J. Pharm. Pharmacol. 1983, 35: 119–121
Communicated August 6, 1982

0022–3573/83/020119–03 $02.50/0

The involvement of the vagal pathway in the antisecretory effect of thyrotropin-releasing hormone on gastric secretion in the dog

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Thyrotropin-releasing hormone (TRH), a tripeptide originally isolated from the hypothalamus, has subsequently been shown to be distributed throughout the central nervous system and also throughout the gastrointestinal tract (Morley et al 1977; Martino et al 1978). Extensive studies on the effects of TRH on gastric secretion in several animal models and man have indicated a wide variety of results often in conflict with each other. Intravenous administration of TRH inhibited pentagastrin-stimulated acid secretion in dogs (Morley et al 1979; Konturek et al 1981) and in man (Dolva et al 1979). In addition, both sham feeding and food-stimulated acid secretion in dogs (Konturek et al 1981), as well as insulin-stimulated acid and pepsin secretion in cats (Gascoigne et al 1980) have been found to be inhibited by TRH. On the contrary, secretory effects of TRH following its intracisternal or intracerebroventricular injection in rats have been observed by Tachè et al (1980) and Morley et al (1981) respectively.

The aim of the present work was to study in dogs the effects of TRH, administered intravenously, on gastric acid and pepsin secretion evoked by various pharmacological stimulants, as well as on bombesin-induced gastrin release.

Materials and methods

Four mongrel dogs, 12–15 kg, were prepared with gastric fistulae and four mongrel dogs of the same weight were prepared with both gastric fistulae and Heidenhain pouches. Food, but not water, was withheld for 18 h before each test. Gastric secretion was stimulated by continuous i.v. infusion (kg^{-1} h^{-1}) of pentagastrin (1 μg), 2-deoxy-D-glucose (2DG) (50 mg), bethanechol (160 μg), histamine (160 μg) and bombesin (1 μg). TRH was given as an i.v. bolus of 20 μg kg^{-1} followed by 20 μg kg^{-1} infused over 30 min during the secretory plateau, while in experiments with bombesin it was administered as a continuous infusion of 20 μg kg^{-1} h^{-1}, 15 min before bombesin. Experiments with pentagastrin, bethanechol and histamine were performed using gastric fistula and Heidenhain pouch dogs, while experiments with 2DG and bombesin were carried out with gastric fistula dogs. The volume of secretion, collected by gravity drainage in 15 min samples, was measured and an aliquot titrated to pH 7 with NaOH 0·01 M by electrometric titration (ETS 822, Radiometer, Copenhagen). Acid output was calculated in m equiv H^+/15 min. Pepsin concentration of gastric samples was measured by a haemoglobin digestion method (Berstad 1970) and pepsin output was expressed as mg of pepsin/15 min. Plasma gastrin concentration was determined by radioimmunoassay using a commercial kit for human gastrin (GASK, Sorin Biomedica, Saluggia) which possesses 100% cross-reactivity with dog gastrin. In this assay, 1–17 human gastrin was used as standard and as immunogen for the production of antiserum. Serial dilutions of dog plasma showed a good parallelism with standard gastrin. The minimum detectable was 8–10 pg ml^{-1}; the intra-assay coefficient variation was less than 10%. In order to avoid the intra-assay variation, samples were also assayed in the same run.

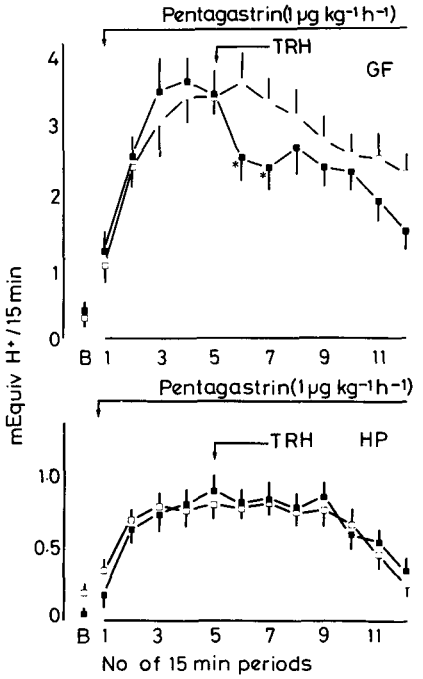

FIG. 1. Effects of TRH (20 μg kg^{-1} i.v. bolus + 20 μg kg^{-1} infused over 30 min) on pentagastrin-stimulated acid secretion from gastric fistula (GF) and Heidenhain pouch (HP) dogs. Control = □——□; TRH = ■——■. Each point represents the mean value of 2 tests in each of the 4 dogs ± s.e. B indicates the mean value at basal conditions. Significant difference from control value: * $P < 0·05$.

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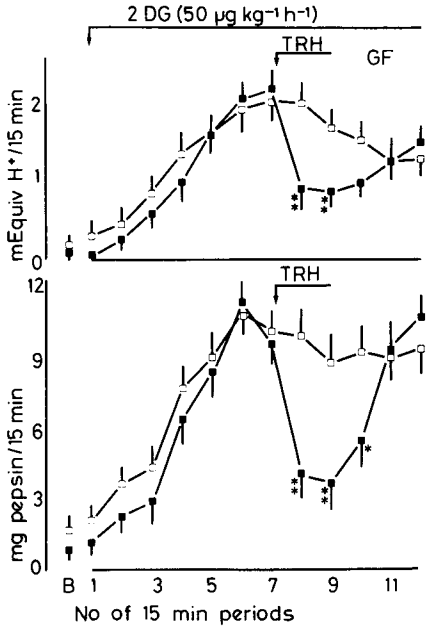

FIG. 2. Effects of TRH (20 μg kg^{-1} i.v. bolus + 20 μg kg^{-1} infused over 30 min) on 2DG-stimulated acid and pepsin secretion from gastric fistula (GF) dogs. Control = □——□; TRH = ■——■. Each point represents the mean value of 2 tests in each of the 4 dogs ± s.e. B indicates the mean value at basal conditions. Significant difference from control value: ** $P < 0.001$; * $P < 0.05$.

The drugs used were: pentagastrin (Peptavlon, ICI), 2-deoxy-D-glucose (Calbiochem), bethanechol hydrochloride (Merck Sharp & Dohme), histamine dihydrochloride (Sigma), synthetic bombesin (Farmitalia-Carlo Erba), thyrotropin-releasing hormone (Biodata). Doses of bethanechol and histamine refer to the salts. Results are expressed as mean values ± s.e. Statistical analysis of data was performed by using Student's *t*-test.

Results

In dogs with gastric fistulae, TRH produced significant inhibition of acid secretion stimulated by pentagastrin (about 35%), whereas secretion from Heidenhain pouches was unaffected (Fig. 1). A more pronounced inhibitory effect of TRH on acid secretion from gastric fistulae was found in experiments with 2DG (about 50%; Fig. 2). Bethanechol produced a well-sustained secretory plateau 60 min after the beginning of the infusion (1·440 ± 0·197 m equiv H^+/15 min from gastric fistulae and 0·880 ± 0·106 m equiv H^+/15 min from Heidenhain pouches; two tests in each of the 4 dogs); under these conditions, TRH had no effect. The continuous infusion of histamine caused a marked increase in acid secretion (at the 60th min, 3·587 ± 0·312 m equiv H^+/15 min from gastric fistulae and 1·038 ± 0·127 m equiv H^+/15 min from Heidenhain pouches; one test in each of the 4 dogs). TRH, infused during the secretory plateau, did not significantly change the acid secretion in response to histamine. The infusion of bombesin produced the maximal increase in acid output and in plasma gastrin levels at the 90th min (1·953 ± 0·224 m equiv H^+/15 min; Δ gastrin = 97·5 ± 13·7 pg ml^{-1}; two tests in each of the 4 dogs); under these conditions, continuous infusion of TRH did not significantly change either acid secretion or gastrin plasma concentration. The infusion of 2DG markedly enhanced pepsin output, which however was significantly reduced by TRH (Fig. 2). By contrast, pepsin output obtained after the infusion of bethanechol (at the 60th min, 5·23 ± 0·47 mg/15 min; two tests in each of the 4 dogs) was not significantly modified by TRH. Pepsin secretion stimulated by pentagastrin, histamine and bombesin was found to behave erratically.

Discussion

The inhibitory action of TRH on gastric secretion in dogs found in the present study appears to be associated with the involvement of the vagal pathway. This hypothesis is consistent with our observations that the inhibitory effects of TRH were evident both against pentagastrin, whose secretory action is partially dependent on parasympathetic gastric fibres (Magee 1975) and, even more so, against 2DG, which acts exclusively through the intact vagus nerve (Eisenberg et al 1966). In addition, the present findings show that the vagally-denervated Heidenhain pouch is less sensitive to the effects of TRH, compared with the innervated stomach. Accordingly, preliminary results have indicated that the dose of TRH necessary to obtain the equivalent percentage reduction of acid secretion from Heidenhain pouches, was twice as high as that needed to produce the same degree of inhibition in the main stomach (Soldani, unpublished data). The low activity of TRH found in the present study on the Heidenhain pouch stimulated by pentagastrin is in contrast with the results of Konturek et al (1981): variations in the sensitivity of animals and/or in the schedule of TRH administration might explain this discrepancy. The vagolytic action of TRH does not appear to involve muscarinic receptors on the oxyntic cell, in the light of the present results concerning bethanechol-stimulated acid and pepsin secretion. Furthermore, the present findings showing the ineffectiveness of TRH against acid secretion caused by histamine indicate that this peptide does not act on gastric H_2 receptors. Moreover, the failure of TRH to affect gastrin release by bombesin suggests that its inhibitory action on acid secretion is independent of serum gastrin levels, in agreement with the results obtained by Konturek et al (1981). The discrepancy in the present study between the results obtained with bombesin and those obtained with pentagastrin might be due to different schedules of TRH administration. The difference in the route of TRH administration can account entirely for the excitatory effects of TRH on

gastric secretion observed in rats by Tachè et al (1980) as opposed to the inhibitory effects found in dogs in the present study. Thus, in accordance with the results of Gascoigne et al (1980) concerning the inhibitory effects of TRH on insulin-stimulated acid and pepsin secretion in cats, the inhibitory action of TRH on gastric secretion in dogs might be the result of an impairment of cholinergic transmission. Further studies are in progress to establish whether the inhibitory effects of TRH on canine gastric secretion are centrally and/or peripherally mediated.

REFERENCES

Berstad, A. (1970) Scand. J. Gastroenterol. 5: 343–348

Dolva, L. O., Hansen, K. F., Berstad, A. (1979) Clin. Endocrinol. 10: 281–286

Eisenberg, M. M., Emas, S., Grossman, M. I. (1966) Surgery 60: 111–117

Gascoigne, A. D., Hirst, B. H., Reed, J. D., Shaw, B. (1980) Br. J. Pharmacol. 69: 527–534

Konturek, S. J., Jaworek, J., Ciezkowski, M., Schally, A. V. (1981) Life Sci. 29: 2289–2298

Magee, D. F. (1975) Gastroenterology 68: 1340–1343

Martino, E., Lenmark, A., Seo, H., Steiner, D. F., Refetoff, S. (1978) Proc. Natl. Acad. Sci. U.S.A. 75: 4265–4267

Morley, J. E., Garvin, T. J., Pekary, A. E., Hershman, J. M. (1977) Biochem. Biophys. Res. Commun. 79: 314–318

Morley, J. E., Steinbach, J. H., Feldman, E., Solomon, T. E. (1979) Life Sci. 24: 1059–1066

Morley, J. E., Levine, A. S., Silvis, S. E. (1981) Ibid. 29: 293–297

Tachè, Y., Vale, W., Brown, M. (1980) Nature (London) 287: 149–151

J. Pharm. Pharmacol. 1983, 35: 121–123
Communicated August 18, 1982

0022–3573/83/020121–03 $02.50/0

Lysosomal enzyme release and ethanol-induced gastric lesions in rats

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Leakage of lysosomal enzymes into cells and the surrounding extracellular space has been implicated in the pathogenesis of gastric ulceration caused by acute stress (Ferguson et al 1972), 5-hydroxytryptamine (5-HT) (Ferguson et al 1973) and vitamin A (Watanabe et al 1981). It is possible that gastric lesions commonly seen in experimental animals after intragastric administration of ethanol are accompanied by labilization of the lysosomal membrane (Dinoso et al 1976; Puurunen et al 1980; Aures et al 1981), and the resulting enzyme release could further exacerbate the mucosal damage. Carbenoxolone and prednisolone have been reported to be lysosomal membrane stabilizers in-vitro (Weissman 1969; Symons & Parke 1980), and were recently shown to provide protection against ethanol-induced gastric injury in rats (Derelanko & Long 1981, 1982). Moreover, prostaglandin E_1, which is capable of stabilizing gastric lysosomes in-vivo and in-vitro (Ferguson et al 1973), is highly active in inhibiting erosive damage on the gastric mucosa due to ethanol and other noxious agents, a property defined as 'cytoprotection' (Robert et al 1979). With pretreatment of PGE_1, 5-HT-induced gastric ulceration in the rat was reduced while the accompanying release of the lysosomal enzyme cathepsin D from the gastric mucosa was partially prevented (Ferguson et al 1973).

Lewis et al (1971) demonstrated that phenylbutazone, which is ulcerogenic in man and animals, enhanced release of acid phosphatase from rat isolated stomachs. In the present study, by using a modified protocol we monitored changes in the release of acid phosphatase from the stomach after intragastric ethanol challenge and ascertained whether prevention of ethanol-induced gastric lesions by various known cytoprotective agents could be linked to inhibition of lysosomal enzyme release. The role of lysosomal enzymes in the reported protective effect through previous exposure to low concentrations of ethanol against subsequent insult with high concentrations of ethanol was also investigated (Robert et al 1978; Code 1981).

Methods

Male Charles River CD rats, 180 to 200 g, were fasted overnight with free access to water before experiments. Before the intragastric administration of 1 ml absolute ethanol, individual animals were treated with one of the following: vehicle (0·4% aqueous methylcellulose) 0·5 ml per rat by mouth or s.c.; prostaglandin E_2 (Sigma), 0.1 mg kg^{-1} by mouth; SC-29333 [(±)-15-deoxy-16α,β-hydroxy-16-methyl prostaglandin E_1 methylester] (Searle, Chicago, Ill., U.S.A.), 30 μg kg^{-1} s.c.; prednisolone (Schering, U.S.A.), 30 mg kg^{-1} by mouth; epidermal growth factor (EGF; Collaborative Research, Waltham, Mass., U.S.A.), 30 μg kg^{-1} s.c.; carbenoxolone (Biogastron, Biorex, London, U.K.), 100 mg kg^{-1} by mouth; 25% ethanol, 1 ml per rat by mouth. All were administered 30 min before absolute ethanol except EGF which preceded ethanol by 10 min. There was always a separate control group receiving only the vehicle.

Ten minutes after absolute ethanol, the animals were killed by a blow to the head. The stomachs were rapidly removed and placed on ice after brief rinsing in 0·9% NaCl (saline). The individual stomachs were opened

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