Role of the Sympathetic Nervous System in Gastric Functional Changes Induced by Thyrotropin-Releasing Hormone in Rats

Masaki Tachibana, Koji Takeuchi and Susumu Okabe

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamasaha, Kyoto 607, Japan

Received September 27, 1994 Accepted November 16, 1994

ABSTRACT—We determined the changes in gastric functions and systemic blood pressure in response to thyrotropin-releasing hormone (TRH) simultaneously in anesthetized rats and examined the role of the sympathetic nervous system in these changes. TRH injected i.c. increased gastric acid secretion, contraction and mucosal blood flow, and produced hemorrhagic lesions in the glandular stomach. These responses to TRH were almost completely inhibited by bilateral cervical vagotomy or atropine. The increased gastric acid secretion and contraction in response to TRH were significantly augmented by pretreatment with yohimbine but not with prazosin. Bilateral adrenalectomy also potentiated the gastric acid secretory and contractile responses to TRH. Neither prazosin, yohimbine nor adrenalectomy had any appreciable effect on the increased gastric mucosal blood flow induced by TRH. TRH-induced gastric mucosal lesions were significantly aggravated by yohimbine and adrenalectomy. In vagotomized rats, TRH significantly suppressed the gastric functional changes induced by electrical stimulation of the vagus nerves. These data suggest that while gastric functional changes and mucosal lesions induced by TRH mainly occur through stimulation of the vagus nerves, these responses are extensively modified by the sympathetic nervous system including the adrenal glands.

Keywords: Thyrotropin-releasing hormone, Gastric function, Systemic blood pressure, Vagus nerve, Sympathetic nerve

Thyrotropin-releasing hormone (TRH) injected intracisternally (i.c.) affects various gastric functions such as acid, pepsin or bicarbonate secretion; motility and mucosal blood flow (GMBF); and induces hemorrhagic damage through vagal activation (1–8). TRH is also known to activate the sympathetic nervous system, leading to increases in the systemic blood pressure and heart rate (9–11). In fact, several investigators examined the influences of surgical and pharmacological manipulations on TRH-induced acid secretion and showed that the increased acid response to central injection of TRH is vagal-dependent and unrelated to changes in the sympathetic nervous system (8, 12, 13). However, since most of these studies on changes in the gastric functions and the cardiovascular system in response to TRH were performed separately, it is difficult to correlate the changes in gastric functions with those of the cardiovascular system. Thus, it is worthwhile to monitor gastric functional and cardiovascular changes simultaneously and correlate these responses in the same animal.

In this study, we measured various gastric functions such as contraction, GMBF and acid secretion in a chambered rat stomach simultaneously with systemic blood pressure, and we examined whether the sympathetic nervous system plays any role in such functions and development of mucosal damage in response to TRH.

MATERIALS AND METHODS

Animals
Male Donryu rats (260–300 g; Charles River, Shizuoka), kept in individual cages with raised mesh-bottoms, were deprived of food but were given free access to tap water until 2 hr before the experiments. Each group comprised 4–6 rats. All of the animals used in this study were anesthetized with urethane administered i.p. (1.25 g/kg), and then they were placed under a heat lamp, to keep their body temperature at 37°C.

Determination of gastric functions and systemic blood pressure
Gastric acid secretion, contraction, GMBF and system-
ic blood pressure were continuously and simultaneously determined for each animal. Briefly, the abdomen was incised, both the stomach and duodenum were exposed, and the pylorus was ligated. A strain gauge (F-041S; Star Medical, Tokyo) was fixed on the serosa of the fundus on the anterior wall along the circular muscle, and then the stomach was mounted in an ex-vivo chamber (14). Gastric contraction was determined by using a force-transducer and by defining the strain gauge free position as 0-g contraction. Quantitative analysis of gastric contraction was performed by determining the number of contractions and by measuring the amplitude of each contraction over a defined period (2, 5 or 10 min). Acid secretion was measured by the pH-stat method (Comtite-7; Hiranuma, Tokyo). The lumen in the chamber was perfused at a flow rate of 1.0 ml/min with saline that was adjusted to pH 7.0 and heated to 37°C. The gastric perfusate was titrated to pH 7.0 against 0.1 N NaOH with an autotitrator. GMBF was measured using a laser Doppler flowmeter (ALF-21; Advance, Tokyo), the probe (type-N, Advance) being placed lightly on the surface of the fundic mucosa with the aid of a balancer (Medical Agents, Tokyo). Systemic blood pressure was monitored using a pressure transducer amplifier system (TP-200TL, AP-100; Nihon Kohden, Tokyo).

**Induction of TRH-induced gastric lesions**

To examine the effect of TRH on the gastric mucosa, it was administered i.c. to the animals at 0.3 - 3 µg/rat in a volume of 5 µl, according to the previous papers (1, 12). Four hours later, the animals were killed. Their stomachs were removed, inflated by injecting 8 ml of 2% formalin, immersed in 2% formalin for 10 min to fix both the inner and outer layers of the gastric wall, and then opened along the greater curvature. The length (mm) of each lesion that developed in the glandular mucosa was determined under a dissecting microscope (Olympus, Tokyo) with a square grid (×10) and summed per stomach.

**Vagotomy and adrenalectomy**

Under urethane anesthesia, bilateral cervical vagotomy and adrenalectomy were performed 30 min or 1 hr before TRH injection, respectively.

**Electrical stimulation of a vagus nerve**

The left and right vagus nerves were separated from the carotid artery and then cut at the cervical level. The peripheral end of the left vagus nerve was stimulated by means of bipolar platinum electrodes with square-wave pulses of 2-msec duration at 3-Hz supermaximal intensity (0.5 mA) (DPS-110-D; Dia Medical Systems, Tokyo).

**Drugs**

Each experiment was started after each gastric functional parameter had become stabilized for >30 min. Atropine sulfate (Wako, Osaka), prazosin hydrochloride (a selective α1-adrenoceptor blocker, Wako) dissolved in saline, and yohimbine hydrochloride (a selective α2-adrenoceptor blocker; Nacalai Tesque, Kyoto) dissolved in saline with a trace of Tween 80 were administered s.c. 20 min before TRH injection. TRH (Pyr-His-Pro-NH2/H2; Peptide Institute, Osaka) was dissolved in saline and administered i.c. The response to vagal stimulation was tested 20 min after TRH administration.

**Statistics**

The data are expressed as means±1 S.E.M. for 4 to 6 rats per group. Statistical analyses were performed by the two-tailed Dunnett’s multiple comparison test or Student’s t-test, and values of P<0.05 were regarded as significant.

**RESULTS**

**Effects of TRH on gastric functions and gastric mucosa**

In the saline-injected group, the acid output, contraction, GMBF and systemic blood pressure values were about 3.5 µEq/10 min, 6 g, 8 ml/min/100 g tissue and 85 mmHg, respectively. TRH injected i.c. at 0.3 µg/rat tended to increase these gastric functions with a significant rise in the systemic blood pressure. At 1 µg/rat, the acid secretion, contraction and GMBF were significantly increased, the former two functions becoming maximal 20 min later (Fig. 1, A and B). At 3 µg/rat, the gastric contraction was slightly decreased compared with that induced at 1 µg/rat, whereas both GMBF and systemic blood pressure became maximal 20 and 30 min later, respectively (Fig. 1, C and D). The maximal acid secretion, contraction, GMBF and systemic blood pressure values induced with 1 or 3 µg/rat of TRH were 16.1±3 µEq/10 min, 12.8±2.4 g, 16.1±0.9 ml/min/100 g tissue and 126.6±3.6 mmHg, respectively. These values returned to the basal ones about 40 min later. However, the increased systemic blood pressure level with 3 µg/rat persisted for more than 90 min. Based on these results, 3 µg/rat of TRH was used in the following experiments.

There was no damage in the gastric mucosa of animals that received saline i.c. In contrast, TRH apparently induced hemorrhagic lesions in the corpus mucosa of the stomach 4 hr later. The total lengths of lesions were 1.9±0.4 mm, 12.0±2.5 mm and 5.8±0.9 mm at 0.3, 1 and 3 µg/rat of TRH, respectively.
Fig. 1. Effects of i.c. administered TRH on gastric acid secretion (A), contraction (B), GMBF (C) and systemic blood pressure (D) in anesthetized rats. Data are means±1 S.E.M. for 4 rats. * Statistically significant difference from the saline group, at P<0.05. ○Saline; ○TRH, 0.3 μg/rat; ▲TRH, 1 μg/rat; ■TRH, 3 μg/rat.

Fig. 2. Effects of vagotomy and atropine on TRH-induced changes in gastric acid secretion (A), contraction (B), GMBF (C) and systemic blood pressure (D) in anesthetized rats. TRH (3 μg/rat) was administered i.c., and bilateral cervical vagotomy or injection of atropine (1 mg/kg, s.c.) was performed 30 min before TRH injection. Data are means±1 S.E.M. for 6 rats. Statistically significant difference at P<0.05: * from the sham-operated group, * from the saline group. ○Sham, ●Vagotomy, ▲Saline, ▲Atropine.
Fig. 3. Effects of prazosin and yohimbine on TRH-induced changes in gastric acid secretion (A), contraction (B), GMBF (C) and systemic blood pressure (D) in anesthetized rats. TRH (3 μg/rat) was administered i.c., and prazosin (0.5 mg/kg) and yohimbine (5 mg/kg) were administered s.c. 30 min before TRH injection. Data are means ± 1 S.E.M. for 6 rats. *Statistically significant difference from the saline group, at P < 0.05. ○Saline + TRH, ●Prazosin + TRH, ▲Yohimbine + TRH.

Fig. 4. Effects of adrenalectomy on TRH-induced changes in gastric acid secretion (A), contraction (B), GMBF (C) and systemic blood pressure (D) in anesthetized rats. TRH (3 μg/rat) was administered i.c., and bilateral adrenalectomy was performed 1 hr before TRH injection. Data are means ± 1 S.E.M. for 6 rats. *Statistically significant difference from the sham-operated group, at P < 0.05. ○Sham + TRH, ●Adrenalectomy + TRH.
Effects of vagotomy and atropine on TRH-induced gastric functional changes and gastric lesions

Gastric acid secretion and contraction stimulated by TRH (3 µg/rat) were significantly inhibited by bilateral cervical vagotomy and pretreatment with atropine (1 mg/kg, s.c.) (Fig. 2, A and B). Vagotomy almost completely inhibited the increase in GMBF caused by TRH (Fig. 2C). Pretreatment with atropine significantly reduced the increase of GMBF in response to TRH, but the degree of the reduction was weak compared with that observed on vagotomy. Both vagotomy and atropine had an insignificant effect on the hypertension induced by TRH (Fig. 2D). The development of gastric damage due to TRH (3 µg/rat) was completely inhibited by vagotomy (0 vs 5.7±1.9 mm in the saline group, N=6) and atropine (0 vs 5.2±0.9 mm in the saline group, N=6).

Effects of yohimbine, prazosin and adrenalectomy on TRH-induced gastric functional changes

Pretreatment with yohimbine (5 mg/kg, s.c.) tended to augment the increases in gastric acid secretion and contraction induced by TRH (Fig. 3, A and B). The maximal responses of acid secretion and contraction induced by yohimbine were observed 20 min after TRH injection (though they were not significant). However, there were significant increases in acid secretion and contraction 30 min later, and these changes persisted for 90 min after TRH injection. In contrast, prazosin (0.5 mg/kg, s.c.) had little or no effect on both the acid secretion and contractile responses (Fig. 3, A and B). Both yohimbine and prazosin had insignificant effects on GMBF (Fig. 3C). The normal systemic blood pressure and that increased by TRH were significantly reduced by prazosin and yohimbine (Fig. 3D).

Adrenalectomy itself had no effect on the gastric acid secretion, contraction, GMBF or systemic blood pressure in the normal animals. The gastric acid secretion and contraction in response to TRH were significantly greater in the adrenalectomized animals than in the sham-operated ones (Fig. 4, A and B). In particular, the enhanced gastric contraction persisted for 90 min. There was no significant difference in GMBF between the adrenalectomized and sham-operated groups in response to TRH (Fig. 4C). The increased systemic blood pressure caused by TRH tended to gradually decrease in the adrenalectomized rats (Fig. 4D).

Effects of yohimbine, prazosin and adrenalectomy on TRH-induced gastric lesions

The mucosal lesions induced by TRH injection were 5.2±0.9 mm in length (N=6). Prazosin administered at 0.5 mg/kg had no effect on the development of TRH-induced lesions (Fig. 5). In contrast, these lesions were significantly worsened by pretreatment with yohimbine at 5 mg/kg; the total lengths of the lesions being 42.0±3.6 mm (N=6). Similarly, the development of gastric lesions in response to TRH was significantly augmented in the adrenalectomized group (21.3±1.9 mm vs 6.8±1.0 mm in the sham-operated group).

Fig. 5. Effects of prazosin, yohimbine and adrenalectomy on TRH-induced gastric mucosal lesions in anesthetized rats. TRH (3 µg/rat) was administered i.c., and the animals were killed 4 hr after TRH injection. Prazosin (0.5 mg/kg) or yohimbine (5 mg/kg) was administered s.c. 30 min before TRH injection, while bilateral adrenalectomy was performed 1 hr before TRH injection. Data are means±1 S.E.M. for 6 rats. *Statistically significant difference from the saline or sham-operated group, at P<0.05.
Effects of TRH on gastric functional changes induced by vagal stimulation

To further investigate the inhibitory influence of TRH mediated by the sympathetic nervous system on gastric functions, we examined the effects of TRH (3 μg/rat, i.c.) on the gastric functional changes induced by direct stimulation of the vagus nerve. The acid secretion was increased by vagal stimulation and reached a peak 30 min later, the acid output being 10.8 ± 0.6 μEq/10 min. The gastric contraction and GMBF were also increased by vagal stimulation for 20 min, the maximal values being 13.1 ± 0.8 g and 186.0 ± 13.5 (4% of basal values), respectively. The vagally stimulated acid secretion, contraction and GMBF were significantly inhibited by TRH (Fig. 6). The maximal acid secretion on vagal stimulation was reduced by 56.5%, and the gastric contraction and GMBF were decreased by 33.6% and 67.7%, respectively. Vagal stimulation had no effect on the systemic blood pressure in either the saline or TRH-pretreated group (data not shown). In addition, the inhibitory effect of TRH on the vagally-stimulated acid secretion was significantly reversed by prior administration of yohimbine; the peak acid output was 9.3 ± 0.7 μEq/10 min (N = 5), which is not significantly different from that (10.8 ± 0.6 μEq/10 min) in the control rats.

DISCUSSION

The present results confirmed the findings of others (1–7, 12–16) that TRH or its analog injected i.c. could enhance gastric functions such as acid secretion, contraction and GMBF. However, the present study further demonstrated that while these functional changes induced by TRH mainly occur through the stimulation of a vagal-cholinergic pathway, these responses are significantly modified by the sympathetic nervous system, including the adrenal gland.

TRH is known to activate the sympathetic nervous system, leading to an increase of the systemic blood pressure or heart rate (9–11). In general, the activation of sympathetic nerves decreases acid secretion and gastric contractility, simultaneously with the reduction of GMBF due to vasoconstriction. In the present study, while the onset of the above functional changes was rapid, the values gradually returned to the basal ones within 50 min. The increases in acid secretion and contraction reached peaks at 1 μg/rat, and GMBF dose-dependently increased, reaching the highest level at 3 μg/rat. As expected from the data of Thieffin et al. (5), vagotomy and atropine markedly inhibited the acid secretion, contraction and GMBF increases caused by TRH. However, yohimbine but not prazosin apparently potentiated all these functions except...
for GMBF. In agreement with the findings of others (9–11), the systemic blood pressure was persistently increased for more than 90 min after TRH injection (3 μg/rat). This increased pressure was not affected by vagotomy or atropine, but markedly reduced by both prazosin and yohimbine. These results strongly suggest that TRH might affect the above gastric functions and systemic blood pressure through different mechanisms. The former might be related to the vagal-cholinergic pathway and the latter to the sympathetic nervous pathway, most probably through the activation of $\alpha_1$- and $\alpha_2$-adrenoceptors.

As shown by the persistent increase in systemic blood pressure and the temporal changes in gastric functions, it appears that the activation of the sympathetic nervous system lasts much longer than that of the cholinergic nervous system. The reason why gastric acid secretion or contraction was not increased with 3 μg/rat of TRH but disappeared gradually appears to be the suppression of vagal activity due to persistent activation of the sympathetic nervous system. The gradual recovery of GMBF can be explained by the same mechanism, i.e., by the suppressed vagal activity. The present results also indicate that the inhibitory effect of sympathetic nerves on vagal activity as to gastric secretion and contraction appears to occur through $\alpha_2$-adrenoceptors. This is consistent with the findings of Yokotani et al. (17, 18), who reported that the gastric acid secretion induced by the electrical stimulation of vagal nerves was inhibited by the stimulation of the splanchnic postganglionic nerves. They further demonstrated that such an inhibitory effect on the gastric acid secretion was not abolished by prazosin but markedly attenuated by yohimbine, thereby suggesting that $\alpha_2$-adrenoceptors are involved in the vagally-stimulated pathway in the gastric wall. Indeed, there is evidence that clonidine, an $\alpha_2$-adrenoceptor agonist, inhibits the gastric acid secretion induced by TRH (16). We also confirmed that electrical stimulation of the unilateral vagus nerve in bilaterally vagotomized rats apparently increased gastric acid secretion, contraction and GMBF, and we found that TRH markedly inhibited these changes caused by vagal stimulation. Since the stimulatory effect of TRH on gastric functions was potentiated by adrenalectomy as well as an $\alpha_2$-blocker, it is most likely that TRH interferes with the enhanced vagal activity through the activation of components of the sympathetic nervous system including the adrenal gland. The present data are not in agreement with those of other studies showing that the acid response to central injection of TRH is vagal-dependent and unrelated to changes in the sympathetic nervous system (8, 12, 13). Although the reason for these different results remains unknown, it may be due to different experimental conditions such as the dose of TRH; we performed the experiment mostly using TRH at 3 μg/rat, i.e., while others examined changes in various gastric functions in response to a much lower dose of TRH (~1 μg/rat). Yet, Flemström and Jedstedt (19) reported that the duodenal bicarbonate response to a low dose of TRH (0.01–1 μg/hr) was significantly augmented by phentolamine, suggesting the modification of this response by the sympathetic nervous system.

The mechanism by which TRH increases GMBF is postulated to involve the release of nitric oxide or prostaglandins through vagal stimulation (5, 20, 21). If TRH activates the sympathetic nervous system, it would be rational that the increase in GMBF in response to TRH should be potentiated by pretreatment with $\alpha$-blockers because of the removal of the suppression by the sympathetic nervous system. However, neither prazosin, yohimbine nor adrenalectomy had any significant effect on the increased GMBF response to TRH. It is most likely that the resistance vessels were dilated by these agents and so the supply of blood to the stomach decreased to an extent that did not allow a further increase in GMBF. The similar phenomenon was observed when acid secretion was stimulated by i.v. histamine; the GMBF was increased only when the hypotensive effect of histamine was blocked by tripelennamine, an H1-antagonist (22). On the other hand, it is possible that TRH stimulates the adrenal glands to release adrenaline which then contributes to the maintenance of a higher blood pressure, because the increased systemic blood pressure gradually reduced in adrenalectomized animals. Yet, the decreasing effect of adrenalectomy on blood pressure was much less potent than those of $\alpha$-blockers. If the adrenal secretion affects the GMBF response to TRH, this effect might be minimal. Determination of circulating catecholamine after TRH treatment would also be necessary for clarifying this point. Thus, the increase of GMBF induced by TRH occurs mainly through vagal stimulation, and the influence of the sympathetic nervous system on this response, if any, may not be detected under the present experimental conditions.

As reported by others (23–25), hemorrhagic lesions were observed in all animals after i.c. injection of TRH (3 μg/rat). These lesions were completely inhibited by vagotomy and atropine, suggesting that the pathological changes are mainly mediated through the vagal pathway. However, these lesions were significantly worsened by pretreatment with yohimbine and adrenalectomy. The underlying mechanism seems to be due to the enhancement by such treatments of acid secretory and contractile responses to TRH, since the influence of the activated sympathetic nervous system (through $\alpha_2$-adrenoceptors) on the vagal-cholinergic pathway was removed. These results also support the above contention that TRH-in-
duced changes in gastric functions are extensively modified by the sympathetic nervous system. Of note was that both yohimbine and adrenalectomy aggravated gastric lesions without affecting the increase in GMBF induced by TRH. These results indicate that GMBF is not a crucial factor in the pathogenesis of TRH-induced gastric lesions.

Taking all the data together, we conclude that while gastric functional changes and mucosal lesions induced by TRH mainly occur through stimulation of the vagocholinergic pathway, these responses are extensively modified by the sympathetic nervous system, including the adrenal glands.

Acknowledgments

The authors wish to thank NJ Halewood for critical reviewing of this manuscript.

REFERENCES

23 Goto Y and Taché Y: Gastric erosions induced by intracisternal thyrotropin-releasing hormone (TRH) in rats. Peptides 6, 153—156 (1985)