. In each of the studies, the treatments were administered in a randomized fashion. Randomization of the treatments was performed by an assistant who did not know the results of the studies.

Effect of intracisternal injection of RX-77368 on gastric mucosal permeability. A 10-µl aliquot containing 1.5, 15, or 150 ng/300 g rat of RX-77368 was injected intracisternally.

Effect of vagotomy and atropine. In another series of experiments, subdiaphragmatic vagotomy was performed by transection of the esophagus between two ligatures immediately below the diaphragm before positioning the gastric cannula. Vagotomy or sham operation was performed 1 h before RX-77368 (15 ng) injection. Thirty minutes after subcutaneous (sc) vehicle or atropine (2 mg/kg) injection. Thirty mula: (PR × $^{51}$Cr-labeled perfusate × 100)/$^{51}$Cr-labeled plasma × stomach weight), where PR is the perfusion rate (milliliters per minute). $^{51}$Cr-labeled perfusate is the radioactivity in the perfusate (in counts per minute (cpm) per milliliter), and $^{51}$Cr-labeled plasma is the radioactivity in the blood plasma (cpm per milliliter). EDTA clearance was expressed in milliliters per minute per 100 grams and the stomach weight was measured in grams (6).
minutes after subcutaneous (sc) vehicle or atropine (2 mg/kg) injection, RX-77368 (15 ng) was injected intracisternally.

**Effect of omeprazole.** Vehicle or omeprazole (40 mg/kg) was injected subcutaneously. At 60 min after omeprazole, RX-77368 (15 ng) was injected intracisternally.

**Effect of luminal perfusion with 0.05 N HCl.** The stomach was perfused with PBS or a solution containing PBS and 0.05 N HCl through the inner cannula at a rate of 1.0 ml/min throughout the experiment. After being stabilized for 30 min, the rats were injected intracisternally with RX-77368 (15 ng).

**Statistical Analysis**

All values are expressed as means ± SE. Unpaired t-tests were used to compare mean values between groups. ANOVA and Duncan’s test were employed for comparison of mean values among three or four groups. Significance was accepted at $P < 0.05$.

**RESULTS**

**Effect of Intracisternal Injection of RX-77368 on Gastric Mucosal Permeability**

A cytoprotective dose (1.5 ng) (25) of RX-77368 did not increase gastric mucosal permeability. However, a dose of 15 ng (submaximal dose) consistently increased mucosal permeability immediately after intracisternal injection of RX-77368 ($P < 0.05$). The permeability peaked within 20 min and gradually returned to control levels within 60 min. A dose of 150 ng (ulcerogenic dose) increased mucosal permeability ($P < 0.01$). However, levels did not return to normal during the 60 min of monitoring (Fig. 1). Gross erosion was not observed at 60 min under any condition.

**Effect of Vagotomy and Atropine on RX-77368-Induced Changes in Mucosal Permeability**

Vagotomy or sham operation was performed 1 h before RX-77368 injection. Vehicle or atropine sulfate (2 mg/kg sc) was administered 30 min before RX-77368 injection. Changes in mucosal permeability in response to vagotomy or atropine pretreatment were assessed by measurement of $^{51}$Cr-labeled EDTA clearance 90 min after intracisternal injection of RX-77368. The RX-77368 (15 ng)-induced increase in permeability was completely blocked by vagotomy ($P < 0.05$) and was significantly blocked by atropine ($P < 0.05$) (Fig. 2).

**Effect of Luminal Perfusion with 0.05 N HCl on RX-77368-Induced Changes in Mucosal Permeability**

Effects of RX-77368 (15 ng) on perfusion with 0.05 N HCl were tested. After luminal perfusion with 0.05 N HCl or PBS, RX-77368 (15 ng) was intracisternally injected into the rats after a 30-min stabilization period. Intragastric perfusion with 0.05 N HCl did not change either the initial $^{51}$Cr-labeled EDTA clearance before RX-77368 injection or clearance during the initial phase, which included the peak value 20 min after RX-77368 administration. However, intragastric perfusion with 0.05 N HCl completely inhibited the recovery of permeability after the peak ($P < 0.05$) (Fig. 3).

**Effect of Omeprazole on 15 ng RX-77368-Induced Changes in Mucosal Permeability**

Omeprazole (40 mg/kg sc) was injected subcutaneously 60 min before RX-77368 injection. Pretreatment with omeprazole...
did not change the basal pH before RX-77368 injection, and no changes in pH were observed after intracisternal RX-77368 (15 ng) injection in omeprazole-treated rats. However, pH decreased from 7 to 3 after RX-77368 (15 ng) injection in rats receiving PBS-only luminal perfusion ($P < 0.05$) (Fig. 4).

Pretreatment with omeprazole did not change the initial $^{51}$Cr-labeled EDTA clearance before RX-77368 injection. Intragastric perfusion with PBS and pretreatment with omeprazole (subcutaneously) did not change the permeability during the initial phase, including the peak value 20 min after RX-77368 injection, but pretreatment with omeprazole quickened the recovery of permeability after the peak ($P < 0.05$) (Fig. 5).

DISCUSSION

TRH has been convincingly established by several groups of investigators as a central vagal stimulator of acid secretion in rats and cats (15). TRH or TRH analogs injected into the cisterna magna increase efferent activity in the gastric branch of the vagus nerve (8a) and increases vagal muscarinic-dependent stimulation of gastric acid, pepsin, histamine, and serotonin secretion, mucosal blood flow (MBF), emptying, and contractility (2, 12, 13, 15, 23, 24). Gastric acid secretion peaks within 20–30 min after TRH injection and returns to the preinjection level in 90–120 min (13, 20). Four hours after TRH or RX-77368 injection into the cisterna magna, erosion appeared on the gastric mucosa but was mitigated by cimetidine or omeprazole (3). These data indicate that gastric acid is an import factor contributing to the ulcerogenic effect of TRH. However, little is known about the early phase (within 60 min) of gastric mucosal change after intracisternal injection of RX-77368, because macroscopic changes are not observed in that period. We report herein the first observations of gastric mucosal damage within 60 min of RX-77368 injection as measured by gastric mucosal permeability using the blood-tolumen $^{51}$Cr-labeled EDTA clearance technique, which has been shown to be an extremely sensitive index of mucosal damage (4–7, 11, 22).

In the present study, RX-77368 increased mucosal permeability in a dose-dependent manner after intracisternal injection (Fig. 1). A cytoprotective dose (1.5 ng) of RX-77368 (25) did not increase mucosal permeability. It has been also reported that such a low dose of TRH analog (1.5 ng) does not increase gastric acid secretion (25). On the other hand, permeability increased and peaked within 20 min of dosing with 15 and 150 ng RX-77368. Permeability gradually returned to control levels within 60 min of administration of the 15- ng dose. However, at the highest dose (150 ng), permeability did not return to baseline levels within 60 min. Previous reports indicated that enhancement of both gastric acid secretion and MBF by intracisternal TRH or RX-77368 injection was abolished by subdiaphragmatic vagotomy (20) or blockade of cholinergic receptors by atropine (25). Even in our present study, the RX-77368-induced increase in permeability was completely abolished by vagotomy and was significantly blocked by atropine (Fig. 2). These data indicate that the effect of RX-77368 was mediated via the vagal-cholinergic pathway. In our experimental protocol, the luminal concentration of HCl actually decreased, because PBS was continuously perfused intragastrically. We then performed experiments in the presence of intragastric perfusion with 0.05 N HCl, a concentration that is similar to physiological conditions. Intragastric perfusion with 0.05 N HCl did not change the clearance during the initial phase, including peak value 20 min after RX-77368 injection, but completely inhibited the recovery of permeability after peak (Fig. 3). These data indicate that intracisternal RX-77368 injection induces first, an increase in gastric mucosal permeability and second, macroscopic lesions in the presence of acid (3).

Although omeprazole had no effect on the increase in clearance during the first 20 min, acid secretion was completely inhibited (Fig. 4) and RX-77368-induced permeability was significantly attenuated in the later period, beginning 30 min after RX-77368 treatment (Fig. 5). We measured the pH of perfusate every 5 min (Fig. 4), and these data were consistent with previous reports (16) on gastric acid secretion. These data strongly suggest that the mechanism responsible for RX-77368-induced increases in permeability is composed of at least two phases: 1) an initial phase, which includes the peak value 20 min after RX-77368 intracisternal injection and which is independent of acid secretion; and 2) the recovery, which is dependent on acid secretion. Sustained increases in acid secretion induced by RX-77368 are important for ulcerogenic effects of the peptide. Although pepsin or histamine secretion, mast cell, or contractility may be related to the initial increase in RX-77368-induced gastric mucosal permeability (2, 9, 23, 24), the specific factors that are related to the initial increase in permeability remain to be investigated in detail.

In conclusion, RX-77368 at ulcerogenic doses increased gastric mucosal permeability, which is mediated via the vagal-cholinergic pathway and is not a secondary change to RX-77368-induced acid secretion. Inhibited recovery of permeability on exposure to ulcerogenic doses of RX-77368 or exposure to HCl plus the submaximal dose of RX-77368 may be crucial for the induction of gastric mucosal lesions by intracisternal injection of a TRH analog.

REFERENCES


