Peripheral mediators involved in gastric hyperemia to vagal activation by central TRH analog in rats

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Király, Ágnes, Gábor Sütő, Paul H. Guth, and Yvette Taché. Peripheral mediators involved in gastric hyperemia to vagal activation by central TRH analog in rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G170–G177, 1998.—Mechanisms mediating the increase in gastric mucosal blood flow (GMBF) induced by the stable thyrotropin-releasing hormone (TRH) analog RX-77368 injected intracisternally at a gastric acid secretory dose (30 ng) were investigated using hydrogen gas clearance in urethane-anesthetized rats. The histamine H2 receptor antagonist pyrilamine (intravenously), capsaicin (subcutaneously, −10 days), and NO-nitro-L-arginine methyl ester (L-NAME, intracisternally) failed to impair the 150% rise in GMBF induced by intracisternal injection of RX-77368. By contrast, atropine (subcutaneously) and NO-nitro-L-arginine (intravenously) completely inhibited the increase in GMBF evoked by intracisternal RX-77368. L-NAME (intravenously) blocked the intracisternal RX-77368-induced increase in GMBF in capsaicin-pretreated rats, and the L-NAME effect was reversed by intravenous L-arginine. These findings indicate that vagal efferent activation induced by TRH analog injected intracisternally at a gastric acid secretory dose increases GMBF through atropine-sensitive mechanisms stimulating L-arginine-nitric oxide pathways, whereas H2 receptors and capsaicin-sensitive afferent fibers do not play a role.

nitric oxide; atropine; histamine; capsaicin; NO-nitro-L-arginine methyl ester; thyrotropin-releasing hormone; blood pressure; acetylcholine; NO-nitro-monomethyl-L-arginine; pyrilamine; gastric vascular resistance

ELECTRICAL STIMULATION of the vagus nerve was largely used to assess mechanisms involved in the vagal regulation of gastric mucosal blood flow (GMBF) (5, 37). The associated increase in gastric acid secretion was originally postulated to account for the gastric hyperemic response to electrical vagal stimulation (5). Further in vivo microscopy studies demonstrated a direct vasodilatory effect on gastric submucosal arterioles mediated by atropine-sensitive (cholinergic) and atropine-resistant mechanisms, particularly at high frequencies of vagal stimulation (5, 37). Electrical activation of the vagal nerve trunk may release transmitters through orthodromic as well as antidromic activation of vagal fibers, particularly at high frequencies (37). Therefore, the use of electrical vagal stimulation may not reflect mechanisms occurring during physiological activation of preganglionic vagal motor neurons in the brain stem.

Convergent findings indicate that medullary thyrotropin-releasing hormone (TRH) is involved in the central vagal regulation of gastric function (33). Endogenous TRH in the brain stem mediates the vagal-dependent gastric acid and motor responses to cold exposure and 2-deoxy-o-glucose in conscious rats (24, 33). Medullary TRH also modulates the resistance of the gastric mucosa, leading to gastric cytoprotection or erosion formation, depending on the intensity and duration of the stimuli activating TRH neurons in conscious or anesthetized rats (12, 34). Preliminary evidence indicates that endogenous TRH released in the dorsal vagal complex by kainic acid-induced excitation of cell bodies in the raphe pallidus (12) stimulates GMBF in urethane-anesthetized rats (11). Therefore, intracisternal injection of TRH or the stable TRH analog induced an increase in the firing of neurons in the dorsal motor nucleus of the vagus and gastric vagal efferent discharges (25, 33), providing a physiologically relevant tool to gain insight into the mechanisms involved in the central vagal regulation of gastric function, including GMBF (16, 36, 38). In earlier studies we showed that TRH or RX-77368 injected into the cisterna magna at various doses increased GMBF independently from the stimulation of gastric acid secretion and prostaglandin generation in urethane-anesthetized rats (15, 35, 36, 38). Previous findings also suggest that different peripheral mechanisms mediate the increase in GMBF, depending on the level of central vagal activation. RX-77368 injected intracisternally at a low dose (1.5 ng), activating gastric vagal efferent discharge (25), increases GMBF and protects against gastric lesions induced by ethanol although it is not sufficient to stimulate gastric acid secretion in rats (13, 15). The hyperemic and cytoprotective responses under these conditions were primarily mediated by an atropine-sensitive stimulation of efferent function of capsaicin-sensitive primary afferent fibers containing calcitonin gene-related peptide (CGRP) (13, 15). By contrast, when RX-77368 was injected at 30 ng, leading to a robust stimulation of gastric vagal efferent discharges and near-maximal acid secretion (25, 32), the increase in GMBF was not altered by capsaicin (−10 days), either alone or combined with intravenous injection of the CGRP receptor antagonist CGRP-(8—37) (16).

In the present study we explored the underlying mechanisms involved in the gastric hyperemic response to a near-maximal acid-secretory dose (30 ng) of intracisternal RX-77368 in urethane-anesthetized rats (32). Such a dose of RX-77368 was shown to produce vagal-dependent gastric secretory or motor responses similar to activation of medullary TRH neurons induced by acute exposure to cold or excitation of midline raphe cell bodies (33). We determined whether gastric hyperemia is muscarinic in nature or whether additional, atropine-resistant, mechanisms also participate, as observed with electrical vagal stimulation at high
frequencies (5, 37). Second, we investigated whether the atropine-dependent response is mediated by histamine and/or nitric oxide (NO) using the histamine receptor antagonist (H₁), pyrilamine, and N⁶-monomethyl-L-arginine (L-NMMA), a NO synthase inhibitor that is devoid of direct muscarinic antagonist properties (1). These substances are potential candidates because they are released or generated through vagal muscarinic-dependent mechanisms in the gastric effluent after intracisternal injection of RX-77368 at 30 ng (29, 41) and have vasodilatory properties in the gastric mucosa (4, 5). We also investigated whether the GMBF response maintained in capsaicin-pretreated rats after intracisternal injection of RX-77368 at 30 ng (16) is sensitive to NO synthase inhibition. Finally, in view of the report that intravenous injection of N⁶-nitro-L-arginine methyl ester (L-NAME) inhibits NO synthase activity in the rat forebrain (8), we also compared the effects of peripheral (intravenous) vs. central (intracisternal) injection of L-NAME to ascertain the peripheral site of action of NO synthase inhibitors.

MATERIALS AND METHODS

Animal Preparations

Male Sprague-Dawley rats (Harlan Laboratories, San Diego, CA), weighing 250–275 g, were housed under conditions of controlled temperature (20 ± 3°C) and illumination (12:12-h light-dark cycle starting at 6 AM). Rats were maintained on Purina Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water ad libitum. Food, but not water, was withdrawn 24 h before the study. All experiments were performed in rats anesthetized with urethane (1.25 g/kg ip).

Drugs and Treatments

The following substances were used: the stable TRH analog RX-77368 (p-Glu-His(3,3'-dimethyl)-Pro-NH₂; Ferring Pharmaceuticals, Feltham, Middlesex, UK) and atropine sulfate, capsaicin, pyrilamine, L-NAME, L-NMMA, L-arginine hydrochloride, and α-arginine hydrochloride, all from Sigma Chemical (St. Louis, MO). RX-77368 was separated into aliquots in 0.1% bovine serum albumin and 0.9% saline at a concentration of 3 µg/10 µl and kept frozen at −70°C. The stock solution of RX-77368, as well as atropine sulfate, pyrilamine, L-NAME, and L-NMMA in powder form were dissolved in 0.9% saline (pH 7.0) before administration. Capsaicin was dissolved in vehicle (absolute ethanol, Tween 80, and isotonic saline; 10:10:80, vol/vol/vol). Unless otherwise stated, the volume for intraperitoneal, subcutaneous, and intravenous bolus injections was 0.3 ml, and for intravenous infusion it was 1.5 ml/h.

Surgical Procedures

All the surgical procedures performed for simultaneous measurements of GMBF and MAP were similar to those previously described (1). After a midline laparotomy, the stomach was exteriorized, the pylorus was ligated, and an incision was made in the forestomach to insert a double-lumen cannula (outer Tygon tube 7 mm diam, inner polyethylene catheter 2 mm diam), which was secured by ligature. Physiological saline (pH 7.0) at room temperature was infused by the inner cannula at a rate of 0.5–0.7 ml/min after washing the stomach. The gastric effluent was collected continuously by flow drainage from the outer tube.

Gastric electrode placement. After a small incision was made on the surface of the anterior wall of the stomach, we inserted a platinum needle electrode from the serosa into the basal portion of the gastric mucosa. The electrode was positioned in a high blood flow area, between the two branches of the left gastric artery, closer to the lesser than the greater curvature of the stomach, as previously described (36). The reference electrode (Ag-AgCl) was placed inside the peritoneal cavity. After placement of the gastric cannula and electrode, we covered the exteriorized stomach with saline-wetted gauze and parafilm, and a heating lamp was placed above the animal. The distance of the lamp was adjusted to maintain body temperature at 36–37°C throughout the study as monitored by a temperature probe inserted into the rectum.

Simultaneous Measurements of GMBF and MAP

After a 1-h equilibration period to achieve a steady state, GMBF was measured by the hydrogen gas clearance technique, as previously described and validated (16). Briefly, each measurement involved a 30-min period, alternating 15 min of saturation with 3% hydrogen gas and 15 min desaturation of the tissue. The gas clearance from the gastric mucosa was analyzed by a computerized monoexponential direct curve-fitting program (20). Values of GMBF (in ml·min⁻¹·100 g⁻¹) reflect changes in blood occurring during the 15- to 30-min desaturation period. MAP (in mmHg) was recorded continuously through the femoral artery for the duration of each experiment. Gastric mucosal vascular resistance (GMVR, in mmHg·ml⁻¹·min⁻¹·100 g⁻¹) was calculated by dividing the MAP value at the beginning of the desaturation period by the respective GMBF. The MAP time point selected for calculation of GMVR was based on the rapid desaturation occurring during the first minutes after removal of the hydrogen from the trachea, which has a maximal influence on the final curve fit measuring GMBF (20).

Experimental Protocols

In all experiments, after one or two basal GMBF measurements, pretreatment was given, and at different time intervals thereafter the TRH analog was injected intracisternally.
at 30 ng. GMBF and MAP measurements were performed at various time intervals before and after intracisternal injection. When pretreatments were injected intraperitoneally or subcutaneously, saline (1.5 ml/h iv) was infused to maintain hydration.

Effect of atropine and pyrilamine. In the first series of experiments, after two basal GMBF and MAP measurements, rats received vehicle (0.9% saline sc) or atropine sulfate (2 mg/kg sc), pyrilamine (1 mg/kg iv bolus followed by infusion of 2 mg·kg⁻¹·h⁻¹ iv), or vehicle (0.9% saline, 0.3 ml iv bolus followed by infusion of 1.5 ml/h iv). Thirty minutes after atropine and 60 min after pyrilamine, RX-77368 (30 ng) was injected intracisternally. GMBF and MAP were monitored and GMVR was evaluated before and up to 120 min after RX-77368 injection.

Effect of capsaicin, L-NMMA, and L-NAME. After two basal measurements L-NMMA (50 mg/kg iv) or vehicle (0.9% saline iv) was administered, and 10 min later RX-77368 (30 ng ic) was injected. The selection of L-NMMA dose was based on an equal rise in blood pressure as L-NAME (28). In a second set of studies rats were injected with capsaicin (25, 50, and 50 mg/kg sc in 0.5 ml) at 12-h intervals. The first capsaicin injection was performed under short enflurane anesthesia. After 10–14 days capsaicin-pretreated rats did not show corneal chemosensory reflex (eye wiping) to a drop of 0.1% NH₄OH instilled into each eye just before the experiment. After two basal measurements, four groups of capsaicin-pretreated rats were injected with either vehicle (2 groups), L-arginine (500 mg/kg iv), or D-arginine (500 mg/kg iv), and 5 min later with L-NAME (except in one vehicle group). Ten minutes after L-NAME, all groups were injected intracisternally with RX-77368 (30 ng), and GMBF and MAP changes were monitored for the following 120-min period. In the third set of experiments, after one basal measurement, L-NAME (500 µg/kg) or vehicle (saline) was injected intracisternally 10 min before intracisternal injection of RX-77368 (30 ng) or vehicle (saline). Each substance was injected intracisternally in a 5-µl volume followed by 5-µl flush of the catheter with saline (total volume 20 µl). The changes in GMBF and MAP were monitored for the 30-min period after intracisternal injection.

Statistics

Results are expressed as means ± SE. Comparisons between two groups were calculated by Student's t-test. Comparisons before and after treatment were analyzed by Student's paired t-test. Multiple comparisons were performed by analysis of variance (ANOVA) followed by Duncan's and Newman-Keuls multiple comparisons test. P < 0.05 was considered statistically significant.

RESULTS

Effect of Atropine and Pyrilamine on GMBF, MAP, and GMVR Changes Induced by RX-77368

The basal GMBF was 54.2 ± 8.0 ml·min⁻¹·100 g⁻¹ (n = 5) (Fig. 1A), MAP 80.4 ± 6.9 mmHg (Fig. 1B), and vascular resistance 1.6 ± 0.3 mmHg·ml⁻¹·min⁻¹·100 g (Fig. 1C) in urethan-anesthetized rats. Values of GMBF, MAP, and GMVR remained stable when measured again at a 30-min interval and after subcutaneous injection of either vehicle or atropine (Fig. 1A, A-C). In the vehicle-injected group, RX-77368 (30 ng ic) evoked a peak increase in GMBF during the first 15- to 30-min period after peptide injection, reaching 138.9 ± 16.2 ml·min⁻¹·100 g⁻¹, which represents a 157% increase from preinjection levels (Fig. 1A). Systemic blood pressure also significantly increased to 116 ± 3 mmHg at 15 min after intracisternal injection of TRH analog, whereas GMVR significantly decreased to 0.9 ± 0.1 mmHg·ml⁻¹·min⁻¹·100 g (Fig. 1, B and C). Thereafter, changes in GMBF, MAP, and GMVR declined, and values reached preinjection basal values at 75- to 90-min periods postinjection (Fig. 1). Atropine (2 mg/kg sc, 30 min) completely abolished RX-77368 (30 ng ic)-induced stimulation of GMBF and decrease in GMVR, whereas the rise in MAP was not significantly modified (Fig. 1). Pyrilamine infusion (1 mg/kg bolus + 2 mg·kg⁻¹·h⁻¹ iv) influenced neither the basal nor RX-77368-induced increase in GMBF and MAP and did not influence the decrease in GMVR (Table 1).

Effect of L-NMMA and Capsaicin Alone or With L-NAME

Intravenous injection of L-NMMA (50 mg/kg, −15 min) completely abolished the increase in GMBF and
Table 1. Effect of pyrilamine on intracisternal RX-77368-induced changes in GMBF, MAP, and GMVR in urethan-anesthetized rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>GMBF (ml·min⁻¹·100 g⁻¹)</th>
<th>MAP (mmHg)</th>
<th>GMVR (mmHg·min⁻¹·100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>11</td>
<td>51.0 ± 3.0</td>
<td>78 ± 3</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>48.7 ± 3.5</td>
<td>76 ± 5</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Vehicle + RX-77368</td>
<td>7</td>
<td>123.5 ± 16.1*</td>
<td>114 ± 3*</td>
<td>0.9 ± 0.1*</td>
</tr>
<tr>
<td>Pyrilamine</td>
<td>4</td>
<td>50.8 ± 8.0</td>
<td>82 ± 3</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Pyrilamine + RX-77368</td>
<td>4</td>
<td>125.4 ± 33.2*</td>
<td>118 ± 6*</td>
<td>0.9 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. After 1 measurement (basal), vehicle (0.3 ml + 1.5 ml/h iv) or pyrilamine (1 mg/kg bolus + 2 mg·kg⁻¹·h⁻¹ iv) was administered to 2 groups of rats. Gastric mucosal blood flow (GMBF) was measured during 15-min period and mean arterial pressure (MAP) at 15 min before and after intracisternal injection of RX-77368 (30 ng). GMVR, gastric mucosal vascular resistance. *P < 0.05 compared with vehicle (ANOVA).

decrease in GMVR elicited by intracisternal injection of RX-77368 (30 ng; Fig. 2, A and C). The rise in MAP induced by intracisternal injection of RX-77368 was further enhanced by L-NMMA in magnitude (+21 mmHg and +20 mmHg at 15 and 45 min, respectively) and duration compared with the vehicle-pretreated group (Fig. 2B). Gastric vascular resistance was significantly increased after intracisternal injection of RX-77368 in the L-NMMA-pretreated group compared with preinjection values (Fig. 2C).

In capsaicin-pretreated rats intracisternal injection of RX-77368 (30 ng) increased GMBF (ml·min⁻¹·100 g⁻¹) and decreased GMVR during the 60-min period postinjection, and then values returned to preinjection levels (Fig. 3, A and C). Intravenous injection of L-NAME (10 mg/kg, −15 min) completely abolished the increase in GMBF and decrease in GMVR induced by intracisternal injection of RX-77368 in capsaicin-pretreated rats (Fig. 3, A and C). L-Arginine (500 mg/kg iv) injected 5 min before L-NAME restored the increase in GMBF and decrease in GMVR induced by intracisternal injection of the TRH analog. The stereoisomer D-arginine (500 mg/kg iv) had no effect (Fig. 3, A and C). The rise in MAP induced by intracisternal injection of RX-77368 in capsaicin-pretreated rats was further enhanced by L-NAME in magnitude (+27 mmHg) and duration (over 105 min) (Fig. 3B). L-Arginine, unlike D-arginine, reversed the amplifying effect of L-NAME on systemic blood pressure while not influencing the rise induced by intracisternal RX-77368 (Fig. 3B).

L-NAME (500 µg/kg) injected into the cisterna magna did not alter changes in GMBF, GMVR, and MAP induced by intracisternal injection of TRH analog (30 ng) (Table 2). L-NAME injected intracisternally alone did not influence basal GMBF but increased MAP and GMVR (Table 2).

DISCUSSION

The stable TRH analog RX-77368 injected intracisternally at 30 ng increased GMBF as shown by the hydrogen gas clearance technique in urethan-anesthetized rats. These results are consistent with previous studies using intracerebroventricular injection of TRH at 1–5 µg or intracisternal administration of RX-77368 at a similar or a lower dose (1.5 ng) and monitoring the increase in gastric blood flow by either the aminopyrine clearance, laser Doppler, or hydrogen gas clearance techniques (23, 35, 36). The GMBF response represents a specific effect of the peptide because intracisternal injection of vehicle did not modify basal GMBF as previously reported (15, 16, 38). Convergent observations indicate that the gastric hyperemic response to central TRH or RX-77368 is mediated by vagal-dependent pathways. First, intracisternal injection of TRH or RX-77368 resulted in a sustained increase in gastric vagal efferent discharges (25). Second, selective microinjection of TRH into the dorsal motor nucleus of the vagus increased GMBF (23). Third, vagotomy abol-
ished the increase in GMBF evoked by TRH injected intracerebroventricularly (38).

In the present study, atropine completely inhibited the threefold increase in GMBF elicited by intracisternal injection of RX-77368 at 30 ng. Previous studies also showed that atropine blocked a twofold stimulation of GMBF induced by intracerebroventricular injection of TRH in urethan-anesthetized rats pretreated with capsaicin. Capsaicin pretreatment (125 mg/kg sc) was given 10–14 days before the experiment. Each column represents means ± SE of 4–9 rats/group. *P < 0.05 compared with respective –30 min basal values (paired t-test); +P < 0.05 compared with vehicle plus RX-77368-treated (ANOVA). GMBF and GMVR values represent 15-min assessment period from time indicated in minutes.

We examined whether the rise in GMBF resulted from a direct effect of acetylcholine or was secondary to the increase in GMBF evoked by TRH injected intracerebroventricularly (38).

A graph showing the influence of N\(^{6}\)-nitro-L-arginine methyl ester (L-NAME) alone or combined with L- or D-arginine on intracisternal RX-77368-induced changes in GMBF, MAP, and GMVR in urethan-anesthetized rats pretreated with capsaicin. Capsaicin pretreatment (125 mg/kg sc) was given 10–14 days before the experiment. Each column represents means ± SE of 4–9 rats/group. *P < 0.05 compared with respective –30 min basal values (paired t-test); +P < 0.05 compared with vehicle plus RX-77368-treated (ANOVA). GMBF and GMVR values represent 15-min assessment period from time indicated in minutes.

Capsaicin-sensitive vagal afferents and/or postganglionic release of nonadrenergic noncholinergic vasodilators such as vasoactive intestinal peptide (VIP) have been postulated to contribute to the atropine-resistant component of gastric hyperemia induced by electrical activation of the vagus (9, 37). We previously showed that the VIP antagonist [4Cl-D-Phe\(^{6}\), Leu\(^{17}\)]VIP, infused intravenously at a dose that prevented an increase in GMBF induced by close intra-arterial infusion of VIP, did not alter the gastric hyperemia to intracisternal injection of RX-77368 at 30 ng (14). Taken together, these findings indicate that the stimulation of GMBF induced by central vagal activation, unlike electrical vagal stimulation, is solely mediated by an atropine-sensitive mechanism.

We examined whether the rise in GMBF resulted from a direct effect of acetylcholine or was secondary to the increase in GMBF evoked by TRH injected intracerebroventricularly (38).
Table 2. Effect of intracisternal injection of L-NAME on RX-77368-induced changes in GMBF, MAP, and GMVR in urethan-anesthetized rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>n</th>
<th>GMBF, ml · min⁻¹ · 100 g⁻¹</th>
<th>MAP, mmHg</th>
<th>GMVR, mmHg · min⁻¹ · 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>20</td>
<td>49.0 ± 2.9</td>
<td>81 ± 3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Vehicle + vehicle</td>
<td>4</td>
<td>50.8 ± 6.4</td>
<td>77 ± 6</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Vehicle + RX-77368</td>
<td>4</td>
<td>123.0 ± 15.2*</td>
<td>114 ± 3*</td>
<td>1.0 ± 0.2*</td>
</tr>
<tr>
<td>L-NAME + vehicle</td>
<td>4</td>
<td>52.5 ± 1.4</td>
<td>117 ± 5*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>L-NAME + RX-77368</td>
<td>8</td>
<td>114.7 ± 9.9*</td>
<td>114 ± 5*</td>
<td>1.1 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. After basal GMBF and GMVR measurements, intracisternal injections of vehicle (5 µl) or N⁵-nitro-L-arginine methyl ester (L-NAME, 500 µg/kg) and 10 min later of vehicle or RX-77368 (30 ng) were performed. GMBF and GMVR were measured during the 15-min period before and 15- to 30-min period after the 2nd injection. MAP was measured 30 min before (basal) and at 15 min after the second injection. *P < 0.05 compared with vehicle + vehicle group (ANOVA).

Discussion

In the present study, the gastric vascular changes induced by intracisternal RX-77368 were prevented by L-NAME, a nitric oxide synthase inhibitor, injected intravenously, completely abolished the gastric hyperemia in response to intracisternal injection of RX-77368 at a 30-ng dose in intact rats (36) and capsaicin-pretreated rats (present study). L-NAME was reported to compete nonselectively at the agonist binding site of muscarinic receptors in in vitro assays (1). However, L-NAME action results from peripheral inhibition of NO synthase activity rather than a possible antimuscarinic action. L-NAME inhibitory action in capsaicin-pretreated rats was reversed in a specific manner by an excess of l-arginine, a substrate for NO synthase, whereas the d-arginine enantiomer was inactive. Moreover, L-NMMA, an inhibitor of endothelial NO synthase, which is devoid of muscarinic antagonist properties (1, 28), also blocked the increase in GMBF and decrease in GMVR induced by RX-77368 in intact rats. Finally, L-NAME injected into the disterna magna did not alter the gastric vascular responses to intracisternal TRH analog. The biological activity of L-NAME injected intracisternally was supported by the increase in basal MAP as previously observed using intracisternal injection of other NO synthase inhibitors (39). Therefore, although systemic injection of L-NAME has been reported to decrease NO synthase in the rat forebrain (8), a peripheral site of action is responsible for intravenous injection of L-NAME-induced blockade of the gastric vascular responses to intracisternal RX-77368.

The source of enhanced NO production most likely originates from the endothelium, where NO is abundantly synthesized (26). In addition, acetylcholine increases GMBF through endothelium-dependent mechanisms (17), and direct superfusion of low doses of acetylcholine onto submucosal arterioles induced vasodilation that is prevented by l-arginine-NO pathway blockers (2). NO synthase is also found in postganglionic neurons of the parasympathetic system that innervate the stomach (3). The neuronal NO serves as a peripheral noncholinergic and nonadrenergic transmitter of gastric relaxation induced by high-frequency electrical vagal stimulation (19). However, because atropine completely abolished the NO-dependent increase in GMBF induced by intracisternal RX-77368, it is unlikely that neuronal NO contributes to the gastric vascular response.

Previous reports established that the hypertensive response to intracisternal TRH is mediated by activation of the sympathetic nervous system and catecholamine release, whereas parasympathetic cholinergic mechanisms do not play a role (21, 30, 38). Likewise, atropine did not alter the rise in MAP induced by intracisternal injection of TRH analog at 30 ng. We also found that the increase in MAP induced by intracisternal RX-77368 was not influenced by an excess of l-arginine. These results indicate that the hypertensive response to intracisternal TRH analog is not related to autonomic mediated alterations of tonic NO generation in peripheral vascular beds (18). As previously observed (36), systemic injection of NO inhibitors enhanced the hypertensive response to intrac-
cisternal RX-77368, and GMVR tends to increase instead of decrease. L-NAME vascular effects after intracisternal RX-77368 were reversed in an enantiomeric manner by an excess of L- but not D-arginine. These results most likely reflect the additional increase in peripheral vascular resistance in various beds because of the removal of the tonic vasodilatory action of NO when NO synthase inhibitors are injected into the circulation in addition to the sympathetic-mediated hypertensive effect of intracisternal injection of RX-77368 (21, 27, 30). Atropine, L-NAME, or L-NMMA blocked the decrease in gastric vascular resistance induced by intracisternal RX-77368, whereas the increase in MAP was not modified by atropine and was further enhanced by NO inhibitors. These findings indicate that the stimulation of GMBF induced by central RX-77368 is not merely the result of changes in systemic arterial pressure but is likely to represent muscarinic NO-mediated vasodilatation occurring locally in the gastric submucosal arterioles.

In summary, the present studies in urethane-anesthetized rats show a primary role of muscarinic-NO pathways, unlike histamine or capsaicin-sensitive afferents, in the gastric hyperemic response to intracisternal injection of RX-77368 at a dose that induces a sustained activation of gastric vaso efferent discharge and near-maximal acid secretion (25, 32). No atropine-resistant component participates in the response. The vasoal stimulation of gastric function observed after RX-77368 at such a dose (32, 33) can be mimicked by endogenous release of mediatory TRH induced by various stimuli (10, 33, 42). Therefore these observations may have relevance to the underlying mechanisms involved in the central vagal regulation of gastric blood flow under conditions of sustained vasmal stimulation.

Perspectives

The present data demonstrate that central vagal efferent activation stimulates GMBF and decreases gastric vascular resistance through cholinergic NO-dependent pathways in intact or capsaicin-treated rats. Further studies are needed to delineate the precise mechanisms by which central vagal efferent cholinergic activation is coupled or gated to the L-arginine-NO pathways in the gastric submucosal arterioles. As recent studies suggest, differential coupling of m1, m2, and m3 muscarinic receptors to activate NO synthase (40) muscarinic receptor subtypes that are involved in the generation of NO, leading to the dilatation of arterioles in the gastric mucosa and increased blood flow, will need to be characterized.

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