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Enhancement by intracerebroventricular thyrotropin-releasing hormone of indomethacin-induced gastric lesions in the rat

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1 Effects of the intracerebroventricular thyrotropin-releasing hormone (TRH) on gastric mucosa were studied in rats.

2 TRH (3 and 10 $\mu g\ rat^{-1}$ i.c.v.) produced slight gastric lesions and also aggravated indomethacin-, aspirin- or 5-hydroxytryptamine (5-HT)-induced gastric lesions, while restraint and cold stress-induced lesions were not influenced by TRH.

3 Bethanechol used at a dose sufficient to produce acid secretion did not influence the gastric mucosa in intact or indomethacin-treated rats.

4 Enhancement of indomethacin-induced gastric lesions by TRH was not inhibited to any significant degree by atropine 0.1 $mg\ kg^{-1}$ s.c., which prevented TRH-induced gastric acid secretion, but tended to be inhibited by phentolamine, 2.5 $mg\ kg^{-1}$ i.p.

5 It is concluded that the enhancement by TRH of indomethacin-induced gastric lesions is due to a combination of the central and peripheral actions of the ulcerogenic agents.

Introduction

The thyrotropin-releasing hormone (TRH) is still a possible candidate for a gut hormone. This hormone is present in both the brain and gut. It has become evident that TRH influences the gastrointestinal tract in animals and man in many ways (Morley, 1979; Jackson, 1982). One of the recently described properties of centrally administered TRH is its ability to increase the motor activity of the gastrointestinal tract (LaHann & Horita, 1977; Smith, LaHann, Chestnut, Carino & Horita, 1977; Tonoue & Nomoto, 1979; Horita & Carino, 1982; LaHann & Horita, 1982). Although a considerable amount of work has been conducted on TRH-induced gastric acid secretion (Taché, Vale & Brown, 1980; Morley, Levine & Silvis, 1981; Maeda-Hagiwara & Watanabe, 1983a), there are few studies about the influence of TRH on the gastric mucosa (Taché, Simard & Collu, 1979).

In the present study, we have examined the influence of intracerebroventricularly administered TRH on various experimental gastric lesions in rats.

Methods

Gastric ulcerations

Male Wistar rats (ST, substrain from Sankyo Lab. Co. Ltd.), weighing 200–250 g, were used after a 24 h fast, but were allowed free access to water. The stomachs were removed 6 h after the administration of indomethacin (20 $mg\ kg^{-1}$ i.p.), aspirin (200 $mg\ kg^{-1}$ p.o.) or 5-hydroxytryptamine (20 $mg\ kg^{-1}$ s.c.). In the stress ulcer experiment, each rat was immobilized in a compartment of the stress cage as described previously (Takagi, Kasuya & Watanabe, 1964; Okabe, Saziki & Takagi, 1970) with a slight modification. The cage was constructed of an aluminium plate enclosed with galvanized wire netting to confine the animals. Rats in the restraining cage were immersed up to their xiphisternum in water (23°C) and 6 h later their stomachs were removed. Animals were anaesthetized with urethane (1.25 $g\ kg^{-1}$ i.p.) 10 min before their stomachs were removed and 5 min later brilliant blue (2% solution)

was injected into the tail vein (1 ml rat^{-1}) to enhance the contrast of the gastric lesions. All of the excised stomachs were inflated with a saline solution (10 ml per stomach) and immersed in a solution of 5% formalin for 10 min to fix the outer surface of the stomach. The stomach was opened by cutting along the greater curvature. The gastric contents were completely removed. The mucosal lesions were measured under a dissecting microscope with a 1 mm square eye grid, and the sum of the lengths of the lesion was recorded as the ulcer index. Since the sensitivity of the gastric mucosa for ulcerogenesis was different between the antrum and corpus, ulcer indexes of both areas were recorded separately. Intracerebroventricular TRH administration was performed under ether anaesthesia by the technique of Noble, Wurtman & Axelrod (1967) with a slight modification (Maeda-Hagiwara & Watanabe, 1983b). TRH or saline (10 µl per rat) was given 5 min before the administration of indomethacin, 5-hydroxytryptamine (5HT) and aspirin, and the start of stress. To minimize leakage of the drug from the ventricle, the needle was left in place for 1 min following the injection. 5-Hydroxytryptamine (5-HT) dissolved in a saline solution was given s.c. Indomethacin and aspirin suspended in a 1% carboxymethylcellulose solution were given intraperitoneally (i.p.) and orally (p.o.), respectively. Animals were randomized and the gastric lesions were measured without the operator being aware of the drug-treatment group to which the rat belonged.

Assay of gastric acid secretion

Male Wistar rats, weighing 200–250 g, were used after a 24 h fast, but were allowed free access to water. A modification of the pylorus ligated stomach of the rat (Shay, Komarov, Fels, Meranze Gruenstein & Siplet, 1945) was used to study the acid secretion. Rats undergoing pylorus ligation under ether anaesthesia were killed 2 h later and the stomachs were carefully excised. TRH and bethanechol were given intraventricularly and subcutaneously respectively, 5 min before the pylorus ligation. The volume of gastric juice was then recorded. The gastric juice was titrated to pH 7 with a 0.1 N NaOH solution for total acid output. The results were expressed in terms of ml 2 h^{-1} and µmol HCl 2 h^{-1}.

Drugs

Drugs used were atropine sulphate (Wako Pure Chem.), aspirin (Iwaki Chem.), bethanechol (Eisai), brilliant blue (Tokyo Kasei), indomethacin (Merck), phentolamine (Regitin, Ciba), 5-HT creatinine sulphate (Merck), thyrotropin-releasing hormone (Protein Res. Foundation) and urethane (Nakarai Chem.).

Statistical analysis

All data are presented as means ± s.e. The data were analysed by Student's *t* test.

Results

Gastric ulcerations

Intracerebroventricular TRH (3 and 10 µg/rat i.c.v.) caused a slight gastric lesion (erosion) in the glandular portion of the stomach 6 h after the treatment, while saline (10 µl/rat i.c.v.) had no influence on the gastric mucosa (Table 1). Bethanechol 1 mg kg^{-1} s.c. did not affect the gastric mucosa. The intraperitoneal injection of indomethacin (20 mg kg^{-1} i.p.) produced gastric lesions as is shown in Table 2. Macroscopically, the mucosa was covered by punctuated or elongated hemorrhages which could easily be cleaned away, leaving small erosions covered by blood clots. Pretreatment with TRH (3 and 10 µg/rat i.c.v.) aggravated the indomethacin-induced gastric lesions in the corpus and antrum (Table 2). Elongated and large lesions covered by blood clots were observed after cleaning away the mucosa, but did not penetrate the muscularis mucosa when hematoxylin-eosin-stained tissue specimens of the lesion area were examined microscopically.

Table 1 Influence of intracerebroventricular thyrotropin-releasing hormone (TRH) on the gastric mucosa

Drugs	*Dose*	*No. of rats*	*Ulcer indexes* (mm) *Antrum*	*Corpus*	*Total index*
Saline	10 µl/rat	9	0	0	0
TRH	10 µg/rat	9	5.2 ± 1.3**	2.4 ± 0.8**	7.7 ± 1.5**
	3 µg/rat	9	0.4 ± 0.3	0.4 ± 0.1	0.7 ± 0.3*
Bethanechol	1 mg kg^{-1}	9	0	0	0

TRH and bethanechol were given intracerebroventricularly and subcutaneously, respectively, under light ether anaesthesia. The stomach was removed 6 h after TRH or bethanechol.
*$P < 0.05$; **$P < 0.01$ compared to saline.

Table 2 Effect of intracerebroventricular thyrotropin-releasing hormone (TRH) on indomethacin-induced ulcers

Drugs	*Dose*	*No. of rats*	*Ulcer indexes* (mm) *Antrum*	*Corpus*	*Total index*
Indomethacin	20 mg kg^{-1}	10	3.8±1.0	23.9±6.5	28.0±5.8
Indomethacin + saline	10 μl/rat	20	3.4±1.2	20.0±3.3	23.3±3.4
Indomethacin + TRH	20 mg kg^{-1} 10 μg/rat	10	12.7±2.8**	46.0±8.2**	58.7±7.5**
+TRH	3 μg/rat	10	12.8±2.7**	18.8±3.4	31.6±4.5

TRH or saline was given intracerebroventricularly immediately before the indomethacin injection (i.p.) under ether anaesthesia. The stomach was removed 6 h after indomethacin.
**$P<0.01$ compared to indomethacin + saline control.

Figure 1 summarizes the influence of TRH given intracerebroventricularly on the other experimental ulcers (aspirin-, 5-HT- and stress-induced gastric lesions). TRH, which made indomethacin ulcers more effective, also aggravated the 5-HT ulcers significantly and tended to enhance the aspirin ulcer. The gastric mucosa of the animals treated with TRH and aspirin definitely showed a much more severe haemorrhage than those following aspirin alone. In other words, the haemorrhage could easily be cleaned away in aspirin only treated rats, while severe erosions were covered by many clots of blood after cleaning away the mucosa in animals pretreated with TRH. However, TRH did not modify the development of the stress-induced ulcers using a dose level which enhanced indomethacin- or 5-HT-induced gastric lesions. In these three ulcer models (aspirin, 5-HT and stress ulcers), TRH had no influence on the antral mucosa, whereas it aggravated the antral lesions in the indomethacin-treated rats.

Figure 2 shows effects of atropine and phentolamine on the aggravation of the indomethacin ulcers induced by TRH. Pretreatment with 1 mg kg^{-1} s.c. of atropine, but not 0.1 mg kg^{-1} s.c., inhibited significantly the gastric lesions produced by TRH and indomethacin. Phentolamine 2.5 mg kg^{-1} i.p. tended to reduce the gastric lesions.

Gastric acid secretion

The basal acid output and gastric juice volume were 324.9±72.4 μmol HCl 2^{-1} ($n=6$) and 2.9±0.5 ml 2 h^{-1} ($n=6$), respectively, in the pylorus ligated rats treated with intracerebroventricular saline. TRH (3

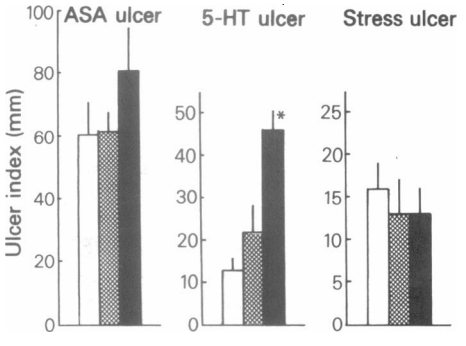

Figure 1 Effects of thyrotropin-releasing hormone (TRH) on various experimental ulcers. The stomachs were removed 6 h after the administration of aspirin (ASA, 200 mg kg^{-1} p.o.), 5-hydroxytryptamine (5-HT) (20 mg kg^{-1} s.c.) or the start of stress. The open columns represent intact animals whereas the other columns represent rats injected with saline (10 μl rat^{-1}) (hatched columns) or TRH (10 μg rat^{-1}) (solid columns), intraventricularly, 5 min before the administration of ASA, 5-HT or the loading of stress. *$P<0.05$ compared to saline controls.

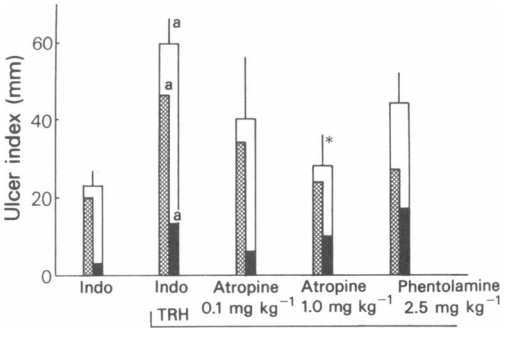

Figure 2 Effects of atropine and phentolamine on gastric lesions induced by indomethacin plus thyrotropin-releasing hormone (TRH). Open column indicates the total index of the lesions in the corpus and antrum, whereas the hatched column indicates the index in the corpus and the solid column indicates the index in the antrum. Atropine or phentolamine was given immediately before the TRH injection. $^{a}P<0.05$ compared to indomethacin (Indo) alone. *$P<0.05$ compared to Indo + TRH.

Table 3 Effect of intraventricular thyrotropin-releasing hormone (TRH) on gastric acid secretion in pylorus-ligated rats

Drugs	Dose	No. of rats	Gastric acid secretion	
			Acid output (μ mol HCl 2 h^{-1})	*Volume* (ml 2 h^{-1})
Saline	10 μl/rat	6	324.9 ± 72.4	2.9 ± 0.5
TRH	10 μg/rat	6	557.4 ± 53.5*	5.4 ± 0.5**
	3 μg/rat	6	418.2 ± 95.5	4.0 ± 0.7
TRH + atropine	10 μg/rat 0.1 mg kg^{-1}	6	122.3 ± 31.3[a]	1.3 ± 0.3[a]
Saline	1 ml kg^{-1}	6	330.0 ± 75.1	3.3 ± 0.5
Bethanechol	1 mg kg^{-1}	6	677.8 ± 137.9*	6.0 ± 0.8*

Drugs except for TRH (intracerebroventricularly) were given subcutaneously immediately before pylorus ligation under ether anaesthesia. Atropine was administered immediately before TRH. Animals were killed 2 h after pylorus ligation and acid secretion was measured.
*$P < 0.05$; **$P < 0.01$ compared to corresponding saline control.
[a]$P < 0.01$ compared to TRH 10 μg/rat.

and 10 μg/rat i.c.v.) increased these gastric secretions as shown in Table 3. Atropine (0.1 mg kg^{-1} s.c.) prevented TRH-induced gastric acid secretion so well that it fell below the baseline. The systemic administration of bethanechol increased gastric secretion in this model.

Discussion

Acid in the stomach is one of the important factors in the development of gastric ulcers. 2-Deoxy-D-glucose, insulin and ergometrine which stimulate gastric acid secretion through the central nervous system (CNS) also aggravate indomethacin-induced ulcers (Maeda-Hagiwara & Watanabe, 1981; Maeda-Hagiwara & Watanabe, 1983b). However, neither bethanechol nor histamine at gastric secretory doses influence indomethacin ulcers (Maeda-Hagiwara & Watanabe, 1983b). It is well known that TRH stimulates gastric acid secretion via the CNS (Taché, Vale & Brown, 1980; Morley, Levine & Silvis, 1981), in rats. TRH alone, but not bethanechol, produced slight gastric lesions in the present study. Although atropine 0.1 mg kg^{-1} s.c. reduced TRH-stimulated gastric acid secretion to below the baseline, the same dose of the compound did not prevent TRH-enhanced indomethacin ulcers to any significant degree. The high doses of atropine (1 or 10 mg kg^{-1}) did not prevent indomethacin ulcers (Watanabe, Watanabe, Maeda-Magiwara & Kanaoka, 1983; Bates, Buckley & Strettle, 1981). It has been demonstrated that TRH acts in the CNS to influence profoundly the muscular activity of the gut (LaHann & Horita, 1977; Smith *et al.*, 1977; Tonoue & Nomoto, 1979; Horita & Carino, 1982; LaHann & Horita, 1982). William & Hinchey (1981) reported a synergism between acid and the gastric contractile activity in the genesis of ulceration of phenylbutazone-treated rats. Therefore, the combination of acid secretion and gastric motility stimulated by TRH may aggravate the drug-induced gastric lesions.

The ulcerogenic action of indomethacin and aspirin has been reported to be potentiated by restraint plus cold stress (Meeroff, Paulsen & Guth, 1975; Brown, Sawrey & Vernikos, 1978). Since rats exposed to cold over a short period have a sharp elevation in serum TSH (Montoya, Wilber & Lorincz, 1979), TRH must be released from the hypothalamus in these conditions. This TRH may modify the indomethacin-, aspirin- or 5-HT-induced ulcers. That is, the central action of TRH may enhance the peripheral effects of the ulcerogenic agents. On the other hand, TRH did not influence cold plus restraint stress-induced ulcers in the present study. The finding is consistent with the studies by Taché *et al.*, (1979) who found that intracerebroventricular TRH did not modify cold stress ulcers, although there are some differences in these experimental conditions. Since experimental conditions in the present stress ulceration are very severe (immobilized rats were immersed in water at 23°C for 6 h), further enhancement of the degree of stress by the central action of TRH appears to be impossible.

Intravenous insulin, intracerebroventricular 2-deoxy-D-glucose or stress all stimulate adrenomedullary adrenaline secretion (Fisher & Brown, 1980). Phentolamine tended to reduce the aggravating effects of TRH on indomethacin-induced ulcers in the present study. Therefore, effects of TRH may be mediated by the adrenal glands. However, the data for the involvement of the adrenal glands in the

aggravation of indomethacin-induced ulcers are conflicting. Adrenalectomy has been shown to aggravate indomethacin- or phenylbutazone-induced ulcers (Abdel-Galil & Marshall, 1968; Urushidani, Kasuya & Okabe, 1979). Whereas, Bhargava, Gupta & Tangri (1973) showed that indomethacin-induced ulcers were apparently suppressed by the removal of the adrenal glands. A synergism between anti-inflammatory agents and acute stress appears to be unrelated to the effects of these agents on the pituitary-adrenal response (Brown *et al.*, 1978). Although the function of the adrenal glands appears to modify indomethacin-induced ulcers in a complicated manner, the exact mechanism of this response is unclear.

It is concluded that this TRH phenomenon is due to the combination of a central action of TRH and peripheral effects of the ulcerogenic agents.

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