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EFFECTS OF THYROTROPHIN-RELEASING HORMONE, AND METHIONINE-ENKEPHALIN ON GASTRIC ACID AND PEPSIN SECRETION IN THE CAT

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1 The effect of intravenous administration of thyrotrophin-releasing hormone (TRH) and methionine-enkephalin on gastric acid and pepsin secretions was investigated in conscious cats prepared with chronic gastric fistulae.

2 TRH, 20 μg kg^{-1} h^{-1}, did not influence unstimulated gastric acid secretion, nor gastric acid secretion stimulated by submaximal doses of pentagastrin or histamine. Pepsin secretion stimulated by pentagastrin was not influenced by TRH.

3 TRH, 20 μg kg^{-1} h^{-1}, significantly reduced the gastric acid and pepsin responses to intravenous infusion of insulin. TRH also significantly reduced the degree of hypoglycaemia seen in response to insulin. TRH, 20 μg kg^{-1} h^{-1}, but not 5 μg kg^{-1} h^{-1}, infused alone resulted in a significant hyperglycaemia.

4 It is concluded that the reduction of insulin-stimulated gastric secretion by TRH is not dependent on the hyperglycaemic action of the peptide. The mechanism of action of TRH on insulin-stimulated secretion is discussed with respect to its site of action.

5 Methionine-enkephalin or the potent analogue, D-Ala^2, Met-enkephalinamide were without effect on unstimulated gastric secretion, or secretion stimulated by pentagastrin, histamine, and insulin. The opiate receptor antagonist, naloxone, did not significantly alter the gastric acid or pepsin response to insulin.

6 It is concluded that there is no evidence that opiates stimulate oxyntic glands directly, nor that the oxyntic cells may possess high affinity binding sites for opiates, nor that endogenous opiates are involved in the control of gastric secretion.

Introduction

Both thyrotrophin-releasing hormone (TRH) and enkephalin were first isolated from brain tissue, but subsequently materials with similar immunological and biological activities have been described in tissues of the gastrointestinal tract (TRH: Morley, Garvin, Pekary & Hershman, 1977; Leppäluoto, Koivusalo & Kraama, 1978; enkephalin: Elde, Hökfelt, Johansson & Terenius, 1976; Hughes, Kosterlitz & Smith, 1977; Polak, Sullivan, Bloom, Facer & Pearse, 1977; Puig, Gascon, Craviso & Musacchio, 1977; Schulz, Wüster, Simantov, Snyder & Herz, 1977; Alumets, Håkanson, Sundler & Chang, 1978). Somatostatin, another peptide first isolated from brain tissue and subsequently identified in the gastrointestinal tract, has profound effects on this system including potent inhibition of gastric acid and pepsin secretion (Gomez-Pan, Reed, Albinus, Shaw, Hall, Besser, Coy, Kastin & Schally, 1975). We were, therefore, interested to investigate the activities of TRH and enkephalin on gastric acid and pepsin secretion in the cat.

Methods

Experiments were carried out in 18 conscious cats that had been prepared with cannulated gastric fistulae under full surgical anaesthesia at least one year earlier. Animals were fasted for 36 h before experiments, with free access to water. Gastric secretion was collected continuously by gravity drainage and divided into 15 min samples. Acid output was determined by electrometric titration of a 1.0 ml sample, or less if the volume was insufficient, to pH 7.0 with 0.1 M NaOH (Radiometer, Copenhagen). Acid outputs were expressed as μequiv H^+ kg body wt^{-1} 15 min^{-1}. Peptic activity of gastric samples was determined by a

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haemoglobin digestion method (Shaw & Wright, 1976) and expressed as μg pepsin ≡ porcine pepsin (3× recrystallized, Pentex Labs., U.S.A.) kg body wt^{-1} 15 min^{-1}. A percutaneous needle (Butterfly 21G, Abbott Labs. Ltd.) was inserted into a cephalic vein and 0.9% w/v NaCl solution (saline) infused at a rate of 12 ml/h. All drugs were added to this infusion. In experiments where blood glucose concentrations were measured a second intravenous needle was inserted into the other cephalic vein. The animals were given approximately 250 u of heparin (mucous; Pularin, Evans Medical Ltd., Liverpool) to facilitate blood sampling. Approximately 0.3 to 0.5 ml of blood was collected in a 5 ml collection tube containing lithium heparin (A.J. Seward, London). A 0.1 ml sample of the blood was deproteinized immediately with 1.0 ml 0.33 N perchloric acid, the suspension centrifuged and 0.2 ml of the supernatant assayed for glucose concentration with a commercial kit (GOD-Perid Method, Boehringer Mannheim GmbH Diagnostica). None of the drugs used in the experiments had any effect on the enzyme-colour complex system. Blood glucose concentrations are expressed as mg/100 ml.

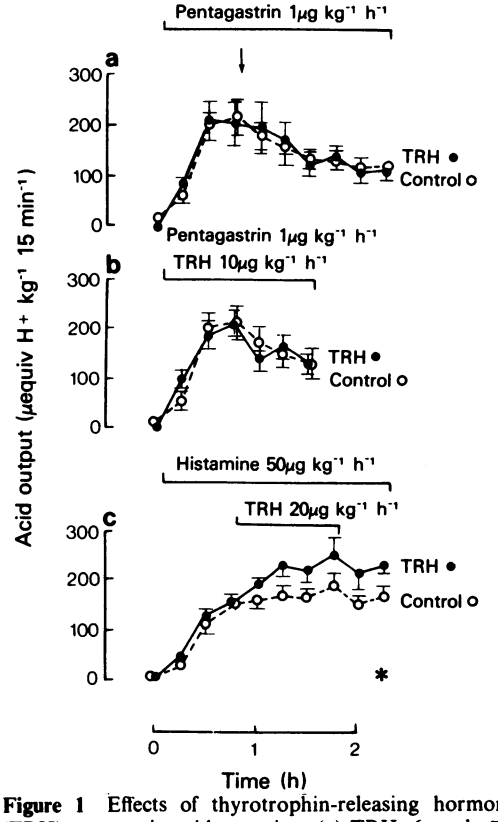

Figure 1 Effects of thyrotrophin-releasing hormone (TRH) on gastric acid secretion. (a) TRH, 6 μg kg^{-1} h^{-1}, was administered as an intravenous injection during stimulation of acid secretion by pentagastrin, 1 μg kg^{-1} h^{-1}. The control response to pentagastrin alone in the same six cats is also illustrated. (b) TRH, 10 μg kg^{-1} h^{-1}, was infused with pentagastrin, 1 μg kg^{-1} h^{-1}, for 1.5 h. (c) TRH, 20 μg kg^{-1} h^{-1}, was infused for 1 h during stimulation of acid secretion by histamine acid phosphate, 50 μg kg^{-1} h^{-1}. Each point is the mean acid secretion in six cats; vertical lines show s.e. mean. The control responses to pentagastrin, and histamine alone are illustrated in the same animals. *Significant difference between mean secretions.

Gastric secretion was stimulated by pentagastrin (Peptavlon; ICI (Pharmaceuticals) Ltd.) 1 μg kg^{-1} h^{-1}, histamine acid phosphate (BDH) 50 μg kg^{-1} h^{-1}, or insulin (Burroughs Wellcome Ltd.) 0.2 u kg^{-1} h^{-1}. Both the chosen doses of pentagastrin and histamine were submaximal for acid secretion. The dose of insulin was chosen as one that gave consistent responses, and the acid secretion is about 50% of that in response to maximal stimulation by pentagastrin (Hirst, Reed, Shaw, Coy & Schally, 1978).

TRH (pGln-His-Pro-NH_2; Roche, Welwyn Garden City) was administered either as an intravenous bolus injection of 6 μg/kg, or a continuous infusion of 5, 10 or 20 μg kg^{-1} h^{-1}. Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and D-Ala^2, Met-enkephalinamide (Tyr-D-Ala-Gly-Phe-Met-NH_2) were synthesized by solid phase methods (Coy, Kastin, Schally, Morin, Caron, Labrie, Walker, Fertel, Berntson & Sandman, 1976), dissolved in saline, and sterilized by microfiltration. Both enkephalins were administered as continuous intravenous infusions. The narcotic antagonist, naloxone (Narcan; Winthrop Labs., England), was administered as a continuous intravenous infusion.

Results are expressed as the mean ± 1 s.e. mean (number). Significance of difference between means was tested by Student's t test for paired data. Significance was set at $P < 0.05$.

Results

Thyrotrophin-releasing hormone

TRH, 6 μg/kg, given as an intravenous injection did not influence the gastric acid secretory response to an intravenous infusion of pentagastrin, 1 μg kg^{-1} h^{-1} (Figure 1a). This method of administration of TRH is used in thyrotrophin secretory tests in man, and similar doses are employed. TRH, 10 and 20 μg kg^{-1} h^{-1}, administered as a continuous intravenous infusion was without effect on pentagastrin (1 μg kg^{-1} h^{-1})-stimulated gastric acid secretion (Figures 1b and 2). Pepsin secretion stimulated by pentagastrin was also not significantly influenced by TRH, 20 μg kg^{-1} h^{-1} (Figure 2). TRH, 20 μg kg^{-1} h^{-1}, did not influence the gastric acid secretion in response to histamine (Figure 1c).

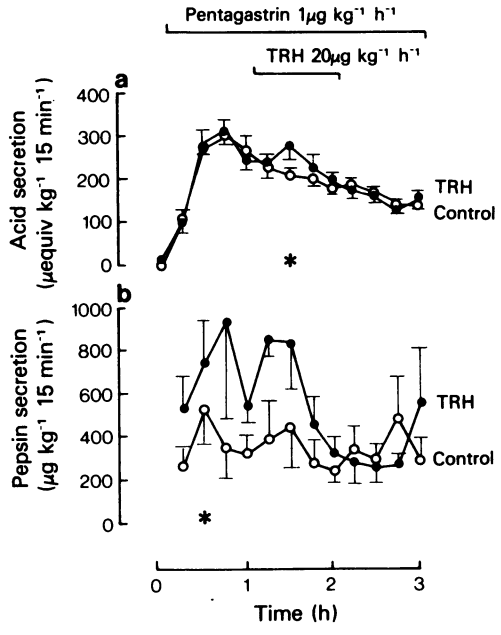

Figure 2 Effect of thyrotrophin-releasing hormone (TRH) on (a) gastric acid and (b) pepsin secretion stimulated by pentagastrin. Pentagastrin, 1 μg kg^{-1} h^{-1}, was infused alone in the control experiments. On a separate occasion TRH, 20 μg kg^{-1} h^{-1}, was infused during the second hour of a 3 h infusion of pentagastrin in the same six cats. Each point is the mean acid secretion in six cats; vertical lines show s.e. mean. *Significant difference between mean secretions.

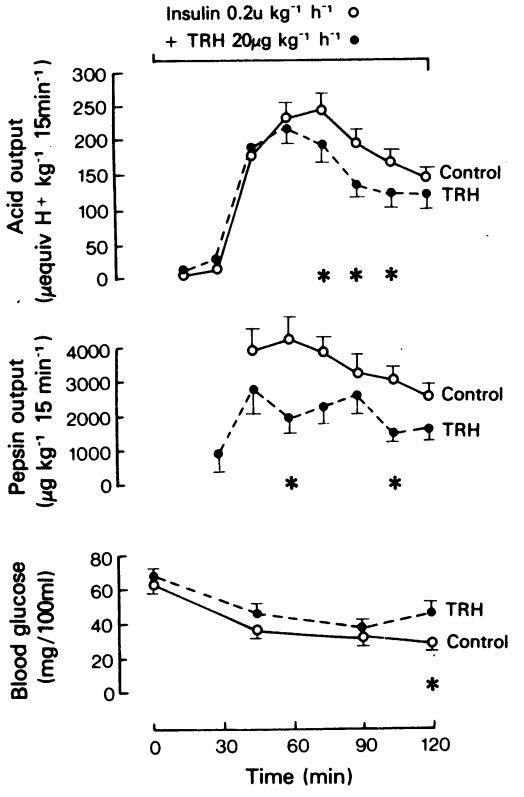

Figure 3 Effect of thyrotrophin-releasing hormone (TRH) on gastric acid and pepsin secretion, and the hypoglycaemia in response to insulin. Insulin, 0.2 u kg^{-1} h^{-1}, was infused alone in the control experiments. TRH, 20 μg kg^{-1} h^{-1}, was added to the insulin infusion for the whole duration in the test experiments. Each point is the mean; vertical lines show s.e. mean. Control and test experiments were carried out in the same animals (acid secretion, $n = 18$; pepsin secretion, $n = 6$; blood glucose concentrations, $n = 13$ to 15). *Significant difference between means.

TRH, 20 μg kg^{-1} h^{-1}, significantly reduced the gastric acid response to insulin-induced hypoglycaemia during the second hour of infusion (Figure 3). Insulin-stimulated pepsin secretion was also reduced by TRH, but this reduction was only significant in two of the six 15 min collection periods (Figure 3). Insulin, 0.2 u kg^{-1} h^{-1}, produces a marked hypoglycaemia. TRH did not influence the degree of hypoglycaemia during the first 90 min of insulin infusion. The blood samples collected after 120 min of infusion of insulin and TRH have a significantly greater mean blood glucose concentration than samples collected at the same time in experiments with insulin alone (Figure 3).

The effect of TRH on blood glucose concentrations was investigated further by infusing TRH alone. TRH, 5 μg kg^{-1} h^{-1}, did not change the blood glucose concentration from fasting levels. However, TRH, 20 μg kg^{-1} h^{-1}, resulted in a significant hyperglycaemia (Figure 4). At all times sampled from 45 min after the start of the TRH infusion, the blood glucose concentration was greater in the experiment with TRH, 20 μg kg^{-1} h^{-1}, compared with 5 μg kg^{-1} h^{-1}. The hyperglycaemia was 33.1 ± 12.1 (6) mg/100 ml greater than the fasting level after 120 min of TRH infusion, a 62.3 ± 24.5 (6)% rise above fasting levels (Figure 4). Gastric acid secretion was unaffected by the infusions of TRH alone.

Methionine-enkephalin

Met-enkephalin infused at rates up to 128 μg kg^{-1} h^{-1} did not influence unstimulated gastric acid secretion (Figure 5). Met-enkephalin, 256 μg kg^{-1} h^{-1}, resulted in copious saliva production and retching in the animals. These toxic effects were immediately reversed upon stopping the enkephalin infusion. Met-enkephalin, 16 to 128 μg kg^{-1} h^{-1}, did not influence

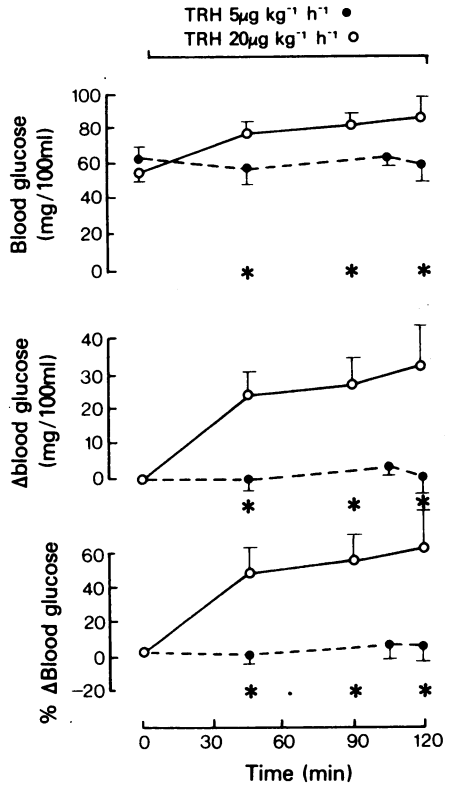

Figure 4 Effect of thyrotrophin-releasing hormone (TRH) on fasting blood glucose concentration. TRH, 5 and 20 μg kg^{-1} h^{-1}, was infused into the same six cats on separate days. Results are expressed as mean of six observations (vertical lines show s.e. mean) in the same six cats for blood glucose concentration, the change in blood glucose concentration from pre-TRH values (Δ blood glucose), and the change in blood glucose concentration expressed as a percentage of the pre-TRH values (%Δ blood glucose). *Significant difference between mean values for TRH 5 and 20 μg kg^{-1} h^{-1}

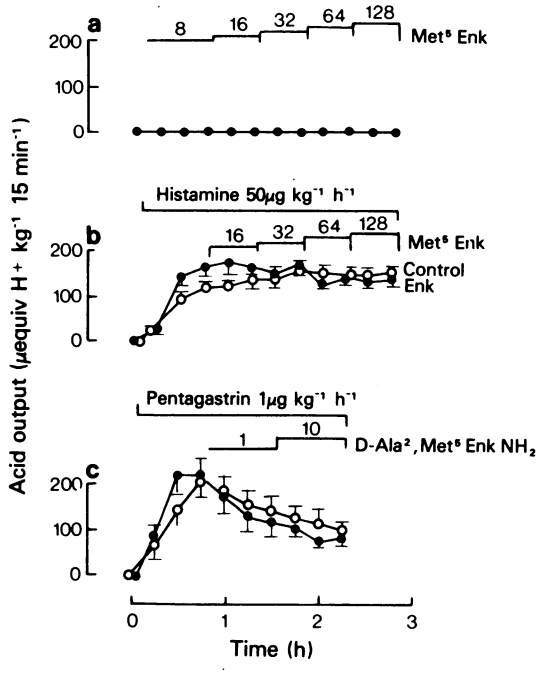

Figure 5 Effect of methionine-enkephalin (Met^5Enk) on gastric acid secretion: (a) methionine-enkephalin was infused alone at doses from 8 to 128 μg kg^{-1} h^{-1} in six animals; (b) methionine-enkephalin was infused at doses from 16 to 128 μg kg^{-1} h^{-1} during stimulation of acid secretion by histamine acid phosphate, 50 μg kg^{-1} h^{-1}. The control response to histamine alone is illustrated in the same six cats. (c) D-Ala^2, methionine-enkephalinamide was infused at 1 and 10 μg kg^{-1} h^{-1} during stimulation of acid secretion by pentagastrin, 1 μg kg^{-1} h^{-1}. The control response to pentagastrin alone is illustrated in the same six cats. Each point is the mean secretion; vertical lines show s.e. mean.

the secretory response to histamine, 50 μg kg^{-1} h^{-1} (Figure 5). The enkephalin analogue, D-Ala^2, Met-enkephalinamide, did not alter the gastric acid secretory response to pentagastrin, 1 μg kg^{-1} h^{-1}, when infused at rates up to 10 μg kg^{-1} h^{-1} (Figure 5). A larger dose of this analogue, 50 μg kg^{-1} h^{-1}, was toxic, having the same side-effects as Met-enkephalin. Again these side-effects were quickly reversed on stopping the enkephalin infusion.

Insulin-stimulated gastric acid and pepsin secretions were not influenced by the simultaneous infusion of Met-enkaphalin, 128 μg kg^{-1} h^{-1} (Figure 6). Blood glucose concentrations were not measured in this experiment.

Naloxone, a narcotic antagonist, did not significantly alter the gastric acid or pepsin response to insulin (Figure 7).

Discussion

Thyrotrophin-releasing hormone

TRH was without effect on gastric acid secretion stimulated by histamine, or on gastric acid and pepsin secretion stimulated by pentagastrin in the cat (Figures 1 and 2). The dose of pentagastrin chosen evokes 30 to 40% of the maximal gastric acid response to pentagastrin (Hirst, Labib & Reed, 1978). In contrast to the lack of effect of TRH reported here in the cat, Morley, Steinbach, Feldman & Solomon

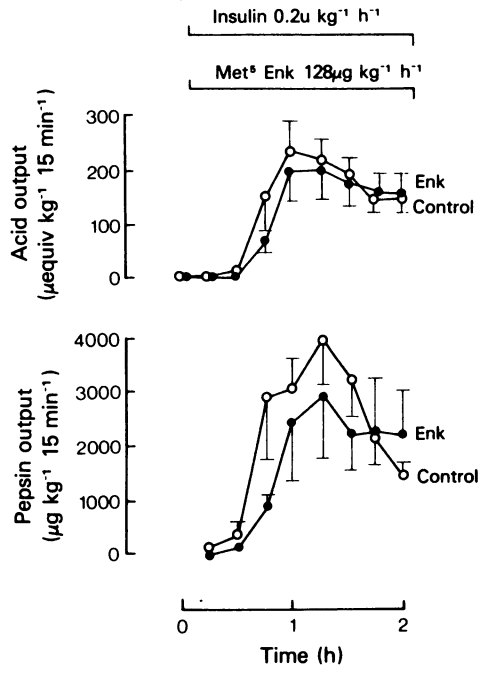

Figure 6 Effect of methionine-enkephalin (Met^{5}Enk) on (a) gastric acid and (b) pepsin secretion stimulated by insulin. Met-enkephalin, 128 μg kg^{-1} h^{-1}, was infused for the entire duration of the insulin, 0.2 u kg^{-1} h^{-1}, infusion. The control response to infusion of insulin alone in the same six cats is also illustrated. Each point is the mean secretion; vertical lines show s.e. mean.

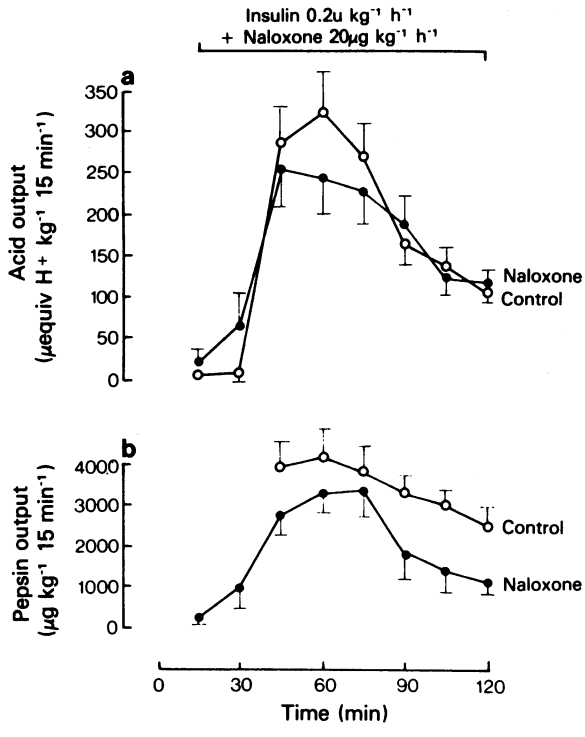

Figure 7 Effect of naloxone on (a) gastric acid and (b) pepsin secretion stimulated by insulin. Naloxone, 20 μg kg^{-1} h^{-1}, was infused for the entire duration of the insulin, 0.2 u kg^{-1} h^{-1}, infusion. The control response to infusion of insulin alone in the same six cats is also illustrated. Each point is the mean secretion; vertical lines show s.e. mean.

(1979) have shown a transient decrease in gastric acid secretion stimulated by a submaximal dose of tetragastrin in dogs. Even more convincingly, Dolva and colleagues have shown that a dose of TRH as low as 8 μg/h, ≃0.11 μg kg^{-1} h^{-1}, significantly reduced gastric acid secretion stimulated by a submaximal dose of pentagastrin in man. In the same studies, pepsin secretion was only significantly reduced by TRH 200 μg/h, ≃2.86 μg kg^{-1} h^{-1} (Dolva, Hanssen & Berstad, 1979; Dolva, Hanssen, Berstad & Frey, 1979).

Insulin stimulates gastric secretion by reducing the blood glucose concentration to levels at which neurones of the vagal nuclei discharge. A pharmacological dose of TRH significantly reduced both the acid and pepsin secreted in response to insulin. Insulin-induced hypoglycaemia was also reduced by TRH (Figure 3). However, the reduction in hypoglycaemia is apparent only after significant inhibition of gastric secretion suggesting that these two effects are not causally related.

The influence of TRH on blood glucose concentrations was investigated further by studying the effect of TRH alone. TRH, 20 but not 5 μg kg^{-1} h^{-1}, resulted in a marked hyperglycaemia in cats (Figure 4). In contrast, TRH is reported to be without effect on fasting blood glucose concentrations in man (Besser, Ratcliffe, Kilborn, Ormston & Hall, 1971; Wildmeister, Daweke, Gries, Grüneklee, Hessing & Horster, 1972), or the hypoglycaemia in response to insulin (Besser *et al.* 1971). Therefore, there appear to be considerable differences in the responses of the cat and man to TRH.

The inhibitory effect of TRH on gastric secretion in the cat stimulated by insulin (an indirect stimulant working via the central nervous system) but not pentagastrin or histamine (direct stimuli) may suggest that the influence of TRH is on the centres in the brain which control the discharge of the neurones in the vagal nuclei. TRH is found in hypothalamic and extra-hypothalamic brain tissue (Brownstein, Palko-

vits, Saavedra, Bassiri & Utiger, 1974; Jackson & Reichlin, 1974; Hökfelt, Fuxe, Johansson, Jeffcoate & White, 1975; Leppäluoto *et al.*, 1978) and it has been suggested that it functions as a neuromodulator in the central nervous system (Brownstein *et al.*, 1974). Intracerebroventricular, but not intravenous administration of TRH, stimulated colonic motility in the rabbit (Smith, La Hann, Chesnut, Carino & Horita, 1977), but others have observed TRH to evoke contractions of the gastric antrum *in vivo* when administered intravenously (Morley *et al.*, 1979), and contractions of gut muscle *in vitro* (Almqvist, 1972). In man, TRH increased basal intragastric pressure, but inhibited gastric motility in response to distension (Dolva & Stadaas, 1979). It is possible therefore that the action of TRH is peripheral. The final mediation of insulin-induced gastric secretion is through the release of acetylcholine. TRH has often been associated with modulating cholinergic transmission (see Kalivas & Horita, 1979) and the inhibition of insulin-stimulated gastric secretion might thus be explained. The lack of inhibition of pentagastrin-stimulated secretion by TRH in the cat would be consistent with the resistance of gastrin-stimulated secretion to atropine in this species (Blair, 1975). These suggestions are speculative and further studies are required to elucidate whether the inhibitory effects of TRH on gastric secretion are centrally or peripherally mediated.

Methionine-enkephalin

Met-enkephalin was without effect on unstimulated gastric acid secretion, and secretion stimulated by histamine, pentagastrin, and insulin (Figures 5 and 6). Met-enkephalin was also without effect on insulin-stimulated pepsin secretion (Figure 6). We used both Met-enkephalin, and D-Ala^2, Met-enkephalinamide. The latter analogue of enkephalin is more potent than Met-enkephalin in a number of biological test systems (Pert, Pert, Chang & Fong, 1976; Coy *et al.*, 1976; Cusan, Dupont, Keldzik, Labrie, Coy & Schally, 1977). In agreement with these findings, the dose of D-Ala^2, Met-enkephalinamide which resulted in toxic side-effects in our animals was at least five times less than observed with Met-enkephalin. We have no reason to believe that the analogue would have a different effect from Met-enkephalin on gastric secretion.

Opiate receptor agonists, particularly morphine, have previously been investigated for their influence on gastric secretion in the dog. Morphine is generally agreed to increase unstimulated gastric secretion when administered intravenously (Yamaguchi, 1974; Magee, 1975; Konturek, Pawlik, Tasler, Thor, Waluś, Król, Jaworek & Schally, 1978a). This response is correlated with an increase in serum gastrin concentration (Yamaguchi, Fuke, Tsujita & Kumada, 1978). However, neither morphine nor Met-enkephalin administered intra-arterially direct to the stomach affected unstimulated gastric secretion (Konturek, Pawlik, Walus, Coy & Schally, 1978b). The reported effects of opiate agonists on stimulated gastric secretion are more confused. Histamine-stimulated acid secretion from Heidenhain pouches (Magee, 1975) and from an *in vivo* chambered preparation (Konturek *et al.*, 1978a, b) was increased by intravenous and intra-arterial administrations, whilst secretion from a gastric fistula was unaffected (Magee, 1975). Pentagastrin-stimulated secretion was reduced (Magee, 1975) or increased (Konturek *et al.*, 1978b) by opiates. Secretion stimulated by 2-deoxy-D-glucose is reported to be reduced by morphine, whereas pilocarpine-stimulated secretion was increased (Magee, 1975).

The confusing nature of the reports on the actions of opiates on gastric secretion may be the result of the multitude of actions of opiates. Opiates have been found to stimulate the release of histamine (Grechishkin, 1968), and gastrin (Yamaguchi *et al.*, 1978), and to increase directly gastric mucosal blood flow (Konturek *et al.*, 1978a, b). These effects are all likely to enhance gastric secretion. In contrast, opiates can inhibit the release of acetylcholine from cholinergic nerve endings in the gut (Paton, 1957; Waterfield, Smokum, Hughes, Kosterlitz & Henderson, 1977), and Magee (1975) has suggested that this action accounts for the inhibition of vagally- and pentagastrin-stimulated gastric secretion. However, Met-enkephalin was without effect on insulin-stimulated secretion in the cat (Figure 6), a finding which is not consistent with Magee's suggestion.

It has been suggested that opiates stimulate the oxyntic glands directly and that the oxyntic cells may possess high affinity binding sites for opiates (Konturek *et al.*, 1978b; Konturek, 1978). Our studies in the cat lend no support to such suggestions. It is unlikely that a marked species difference between the dog and cat can explain the difference between our results in the cat and those of others in the dog since the various reports in the dog are often conflicting (see above). The failure of naloxone to influence the secretory response to insulin (Figure 7) may be evidence that endogenous opiates are not involved in the control of gastric secretion, even secretion mediated by the vagal nerves. However, this evidence cannot be taken as definitive since opiate antagonists administered systemically are known to produce little effect on systems well-characterized to contain opiate receptors (Hughes & Kosterlitz, 1977).

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