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The role of thyrotropin-releasing hormone (TRH) in the pathogenesis of water-immersion stress in rats —Inhibition of TRH release from the stomach by atropine, ranitidine or omeprazole—

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Summary: The role of thyrotropin-releasing hormone (TRH) in the development of gastric erosions and ulcers induced by water-immersion stress was studied. Intraperitoneally administered bethanechol induced a decrease in the gastric wall immunoreactive TRH (ir-TRH) concentrations and an increase in gastric juice ir-TRH concentrations in a dose-related manner, while atropine induced an increase in gastric wall ir-TRH concentrations and a decrease in gastric juice ir-TRH concentrations under non-stress condition. Intraperitoneally administered omeprazole did not influence gastric wall ir-TRH concentrations but elevated gastric pH. Water-immersion stress induced a decrease in gastric wall ir-TRH concentrations and an increase in gastric juice ir-TRH concentrations with a decrease in gastric pH prior to ulcer formation. Pretreatment with atropine or ranitidine inhibited the development of stress ulcers, reduced changes in ir-TRH concentrations in the gastric wall and gastric juice, and induced an increase in gastric pH. Omeprazole inhibited stress ulcer formation and changes in gastric wall and gastric juice ir-TRH concentrations. These results suggest that TRH release from the stomach wall into gastric juice is of importance in the pathogenesis of stress ulcer and that its release is mediated by both muscarinergic and histaminergic (H_2) systems. Furthermore, omeprazole has an inhibitory effect on TRH release under stress ulcer. *Gastroenterol Jpn 1993;28:1-9.*

Key words: *atropine; ranitidine; omeprazole; thyrotropin-releasing hormone; water-immersion stress.*

Introduction

Thyrotropin-releasing hormone (TRH), one of the brain-gut peptides, is widely distributed throughout the central nervous system and gastrointestinal tract[1]. When centrally administered, TRH has an ulcerogenic effect by stimulating gastric acid secretion and motility[2,3]. A recent report demonstrated that TRH exists in basal granulated cells in the gastric mucosa[4]. More recently, we documented that water-immersion stress induced TRH release from the stomach wall into gastric juice[5], and that this TRH has some physiological significance[6]. In the stress-induced ulcer, the muscarinergic system and histaminergic system are well-known factors controlling gastric mucosal injury and protection[7,8]. For the treatment of peptic ulcer, muscarine receptor antagonists and histamine (H_2) receptor antagonists and recently proton pump inhibitors like omeprazole are used mainly to inhibit gastric acid secretion. However, these drugs possess other effects on gastric functions: muscarinergic agents alter concentrations of several brain-gut peptides[9]; histamine stimulates TRH release via H_2 receptor[10]; TRH stimulates antral motility with hista-

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minergic involvement[11]; and omeprazole has cytoprotective effects[12]. Thus, it is possible that peptidergic, muscarinergic and histaminergic systems are mutually related to the pathogenesis of stress ulcers, although at present little is known about the effects of such drugs on TRH concentrations in the stomach. A study concerning the effect of these drugs on immunoreactive TRH (ir-TRH) concentrations in the stomach under water-immersion stress might provide some clues as to the role of TRH in the pathogenesis of stress-ulcer. Therefore, we evaluated changes in gastric ir-TRH concentrations, gastric juice pH (gastric pH) and ulcer formation, when known inhibitors of acid secretion, atropine, ranitidine and omeprazole were administered under water-immersion stress in rats.

Materials and Methods

1. Animals

Male Sprague-Dawley rats (SLC, INS. Shizuoka, Japan), weighing 200–250 g, were housed under conditions of controlled temperature (23±2°C), humidity (60±5%) and illumination (9 AM to 9 PM), and fed laboratory chow and water *ad libitum*. For 24 hours before the experiments rats were allowed free access to water, but were deprived of food.

2. Drugs

The following drugs were used: bethanechol chloride (Sigma Chemical Company, St. Louis, Mo USA), atropine sulphate (Sigma Chemical Company), ranitidine hydrochloride (Japan Glaxo Co., Ltd., Tokyo), and omeprazole (Yoshitomi Pharmaceutical Industries, Ltd., Osaka). Except for omeprazole, the drugs were dissolved in saline. Omeprazole was first dissolved in 10% polyethylene glycol and then diluted with saline.

3. Experimental procedure

All experiments were performed according to the same time schedule in a temperature-controlled room (23°C). Saline or tested drugs were injected intraperitoneally (i.p.). Rats were decapitated by a guillotine under light ether anesthesia.

a) Controls

Untreated rats and saline-treated rats were decapitated, and used as controls for the drug-treated groups (n=6 per group).

b) Bethanechol, atropine or omeprazole treated group

Bethanechol group: Forty-eight rats were administered bethanechol (5 or 20 mg/kg) and the 6 rats of each subgroup were respectively decapitated at 10, 30, 60 and 120 min after the injection. The other 6 rats receiving bethanechol (1 mg/kg) were also decapitated at 30 min after injection.

Atropine group: Thirty rats were given atropine (10 mg/kg) and the 6 rats of each subgroup were respectively decapitated at 10, 20, 30, 60 and 120 min after injection. The other 12 rats receiving atropine (1 or 5 mg/kg) were also decapitated at 20 min after injection (n=6 per group).

Omeprazole group: Twenty-four rats were administered omeprazole (4 mg/kg) and the 6 rats of each subgroup were respectively decapitated at 20, 30, 60 and 120 min after injection. The other 24 rats received omeprazole (0.1, 0.2, 2 or 8 mg/kg) and were also decapitated at 30 min after injection (n=6 per group).

c) Water-immersion stress

From 20 min after the administrations of saline, rats were restrained in water for 30, 60 or 120 min according to the water-immersion method devised by Takagi[13] and the 6 rats of each subgroup were decapitated after the restraint was completed. The ulcer index, gastric pH, gastric wall and gastric juice ir-TRH concentrations were measured.

d) Pretreatment with atropine, ranitidine, or omeprazole under water-immersion stress

Twenty min after the administrations of atropine (1, 5 or 10 mg/kg), ranitidine (1 or 10 mg/kg) or omeprazole (0.1, 1 or 4 mg/kg), rats were restrained in water for 30, 60 or 120 min according to the water-immersion method and 6 rats of each subgroup were decapitated after the restraint. The ulcer index, gastric pH and ir-TRH concentrations in the gastric wall and gastric juice were also measured.

4. Preparation of the stomach and gastric juice, and measurement of the ulcer index and gastric pH

Immediately after decapitation, the abdomen was opened by a midline incision. After ligation of the pyloric ring, the stomach was carefully removed and the gastric juice was withdrawn with a cannula to serve as the samples for pH measurement and TRH assay. The stomach was then cut along the greater curvature. In the water-immersion stress experiment, the mucosal surface was extended and photographed to measure the ulcer index. Then the stomach was used as the sample for the TRH assay. All specimens were immediately weighed and stored at −20°C until assayed[14]. Using the photographs of the mucosa, the ulcer index was expressed as total area (mm^2) of the individual erosions and ulcers observed after water-immersion stress according to the method described previously[5,15]. Gastric pH was measured with a digital pH meter (CG817, Shott Geraete, Hofheim, Germany).

5. Extraction and Assay of TRH

The extraction of TRH from the stomach wall and gastric juice was performed by the method described previously[14]. The recovery rate was 70.3±4.0% (mean ± standard deviation, n=6). After extraction, TRH concentrations in the gastric wall and gastric juice were measured by a radioimmunoassay (RIA) described previously[5,14,16]. Gastric wall ir-TRH concentrations were expressed as pg/mg wet weight (pg/mg w.w.), and those in gastric juice were expressed as pg/ml.

6. Statistical analysis

Data were expressed as mean ± standard error (SE). Statistical analysis was performed using analysis of variance (general linear model procedure) followed by Dunnett's test for ulcer index and ir-TRH concentrations (two-sided test). We used Kruskal-Wallis' H test followed by Wilcoxon's U test for gastric pH (two-sided test). Dose relation was examined using linear regression analysis for ir-TRH concentrations (two-sided test) and using Jonkheere's test for gastric pH (one-sided test). Significance was set at the level of $P<0.05$.

Results

1. Gastric pH and ir-TRH concentrations in the gastric wall and gastric juice in control rats

The gastric juice pH was 2.82±0.22. The gastric wall ir-TRH concentration was 0.69±0.04 pg/mg w.w. and that in gastric juice was 40.0±2.2 pg/ml in control rats. Neither the gastric pH nor ir-TRH concentrations in the gastric wall and gastric juice after saline injection changed significantly over 120 min.

2. Effect of bethanechol, atropine, or omeprazole administration on gastric pH and ir-TRH concentrations in the gastric wall and gastric juice

Bethanechol (20 mg/kg) administration caused a significant decrease in gastric pH. Both 5 and 20 mg/kg of bethanechol induced a significant decrease in gastric wall ir-TRH concentrations with a nadir at 30 min, and a significant increase in gastric juice ir-TRH concentrations with a peak at 30 min (**Figure 1**). Atropine (10 mg/kg) significantly increased the gastric pH, and gastric wall ir-TRH concentrations with a peak at 20 min, and significantly decreased gastric juice ir-TRH concentrations with a nadir at 20 min (**Figure 2**). Gastric pH was significantly increased by 4 mg/kg omeprazole. There were no significant changes in ir-TRH concentrations in the gastric wall or gastric juice when 4 mg/kg of omeprazole was injected (**Figure 3**). Gastric wall ir-TRH concentrations decreased significantly by bethanechol in a dose-dependent manner, and increased dose-dependently by atropine (**Figure 4**). No changes in the gastric wall ir-TRH concentrations were caused in the groups treated with 0.1–8 mg/kg omeprazole at 30 min (data not shown).

3. Effect of water-immersion stress on ulcer index, gastric pH, and ir-TRH concentrations in the gastric wall and gastric juice

Macroscopic ulcer formation was first observed after 60 min of water-immersion stress, and the ulcer index increased significantly at 120 min. Gastric pH was significantly decreased by water-immersion stress for 30 min and remained low until 120 min. Gastric wall ir-TRH concentrations

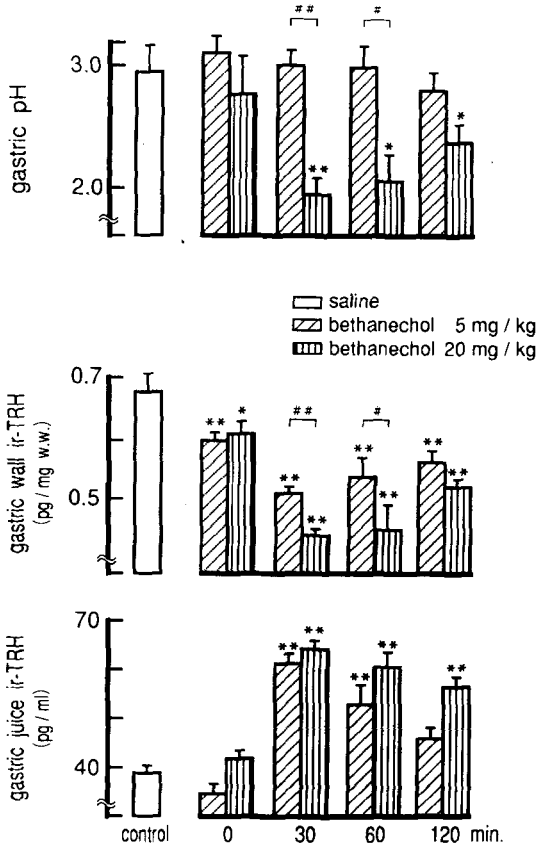

Figure 1. Effects of bethanechol (5 or 20 mg/kg) on gastric pH and ir-TRH concentrations in the gastric wall and gastric juice. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$ vs saline treated group. #, ##: $P<0.05$ and $P<0.01$.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations.

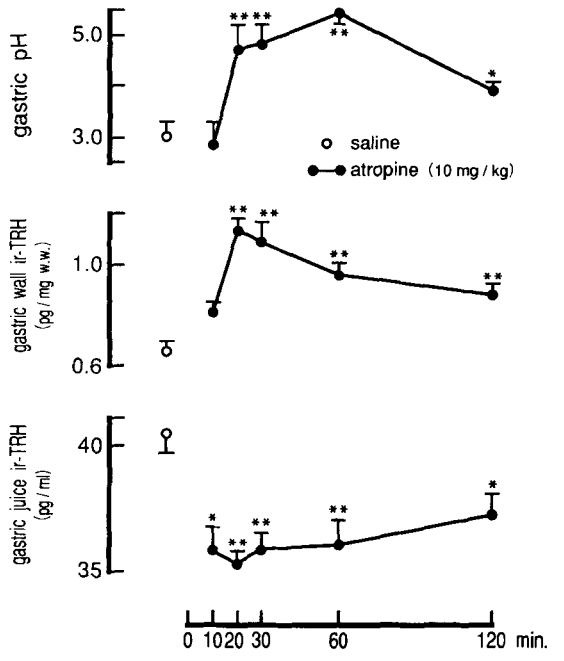

Figure 2. Changes in gastric pH and ir-TRH concentrations in the gastric wall and gastric juice after atropine (10 mg/kg) administration. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$ vs saline treated group.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations.

were significantly decreased by water-immersion stress for 30 min and the lower levels continued to 120 min. In gastric juice, on the other hand, levels were significantly increased by water-immersion stress for 30 min and the higher levels continued to 120 min (**Figure 5**).

4. *Effect of atropine, ranitidine, or omeprazole pretreatment on ulcer index, gastric pH and ir-TRH concentrations in the gastric wall and gastric juice under water-immersion stress*

The increase in ulcer index induced by water-immersion stress was inhibited by atropine, ranitidine and omeprazole pretreatment (**Figure 6**). The decrease in gastric pH and gastric ir-TRH

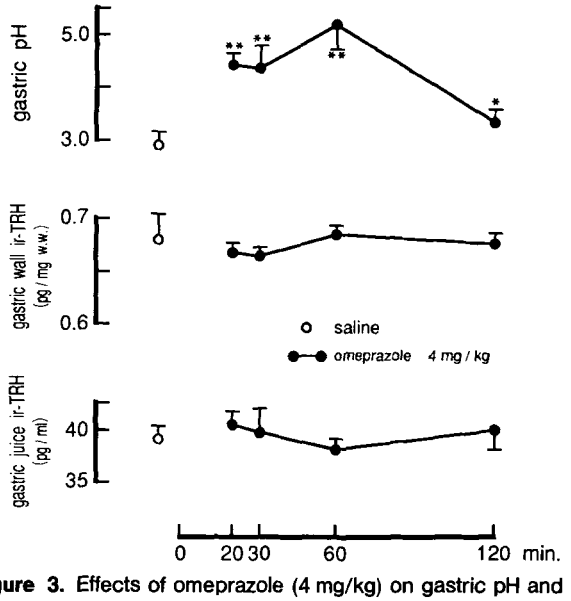

Figure 3. Effects of omeprazole (4 mg/kg) on gastric pH and ir-TRH concentrations in the gastric wall and gastric juice. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$ vs saline treated group.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations.

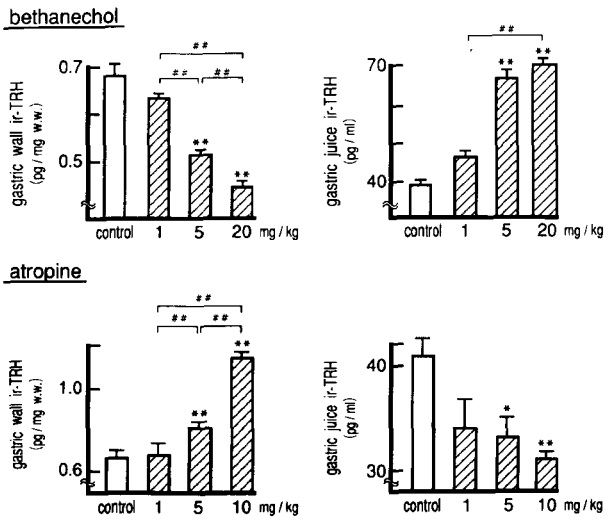

Figure 4. Dose relation of bethanechol and atropine to ir-TRH concentrations in the gastric wall and gastric juice. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$ vs saline treated group. ##: $P<0.01$.; by Dunnett's test (two-sided). Dose-relation was detected by linear regression analysis (two-sided) for bethanechol to ir-TRH concentrations of gastric wall ($P<0.01$) and of gastric juice ($P<0.01$); for atropine to ir-TRH concentrations of gastric wall ($P<0.01$) and of gastric juice ($P<0.05$).

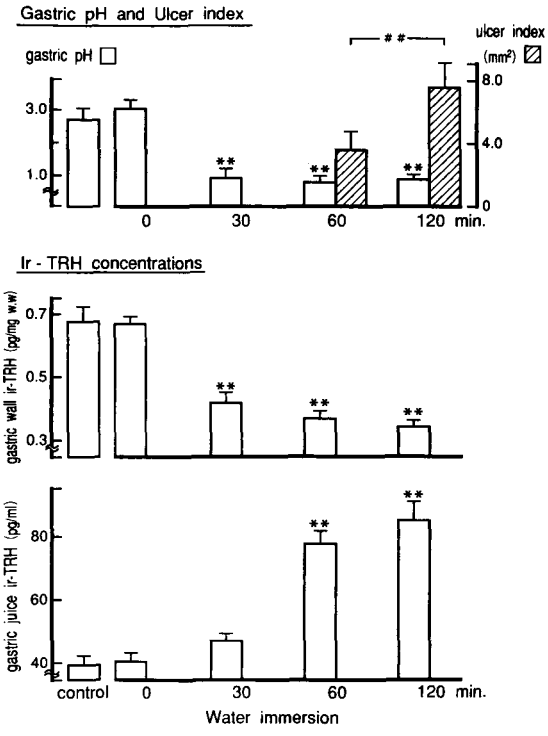

Figure 5. Effects of water-immersion stress on gastric pH, ulcer index and ir-TRH concentrations in the gastric wall and gastric juice. Values are expressed as mean±SE (n=6). **: $P<0.01$ vs non-stress group. ##: $P<0.01$.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ulcer index and ir-TRH concentrations.

concentrations under water-immersion stress was also significantly diminished in a dose-dependent manner after 30–120 min of stress, and the increase in gastric juice ir-TRH concentrations under 60 and 120 min of water-immersion stress was significantly inhibited by pretreatment of atropine (**Figure 7**). Pretreatment with ranitidine inhibited decrease in gastric pH, and changes in ir-TRH concentrations in the gastric wall and gastric juice in a dose-dependent manner (**Figure 8**). The decrease in gastric pH under water-immersion stress was inhibited by omeprazole pretreatment. Omeprazole (1 and 4 mg/kg) pretreatment significanly inhibited the decrease in gastric wall ir-TRH concentrations and the increase in gastric juice ir-TRH concentrations under stress (**Figure 9**).

Discussion

Recent studies have demonstrated the presence of TRH in the stomach wall and gastric juice[5] and TRH has ulcerogenic effects in the stomach[6]. However, it is unknown whether anti-ulcer drugs cause any changes in gastric TRH concentrations. Accordingly, we evaluated the effects of typical anti-ulcer drugs such as atropine, ranitidine and omeprazole on gastric TRH concentrations.

In the present study, the doses of drugs administered were pharmacological and similar to those used in the previous reports[10,17,18,19]. In a preliminary experiment, the rats recovered around 20 min after light ether anesthesia, and the effects of these drugs on gastric ir-TRH concentrations also peaked around 20 min after i.p. injection. Therefore we administered each drug 20 min before applying water-immersion stress. For the mesurement of the gastric wall and gastric juice TRH levels, a previously reported RIA method[14,16] was applied. The elution profile of ir-TRH from the

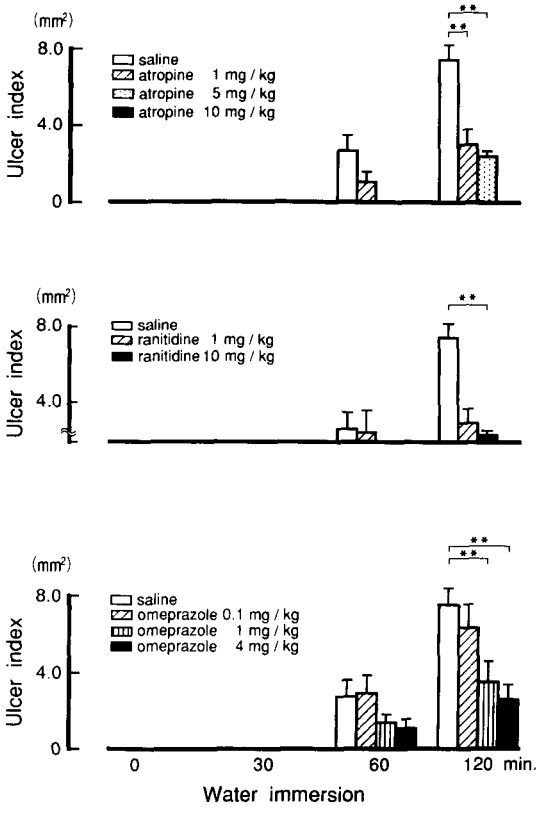

Figure 6. Effects of atropine, ranitidine and omeprazole pretreatment on ulcer index under water-immersion stress. Values are expressed as mean±SE (n=6). **: P<0.01.; by Dunnett's test (two-sided).

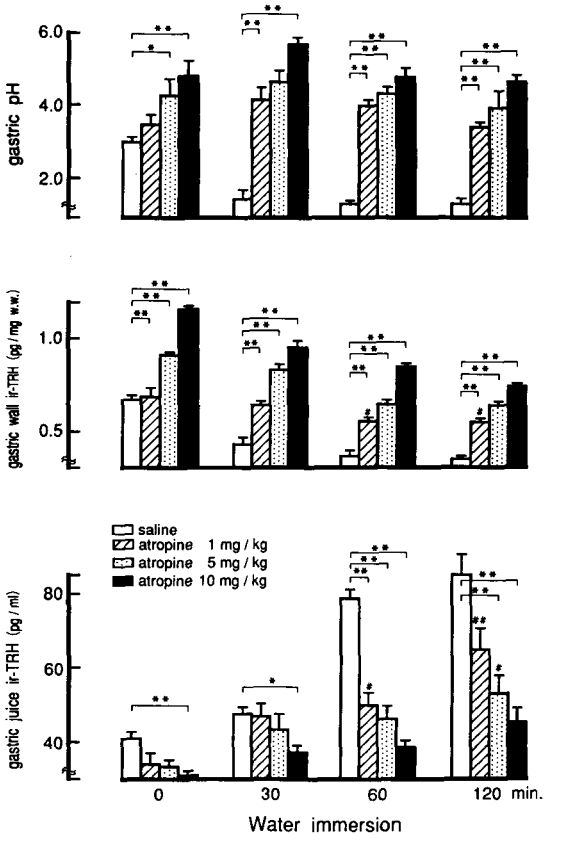

Figure 7. Changes in gastric pH and ir-TRH concentrations in the gastric wall and gastric juice under water-immersion stress after atropine pretreatment. Values are expressed as mean± SE (n=6). *, **: P<0.05 and P<0.01 #, ##: P<0.05 and P<0.01 vs non-stress (saline treated) group.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations. Dose relation was detected by Jonckheere's test (one-sided) for gastric pH at 60 min (P<0.01); by linear regression analysis (two-sided) for gastric wall ir-TRH concentrations at 60 min (P<0.01) and for gastric juice ir-TRH concentrations at 60 min (P<0.01).

stomach was the same as that of synthetic TRH on a Sephadex G10 column, and the dilution curve of the acid-methanol extract of the stomach is parallel to the standard curve for this RIA system. In addition, TRH is known to be stable in gastric juice[5]. These data indicate that the present experimental conditions and the method of TRH measurement can be used to investigate the effects of anti-ulcer agents on gastric and gastric juice TRH concentrations under water-immersion stress in rats.

In the untreated rats, the ir-TRH concentration in the gastric wall was 0.69±0.04 pg/kg w.w. and that in gastric juice was 40.0±2.2 pg/ml. These results are similar to those of previous reports[1,10,15,20].

Bethanechol decreased gastric wall ir-TRH concentrations dose-dependently and increased them in the gastric juice at doses of 5 and 20 mg/kg. It also induced a decrease in gastric pH at 20 mg/kg. In contrast, atropine induced an increase in gastric ir-TRH concentrations and a decrease in gastric juice in a dose-dependent manner. These results indicated bethanechol induced TRH release and atropine inhibited TRH release from the stomach wall into gastric juice, suggesting that ir-TRH release from the stomach wall was mediated by muscarinergic agents dose-dependently.

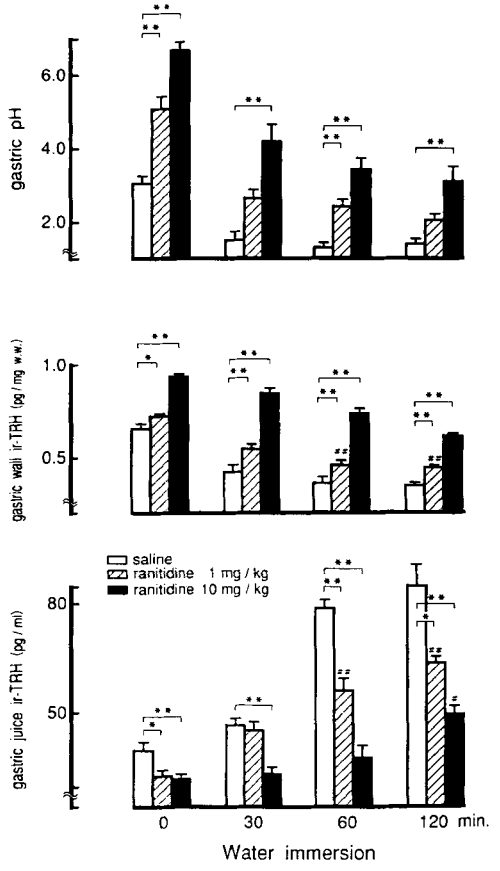

Figure 8. Effects of ranitidine pretreatment on gastric pH and ir-TRH concentrations in the gastric wall and gastric juice under water-immersion stress. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$. #, ##: $P<0.05$ and $P<0.01$ vs non-stress (saline treated) group.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations. Dose relation was detected by Jonckheere's test (one-sided) for gastric pH at 60 min ($P<0.01$); by linear regression analysis (two-sided) for gastric wall ir-TRH concentrations at 60 min ($P<0.01$) and for gastric juice ir-TRH concentrations at 60 min ($P<0.01$).

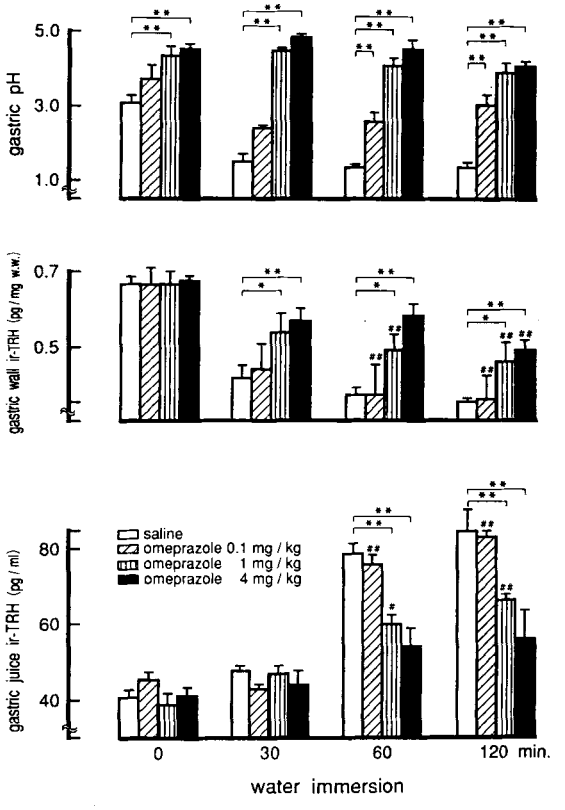

Figure 9. Omeprazole pretreatment inhibits changes in gastric pH and ir-TRH concentrations in the gastric wall and gastric juice under water-immersion stress. Values are expressed as mean±SE (n=6). *, **: $P<0.05$ and $P<0.01$. #, ##: $P<0.05$ and $P<0.01$ vs non-stress (saline treated) group.; by Wilcoxon's U test (two-sided) for gastric pH and by Dunnett's test (two-sided) for ir-TRH concentrations.

The data is agreeable to our previous *in vitro* study in which ir-TRH release was stimulated by acetylcholine and inhibited by atropine[21]. In contrast, omeprazole administration did not influence ir-TRH concentrations in the gastric wall and gastric juice but induced a significant increase in gastric pH. Considering that omeprazole induced an increase in gastric pH without changing ir-TRH release while bethanechol induced ir-TRH release with altering gastric pH, TRH release was not necessarily related to gastric pH in contrast to the close relation of somatostatin to gastric pH[22].

Under water-immersion stress, gastric pH was characteristically decreased, ir-TRH was released from the stomach wall into gastric juice after 30 min, and macroscopic ulcers developed after 60 min. Thus, ir-TRH release occurred prior to stress ulcer formation.

Pretreatment with ranitidine, as well as atropine, dose-dependently inhibited ulcer formation, the fall in gastric pH and changes in gastric ir-TRH concentrations induced by water-immersion stress. The parasympathetic system is stimulated by stress, followed by activation of the histaminergic system[23]. Nakada et al[10] demonstrated that

intraperitoneally administered histamine stimulated TRH release from the stomach wall to gastric juice via H_2 receptor. These findings suggested that changes in ir-TRH concentrations induced by water-immersion stress are mediated via both the muscarinergic and histaminergic systems. When administered intraluminally, TRH decreases gastric pH and somatostatin concentrations in the stomach[6]. TRH also reduces mucosal prostaglandin E_2 levels[15] and increases antral motility[11]. These data combined with those from previous findings suggest that TRH may be related to the pathogenesis of stress ulcers and that its effect on ulcer formation is mediated via both the muscarinergic and histaminergic (H_2) systems.

Omeprazole did not influence ir-TRH concentrations in the non-stress state, but inhibited ir-TRH release in responce to water-immersion stress. Ten weeks of high-dose omeprazole therapy decreased gastrin concentrations and increased somatostatin concentrations in the rat stomach while other peptides did not change even with such long-term treatment[24]. Creutzfeldt et al[25] have reported that functional and morphological changes of G cells and D cells under long-term omeprazole treatment are secondary to the elevated levels of gastric pH. To date, the direct relation of proton pump inhibitors to endocrine cells or brain-gut peptides has not been demonstrated in contrast to the close relationship of muscarinergic and histaminergic systems to endocrine cells and brain-gut peptides. These results are compatible with our finding that omeprazole did not influence TRH release under non-stress state. On the other hand, omeprazole has a protective action against gastric mucosal damage[12] as well as inhibiting gastric acid secretion. Furthermore, omeprazole does not change gastric mucosal blood flow under non-stress condition[17] but inhibits a decrease in gastric mucosal blood flow under shock conditions in rats[26]. Thus, it is possible that omeprazole exerted an inhibitory effect on stress ulcer formation by inhibiting TRH release under water-immersion stress.

In the present study, pretreament of atropine, ranitidine or omeprazole may influence somatostatin concentrations; Atropine and ranitidine inhibit somatostatin release under a condition with increased gastric pH[22,27]. Omeprazole decreases somatostatin synthesis and secretion[28]. In addition, water-immersion stress decreases somatostatin levels in the antrum but increases its levels in the corpus[29]. Thus, pretreatment with these drugs and water-immersion stress might modify somatostatin release from the stomach wall and altered somatostatin concentrations might influence changes in TRH concentrations. Further studies are necessary to elucidate mutual relationship between TRH and other regulatory peptides including somatostatin.

Accumulated evidences have shown that TRH has an ulcerogenic effect in the stomach[11,15]. In particular, directly-administered TRH in gastric juice decreases gastric pH and somatostatin concentrations[6]. The present study provided the further evidence that anti-ulcer drugs may act on gastric acid secretion partly via inhibition of TRH release into gastric juice.

In conclusion, TRH in the stomach may play a crucial role in the pathogenesis of stress ulcers, with atropine, ranitidine and omeprazole inhibiting ulcer formation by preventing the release of TRH from the stomach wall into the gastric juice.

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References

1. Leppäluoto J, Koivusalo F, Kraama R. Thyrotropin-releasing factor: Distribution in neural and gastrointestinal tissues. Acta Physiol Scand 1978;104:175-179.
2. Hernandez DE. Neuroendocrine mechanisms of stress ulceration: Focus on thyrotropin-releasing hormone (TRH). Life Sci 1986; 39:279-296.
3. Taché Y, Vale W, Brown M. Thyrotropin-releasing hormone–CNS action to stimulate gastric acid secretion. Nature 1980; 287:149-151.
4. Tsuruo Y, Hökfelt T, Visser TJ, et al. TRH-like immunoreactivity in endocrine cells and neurons in the gastrointestinal tract of the rat and guinea pig. Cell Tissue Res 1988;253:347-356.
5. Iizuka A, Morise K, Maeda Y, et al. Thyrotropin-releasing hormone levels under water-immersion stress in rats. Jpn J Gastroenterol 1988;85:1069-1076. (in Japanese)
6. Furusawa A, Morise K, Maeda Y, et al. Effect of luminal administration of thyrotropin-releasing hormone on gastric pH and intra-

luminal release of somatostatin in rats. Gastroenterol Jpn 1992; 27:165-171.
7. Yano S, Akahane M, Harada M. Role of gastric motility in development of stress-induced gastric lesions of rats. Japan J Pharmacol 1978;28:607-615.
8. Guth PH, Hall P. Microcirculatory and mast cell changes in restraint-induced gastric ulcer. Gastroenterology 1966;50:562-570.
9. Nishi S, Seino Y. Kitano N, et al. Effects of naloxone on basal and vagus nerve-induced secretions of GRP, gastrin and somatostatin from the isolated perfused rat stomach. Life Sci 1987;41:1787-1793.
10. Nakada K, Mitsuma T, Furusawa A, et al. The effects of histamine on the concentrations of immunoreactive thyrotropin-releasing hormone in the stomach and hypothalamus in rats. Gastroenterol Jpn 1990;25:425-431.
11. Bruce LA, Behsudi FM, Fawcett CP. Histaminergic involvement in thyrotropin-releasing hormone stimulation of antral tissue in the rat. Gastroenterology 1979;76:908-912.
12. Kontrek SJ, Brzozowski T, Radecki T. Protective action of omeprazole, a benzimidazole derivative, on gastric mucosal damage by aspirin and ethanol in rats. Digestion 1983;27:159-164.
13. Takagi K, Okabe S. The effect of drugs on the production and recovery processes of the stress ulcer. Jap J Pharmac 1968;18:9-18.
14. Mitsuma T, Hirooka Y, Nihei N, et al Radioimmunoassay of thyrotropin releasing hormone in human serum and its clinical application. Acta Endocrinol 1976;83:225-235.
15. Maeda Y, Morise K, Kusugami K, et al. Effects of water-immersion-induced stress and intraperitoneal administration of brain-gut peptides upon immunoreactive thyrotropin-releasing hormone and prostaglandin E_2 concentrations in the rat stomach. Gastroenterol Jpn 1991;26:417-423.
16. Mitsuma T, Hirooka Y, Nihei N. Measurement of TRH in the urine by a TRH radioimmunoassay. Folia Endocrinol Jap 1974;50:1005-1014. (in Japanese)
17. Mattsson H, Andersson K, Larsson H. Omeprazole provides protection against experimentally induced gastric mucosal lesions. Eur J Pharmacol 1983;91:111-114.
18. Harrison SD, Bosin TR, Maickel RP. Physiological disposition of atropine in the rat. Pharmac Biochem Behav 1974;2:843-845.
19. Wong WS, Rahwan RG, Stephens RL. Examination of the potential antiulcer activity of the calcium antagonist proryl- methylene dioxyindene. Pharmacology 1990;41:215-223.
20. Morley JE, Garvin TJ, Pekary AE, et al. Thyrotropin-releasing hormone in the gastrointestinal tract. Biochem Biophys Res Comm 1977;79:314-318.
21. Mitsuma T, Sun DH, Nogimori T, et al. Effects of acetylcholine on release of immunoreactive thyrotropin-releasing hormone from the rat stomach *in vitro*. J Aichi Med Univ Assoc 1987;15:549-554.
22. Schubert ML, Edwards NF, Makhlouf GM. Regulation of gastric somatostatin secretion in the mouse by luminal acidity: A local feedback mechanism. Gastroenterology 1988;94:317-322.
23. Kim SE. Vagal influence on gastrointestinal histamine in the rat. Arch Jpn Chir 1981;50:14-28.
24. Allen JM, Bishop AE, Daly MJ, et al. Effect of inhibition of acid secretion on the regulatory peptides in the rat stomach. Gastroenterology 1986;90:970-977.
25. Creutzfeldt W, Stöckmann F, Conlon JM, et al. Effect of short-and long-term feeding of omeprazole on rat gastric endocrine cells. Digestion 1986;35 (Suppl. 1):84-97.
26. Kawano S, Tsuji S, Masuda E, et al. Protective effect of proton pump inhibitor on the gastric mucosa. Jpn J Clin Med 1992;50:33-38. (in Japanese)
27. Simoens C, Woussen-Colle MC, Graef JD. Effect of cimetidine, ranitidine and omeprazole on postprandial gastrin and somatostatin release in conscious dogs. Regul Pept 1988;22:285-293.
28. Wu SV, Giraud A, Mogard M, et al. Effects of inhibition of gastric secretion on antral gastrin and somatostatin gene expression in rats. Am J Physiol 1990;258:G567-G573.
29. Tsutsui M, Matsubara Y, Setsu Y, et al. Somatostatin concentration of gastric corpus and antrum in rats with water immersion stress. Proceedings of the third gut hormone conference. Japanese society of gut hormones. Tokyo: Igaku Tosho Syuppan ltd, 1984;477-482. (in Japanese)