Central Nervous System Action of Thyrotropin-Releasing Hormone to Increase Gastric Mucosal Blood Flow in the Rat

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The central nervous system effects of thyrotropin-releasing hormone (TRH) on gastric acid secretion and mucosal blood flow were studied in rats. Corpus mucosal blood flow was measured by the hydrogen gas clearance technique and acid output by a continuous gastric perfusion method in fasted, urethane-anesthetized rats. Thyrotropin-releasing hormone (1 or 5 μg) injected into the cerebral lateral ventricle induced concomitant increases in gastric acid secretion and mucosal blood flow. Intravenous infusion of step doses of TRH (60 and 180 μg/kg · h) had no effect on these parameters. Bilateral vagotomy and atropine (0.15 mg/kg) completely blocked the effects of intracerebroventricular injection of TRH (5 μg) on gastric acid secretion and mucosal blood flow. In contrast, intravenous omeprazole (20 μmol/kg) completely inhibited the increase in gastric acid secretion but not the increase in mucosal blood flow elicited by intracerebroventricular administration of TRH (5 μg). These results demonstrate that TRH acts in the brain to stimulate gastric acid secretion and mucosal blood flow through vagal dependent pathways and peripheral muscarinic receptors. Part of the effect of central TRH on gastric mucosal blood flow is not secondary to the stimulation of acid secretion and appears to represent a direct cholinergic vasodilatory response.

The role of the central nervous system in the regulation of gastric function has long been recognized (1). Recently, a growing number of neuropeptides has been shown to influence gastric secretory and motor function through central nervous system-mediated modulation of the autonomic nervous system (2,3). Among these peptides, thyrotropin-releasing hormone (TRH), a tripeptide originally isolated from the hypothalamus (4), has been reported to act in the brain to stimulate gastrointestinal secretion, motility, transit, and ulcer formation in conscious or anesthetized rats, rabbits, or cats (3,5,6). Convergent pharmacologic, surgical, and electrophysiologic evidence indicates that TRH actions on gut function are mediated through activation of the parasympathetic outflow and peripheral muscarinic receptors (5,6). These neuropharmacologic observations are well correlated with neuroanatomic studies demonstrating high levels of TRH-like immunoreactivity and TRH receptors in hypothalamic (paraventricular nucleus) and medullary nuclei (dorsal vagal complex, nucleus ambiguus) (7–12).

Although it is well established that gastric mucosal blood flow (MBF) is altered by electrical stimulation of the vagus nerves in various species (13–15), little is known about the brain peptidergic regulation of gastric MBF as compared with gastric motility (3) or acid secretion (2). Okuma et al. (16) reported a dose-dependent increase in MBF and acid secretion after central injection of TRH. However, the aminopyrine clearance technique used for measuring blood flow precludes definite conclusions as the technique is known to reflect changes in acid secretion as well as gastric MBF. The limitations of this technique have been reviewed recently (17).

The aims of the present study were to investigate the following hypotheses: (a) the injection of TRH into the intracerebroventricular system of anesthetized rats increases gastric MBF, as determined by a technique known to reflect only changes in gastric...
blood flow (hydrogen gas clearance technique); and (b) TRH action is mediated by vagal-cholinergic pathways and is independent of enhanced gastric acid secretion.

Materials and Methods

Animals

Male Sprague-Dawley rats (Hilltop, Scottsdale, Pa.) weighing 200–300 g were housed under conditions of controlled temperature (20 ± 1°C) and lighting (6 AM–6 PM). They were maintained ad libitum on Purina Laboratory Chow (Ralston Purina, St. Louis, Mo.) and tap water for a few days, then fasted overnight with free access to water. All experiments were performed in rats anesthetized with urethane (1.25 g/kg) given subcutaneously.

Drugs and Treatment

The following substances were used: TRH (Peninsula Laboratories, Belmont, Calif.), atropine sulfate solution (Lilly, Indianapolis, Ind.), and omeprazole (Ab Hassle, Mölndal, Sweden). Thyrotropin-releasing hormone, in lyophilized form, was dissolved in 0.9% saline, containing 0.1% bovine serum albumin. Atropine was diluted in saline and omeprazole was dissolved in 70% ethanol, and this stock solution (10 mg/ml) was diluted in bicarbonate buffer (0.56 mg/ml) before the experiment.

Drugs were administered without disturbing the animal preparation using brain guide cannula and intravenous catheters positioned before performing gastric surgery. Rats anesthetized with urethane were mounted on ear bars of a stereotaxic apparatus and the guide cannula (20-gauge needle protruding 4 mm from a stainless steel parallelipped that was 7 mm long, 6 mm wide, and 4 mm high) was inserted through the skull into the lateral ventricle using the following coordinates from the bregma: 0.8 mm posterior and 1.5 mm lateral. The stainless steel parallelipped was secured to the skull with glue (Krazy Glue, Pen) and the orifice of the guide cannula was closed by inserting a 22-gauge needle. At the time of the injection, the needle was removed and an L-shaped stainless steel cannula (22 gauge) connected via polyethylene tubing to a 50-μl Hamilton microliter syringe was inserted into the guide cannula (tip resting 1 mm below that of the guide cannula). Intracerebroventricular injections of TRH or vehicle were administered by pressure ejections in 10 μl. The accuracy of the lateral ventricle injections was verified at the end of each experiment by injecting methylene blue under the same conditions.

The intravenous administrations were performed through a 23-gauge needle inserted into one of the femoral veins. Intravenous infusions were given at a constant rate of 1.8 ml/h or bolus injections in 0.5 ml.

Measurements of Mean Arterial Blood Pressure, Gastric Acid Secretion, and Gastric Mucosal Blood Flow

After positioning the guide cannula for intracerebroventricular injection, a tracheotomy was performed and PE-260 tubing was inserted into the trachea to ensure a patent airway for administration of 3% hydrogen in air. Rats were not ventilated by a respirator but were allowed to breathe the gas on their own. The right carotid artery was cannulated with PE-50 tubing for monitoring systemic blood pressure. Results are expressed as mean arterial blood pressure in millimeters of mercury.

For the measurement of gastric acid secretion, a laparotomy was performed and the stomach exteriorized. An incision was made in the forestomach and the gastric content was gently washed out with physiologic saline at room temperature. The pylorus was ligated and a double-lumen cannula (diameters of the outer Tygon tubing and inner polyethylene catheter were 7 and 2 mm, respectively) was inserted through the incision in the forestomach and secured there by a ligature. Physiologic saline at room temperature was infused via the inner cannula at a rate of 0.8 ml/min. Gastric effluent was collected continuously by flow drainage from the outer tubing and separated in 15-min collection periods. Acid output of the perfusate collected for 15 min (micromoles per 15 min) was determined by titration with 0.1 N NaOH to pH 7.0 with an automatic titrator (Radiometer, Copenhagen, Denmark).

For the measurement of gastric MFB, a limited excision (~3 mm in diameter) was made into the serosa and muscularis externa of the anterior corpus to expose the submucosa and the basal part of the mucosa. A ring-shaped platinum electrode was placed in contact with the exposed basal portion of the mucosa to measure the deep MFB (18). The diameters of the platinum wire and ring were 125 μm and 1 mm, respectively. An Ag-AgCl reference electrode was placed inside the peritoneal cavity. Gastric MFB was measured using the hydrogen gas clearance technique and expressed in milliliters per minute per 100 g. The use of the hydrogen gas clearance technique for measurement of gastric MFB has been validated previously (19). The 3% hydrogen in air was premixed in a cylinder and administered via a tube placed over the tracheotomy tubing (10). Current is generated at the surface of the platinum electrode by oxidation of molecular hydrogen to hydrogen ions and electrons. The magnitude of this current, measured using a polarographic and amplifying unit (Val Tech Electronics, Sherman Oaks, Calif.), is proportional to the concentration of molecular hydrogen in contact with the electrode. When rats breathe the 3% hydrogen in air, the current increases and reaches a plateau in about 10–15 min, indicating that the tissue is saturated with hydrogen. When the source of hydrogen is removed, the current tracing gradually falls and returns to baseline. As hydrogen can be removed only by blood flow perfusing the tissue, the rate at which desaturation occurs provides an estimate of blood flow. For uniformity, saturation and desaturation curves were recorded for 15 min each. The desaturation curves were analyzed by a computerized monoeponential program previously described (20). Data were collected and stored online on a floppy disk using an ADALAB analog to digital converter and an Apple IIe computer. Gastric MFB was then determined by a computer program that involved multiple iterations of a nonlinear least-squares regression.
Experimental Design

Throughout the experiment, temperature was monitored by a rectal thermometer and maintained at 36°–37°C by heat lamp. Each study was performed on separate groups of rats.

Study 1. Effect of intracerebroventricular injection of thyrotropin-releasing hormone on gastric acid secretion and corpus mucosal blood flow. After a 15-min stabilization period, basal MBF was measured. Then, TRH (1 or 5 μg) or vehicle was injected intracerebroventricularly. Corpus MBF was measured twice during the following hour (15–30 and 45–60 min after the injection), gastric acid output was determined every 15 min, and mean arterial blood pressure was recorded continuously.

Study 2. Effect of intravenous injection of thyrotropin-releasing hormone on gastric acid secretion and corpus mucosal blood flow. Physiologic saline was infused intravenously during the first 45 min of the experiment. Then, each animal received an intravenous infusion of vehicle followed by step doses of TRH (60 and 180 μg/kg·h). The duration of each infusion was 30 min. Corpus MBF was measured during the last 15 min of each infusion. Gastric acid output was measured every 15 min. Mean arterial blood pressure was recorded continuously throughout the experiment.

Study 3. Effect of intracerebroventricular injection of thyrotropin-releasing hormone on gastric acid secretion and corpus mucosal blood flow in vagotomized rats. Subdiaphragmatic vagotomy was performed by transection of the esophagus between two ligatures immediately below the diaphragm before positioning the gastric cannula and the platinum electrode. After basal MBF measurement, vagotomized rats received 5 μg of TRH or vehicle intracerebroventricularly and MBF was monitored during the 15–30- and 45–60-min periods after the injection. Gastric acid secretion was measured every 15 min and mean arterial blood pressure was monitored continuously.

Study 4. Effect of atropine and omeprazole on intracerebroventricular thyrotropin-releasing hormone-induced stimulation of gastric acid secretion and mucosal blood flow. After determination of basal acid secretion and MBF, atropine (150 μg/kg), a cholinergic muscarinic receptor antagonist, omeprazole (20 μmol/kg), a specific inhibitor of the hydrogen-potassium-stimulated adenosine triphosphatase of the parietal cells, or vehicle (ethanol/bicarbonate) was slowly injected intravenously over 5 min. Fifteen minutes later, TRH (5 μg) was injected intracerebroventricularly. Hydrogen gas clearance curves were generated during the 15–30-min and 45–60-min periods after TRH injection. Gastric acid secretion was measured every 15 min and mean arterial blood pressure was recorded continuously.

Data Analysis

Results

Study 1. Effect of Intracerebroventricular Injection of Thyrotropin-Releasing Hormone on Gastric Acid Secretion and Corpus Mucosal Blood Flow

Intracerebroventricular injection of TRH at 1 or 5 μg increased both gastric acid secretion and MBF, whereas vehicle had no significant effect (Figure 1). The peak increments of gastric acid secretion and MBF were observed 30 min after TRH injection. Gastric acid secretion was still significantly elevated 60 min after intracerebroventricular injection of the peptide at both doses. The elevation of gastric MBF was also maintained for 60 min but only at the highest TRH dose (Figure 1).

Injection of TRH into the lateral ventricle induced within 1 min a 10%–15% significant peak increase in mean arterial blood pressure (basal: 108 ± 6 mmHg, 1 μg TRH: 122 ± 5 mmHg, n = 7, p < 0.05; basal: 105 ± 5 mmHg, 5 μg TRH: 119 ± 6 mmHg, n = 7, p < 0.05; basal: 107 ± 7 mmHg, vehicle: 106 ± 7 mmHg, NS, n = 7; Wilcoxon test). The hypertensive effect...
Study 2. Effect of Intravenous Injection of Thyrotropin-Releasing Hormone on Gastric Acid Secretion and Corpus Mucosal Blood Flow

Intravenous infusion of vehicle followed by step doses of TRH (60 and 180 µg/kg·h) did not influence gastric MBF as compared with basal values (Figure 2). Basal gastric acid secretion was low in urethane-anesthetized rats and tended to decrease spontaneously during the course of the experiment. This decrease was observed at the beginning of the experiment and during vehicle infusion and was not further changed following step-dose infusion of TRH (Figure 2). Intravenous infusion of TRH did not significantly modify mean arterial blood pressure (data not shown).

Study 3. Effect of Intracerebroventricular Injection of Thyrotropin-Releasing Hormone on Gastric Acid Secretion and Corpus Mucosal Blood Flow in Vagotomized Rats

In subdiaphragmatic vagotomized rats, intracerebroventricular injection of TRH (5 µg) had no effect on gastric MBF and acid secretion when compared with the vehicle-treated group (Figure 3). Vagotomy did not suppress the transient hypertensive effect elicited by intracerebroventricular TRH. A 19.6% ± 2% significant (p < 0.01, Wilcoxon test) peak increase in mean arterial blood pressure was observed immediately after TRH injection (89 ± 3 mmHg before and 92 ± 2 mmHg after intracerebroventricular injection of vehicle, n = 7, and 94 ± 5 mmHg before and 113 ± 5 mmHg after intracerebroventricular injection of 5 µg of TRH, n = 7); thereafter values returned to control levels within 15–20 min (data not shown).

Study 4. Effect of Atropine and Omeprazole on Intracerebroventricular Thyrotropin-Releasing Hormone-Induced Stimulation of Gastric Acid Secretion and Mucosal Blood Flow

Both atropine (150 µg/kg) and omeprazole (20 µmol/kg) completely prevented the increase in gastric acid secretion elicited by 5 µg of TRH injected intracerebroventricularly (Figure 4). However, whereas atropine also blocked the effect of TRH on gastric MBF, a persistent significant increase in MBF from 31 ± 2 to 45 ± 8 ml/min·100 g body wt was observed in the omeprazole-pretreated group (Figure 4). Atropine and omeprazole did not influence the transient hypertensive effect of TRH. Mean arterial blood pressure in vehicle- (n = 6), atropine- (n = 6), or omeprazole- (n = 5) pretreated rats was 102 ± 7, 97 ± 4, and 92 ± 4 mmHg, respectively, before and 120 ± 8, 124 ± 4, and 110 ± 7 mmHg after intracerebroventricular injection of 5 µg of TRH (p < 0.05, Wilcoxon test, compared with respective basal values).

Discussion

The present study demonstrates that the intracerebroventricular injection of TRH (3–14 nmol) stimulates gastric MBF as determined by a technique known to measure only blood flow. Peptide action
was prolonged for up to an hour after injection. By contrast, TRH infused intravenously at doses equal to or higher than doses effective when given intracerebroventricularly did not alter gastric MBF. These results indicate that the stimulation of gastric MBF induced by intracerebroventricular TRH is mediated through the central nervous system. In agreement with previous findings (16,21,22), we also observed marked and long-lasting concomitant stimulation of gastric acid secretion in response to the intracerebroventricular injection of TRH. The enhancement in both gastric acid secretion and MBF involved vagal-cholinergic mechanisms as the stimulatory effect was abolished by subdiaphragmatic vagotomy or blockade of cholinergic receptors by atropine. These findings further support the role of vagal cholinergic pathways in the gastric response to centrally administered TRH. Vagotomy and atropine were previously reported to suppress the stimulation of gastric acid secretion, emptying, and motility induced by TRH injected into the cerebrospinal fluid or dorsal vagal complex (23–26). Electrophysiologic studies have provided evidence that intracerebroventricular or intracisternal injection of TRH stimulates cervical and gastric vagal efferent discharges in the rat (27,28). Moreover, mapping studies using microinjection of TRH into discrete brain nuclei have further identified the dorsal vagal complex and nucleus ambiguous as sites of action for TRH-induced vagally mediated increase in gastric acid secretion and contractility in rats and cats (16,23,29–32). The dorsal vagal complex has also been identified as a site of action for TRH-induced stimulation of gastric MBF measured by the aminopyrine clearance technique (16). As >90% of the preganglionic neurons in the dorsal motor nucleus of the vagus contribute projections to the stomach through the descending branch of the vagi (33,34) and 65% of the total medullary TRH immunoreactivity and high concentrations of TRH receptors have been located in the nuclei of the dorsal vagal complex (8,10,11), TRH may play a physiologic role in mediating the parasympathetic activation of gastric function associated with cephalic stimuli or vago-vagal reflexes.

Previous studies indicate that electrical stimulation of the vagus nerves increases gastric MBF. Martinson (13) reported that electrical stimulation of high threshold vagal fibers induced a concomitant augmentation of gastric MBF and acid secretion in the cat. After atropine administration, a persistent increase in gastric MBF up to 30% was still observed during high threshold stimulation, whereas acid secretion was completely blocked (13). Using an in vivo microscopy technique in the rat, Guth and Smith (14) observed vasodilation of gastric submucosal arterioles within 10 s following electrical vagal stimulation. Such a rapid onset of the vascular response strongly suggests a direct vasodilator effect of vagal stimulation not related to the stimulation of gastric acid secretion (14). The atropine-resistant vascular response to electrical vagal stimulation was confirmed in rats using a cross-thermocouple technique (15) to measure gastric MBF and in vivo microscopy (35). In the present study, the increase in gastric MBF induced by central injection of TRH was completely abolished by atropine pretreatment. Swan and Jacobson (36) used insulin and 2-deoxy-D-glucose to produce vagal activation in the dog. The augmentations of gastric blood flow and acid secretion were both totally blocked by atropine. Taken together, these data suggest that different groups of vagal fibers may be activated by electrical stimulation of the peripheral vagus and by central vagal activation using intracerebroventricular injection of TRH, peripheral administration of insulin or 2-deoxy-D-glucose. Perhaps peptidergic sensory afferent fibers that permit retrograde conduction are recruited by peripheral electrical stimulation of the vagus nerves but not by central vagal stimulation elicited by TRH, insulin, or 2-deoxy-D-glucose (27,28,37).

As atropine simultaneously blocked both gastric acid secretion and MBF in response to TRH injected into the lateral ventricle, the increase in gastric MBF
can be explained either by a direct cholinergic vasodilator effect of the vagus nerve on gastric microvessels or by an effect secondary to the stimulated acid secretion. Omeprazole, a selective proton pump in hibitor at the parietal cell, completely inhibited intracerebroventricular TRH induced stimulation of gastric acid secretion, whereas a significant increase in MBF was still maintained. In a previous study, omeprazole was found to block both gastric acid secretion and MBF stimulated by intravenous infusion of pentagastrin using the hydrogen gas clearance technique (18). These results indicate that omeprazole could produce a reduction in gastric MBF secondary to its antisecretory action. Taken together, these data suggest that the TRH-induced increase in MBF is, in part, mediated by a direct cholinergic vasodilator effect of increased vagal efferent activity, independently from the stimulated gastric acid secretory process. It is unlikely that changes in gastric MBF in response to intracerebroventricular TRH reflect alterations of mean arterial blood pressure as the hypertensive effect of TRH was transient and not abolished by subdiaphragmatic vagotomy or atropine.

Although the central regulation of gastric MBF is still poorly understood, it is clear that the autonomic nervous system plays an important role in the peripheral regulation of gastric MDF (38). Brain neuropeptides that modulate the autonomic output from the central nervous system have potential effects on gastric blood flow. In this study, TRH was shown to act in the brain to increase gastric MBF. This effect appears to be mediated through vagal-cholinergic pathways and is independent of enhanced gastric acid secretion and alteration of mean arterial blood pressure.

References


