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PEPSIN SECRETION IN ANAESTHETIZED CATS STIMULATED BY PENTAGASTRIN AND GASTRIN II IN THE PRESENCE OR ABSENCE OF SECRETIN

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SUMMARY

1. In fasting anaesthetized cats pentagastrin and gastrin II, infused alone in doses which evoked a large acid response, did not stimulate the secretion of pepsin. However, peptic secretion increased significantly when either acid stimulant was infused simultaneously with a dose of Boots' secretin, in itself below the threshold for peptic stimulation.

2. The potentiation by pentagastrin and gastrin II of the peptic response to secretin was similar to the potentiation observed when caerulein, histamine and N-methyl histamine are given with secretin, an effect we have attributed to a non-specific increase of gastric mucosal blood flow which accompanies the infusion of these acid stimulants and effectively increases the concentration of secretin delivered at the site of the chief cell in the gastric mucous membrane.

INTRODUCTION

In anaesthetized cats caerulein, histamine and N-methyl histamine do not stimulate the secretion of pepsin when infused intravenously on their own. However, these acid stimulants potentiate the peptic response to an infusion of Boots' secretin, itself below the threshold of peptic stimulation, by increasing gastric mucosal blood flow and doubling the effective concentration of secretin delivered to the peptic cell (Braganza, Gibbs & Howat, 1975). Experiments using pentagastrin (*t*-butyloxycarbonyl-β-Ala.Try.Met.Asp.Phe NH_2) and gastrin II have been conducted to evaluate the peptic responses of anaesthetized cats to these polypeptides which share with caerulein the C-terminal tetrapeptide amide Try.Met.Asp.Phe-NH_2.

METHODS

Cats of 3 kg average wt. were fasted for 24 hr, anaesthetized with chloralose (75 mg/kg body wt.) and prepared according to techniques detailed previously (Howat & Schofield, 1954; Braganza *et al.* 1975). The splanchnic nerves were sectioned extraperitoneally. The stomach was isolated from the duodenum by a pyloric ligature and drained by an indwelling oesophageal catheter. The pancreatic duct was cannulated and the common bile duct was occluded. 25 ml. 0·005 N-HCl, warmed to 37 °C, was instilled into the stomach at the start of each collection period and withdrawn by syphonage after 15 min. The volume of each specimen was recorded to the nearest 0·5 ml., total acidity was estimated by titration with 0·05 N-NaOH and peptic activity was measured by the method of Hunt (1948). The volume of pancreatic juice secreted each 15 min was recorded to the nearest 0·05 ml., bicarbonate concentration was measured by back titration

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after treatment with 0·1 N-HCl and lipase activity by the pH stat method of Marchis-Mouren, Sarda & Desnuelle (1959).

The doses of pentagastrin and gastrin II employed are given in Table 1. The test substances were dissolved in sterile isotonic saline and given by bolus I.V. injection or by constant I.V. infusion for 45 min. An interval of 60–90 min was allowed between infusions to permit a return to

TABLE 1. Design of the study

Five cats in each group

Group		
1	Pentagastrin	5 μg/kg I.V.
2	Pentagastrin	0·02 μg/kg per min I.V.
3	Gastrin II	0·02 μg/kg per min I.V.
4	Pentagastrin + secretin (Boots) I.V.	0·02 μg/kg per min I.V.
5	Gastrin II + secretin (Boots) I.V.	0·02 μg/kg per min I.V.

One experiment of each

(1) Pentagastrin 0·2 μg/kg per min infused alone and with secretin
(2) Gastrin II 0·02 μg/kg per min infused alone and with secretin
(3) Gastrin II 0·2 μg/kg per min infused alone and with secretin

basal conditions. In approximately half the experiments secretin (Boots) was given either by repeated I.V. injections or by constant I.V. infusion. The dose of secretin (usually 4 Crick Harper Raper (CHR) units/kg per hr) was selected to ensure a yield of between 0·5 and 1·5 ml. pancreatic juice each 15 min.

The total output of acid or pepsin secreted in response to each test infusion was derived by subtracting twice the sum of the three 15 min outputs preceding the infusion from the sum of outputs of the three 15 min periods of the infusion and three subsequent 15 min periods, by which time the acid responses had returned to prestimulation values. The significance of the differences in the mean outputs of acid and pepsin after stimulation compared to the respective basal outputs were estimated by paired t tests. The total outputs of acid and of pepsin secreted in response to each stimulant given with and without secretin were compared by applying to each group a t test for uncorrelated means after it had been ascertained that the variances did not differ significantly. A result was considered significant when $P < 0·05$ (two-tailed tests).

RESULTS

Experiments without secretin (Figs. 1 and 2)

The rapid I.V. injection of 5 μg pentagastrin/kg (Fig. 1) resulted in a peak acid output of 0·4 ± 0·02 m-mole HCl within 15 min of stimulation (mean ± S.E. of mean of five expts.), a value significantly higher than the prestimulation output, 0·17 ± 0·01 m-mole HCl per 15 min in these experiments ($P < 0·001$). The output of acid remained high for 45 min after injection. The peak output of pepsin after stimulation, 68·2 < 10·1 units (mean ± S.E. in five experiments), was also significantly higher than the basal value, 23·4 ± 2·9 units, in these experiments, but the peptic response was transient and not discernable after the first 15 min following stimulation (Fig. 1).

The patterns of acid responses to infusions of pentagastrin and of gastrin II (0·02 μg/kg per min) were similar (Fig. 2). The peak acid outputs occurred in the period 30–45 min after the start of the infusions, and acid outputs remained significantly higher than the corresponding basal values for 45 min after the infusions were terminated. The total output of HCl after pentagastrin, 1·46 ± 0·33 m-mole

(mean ± S.E. of mean of five experiments), did not differ from that after gastrin II, 1·07 ± 0·33 m-mole (mean ± S.E. of mean of five experiments, $0·2 < P < 0·3$). Neither gastrin II nor pentagastrin increased the output of pepsin (Fig. 2), nor was the pancreas, which in a fasting cat does not secrete, stimulated.

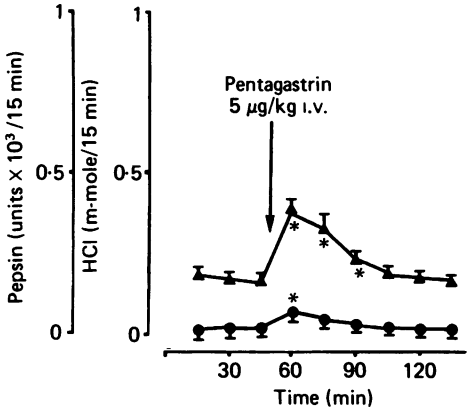

Fig. 1. Acid and peptic responses to a bolus I.V. injection of pentagastrin 5 μg/kg (mean ± S.E. of mean in five cats). ▲, HCl; ●, pepsin; ★, $P < 0·05$.

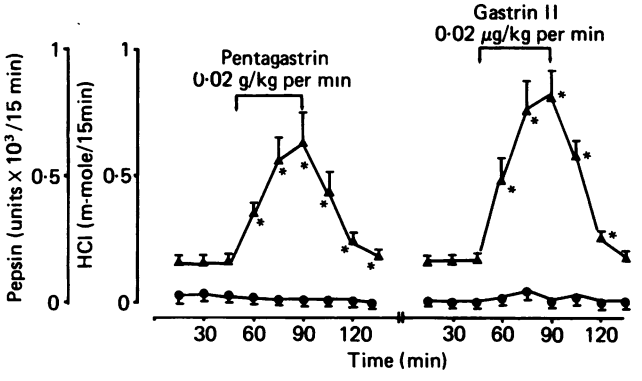

Fig. 2. Acid and peptic responses to infusion of pentagastrin 0·02 μg/kg per min (five cats, mean ± S.E. of mean), and gastrin II 0·02 μg/kg per min (five cats, mean ± S.E. of mean). ▲, HCl; ●, pepsin; ★, $P < 0·05$.

Experiments with and without secretin (Figs. 3 and 4)

In one experiment with pentagastrin and two with gastrin II, the acid stimulant was delivered twice in the same cat, initially on its own and later against a background infusion of secretin which induced and maintained a flow of pancreatic juice. In Expt. C81 (Fig. 3) pentagastrin given on its own (0·2 μg/kg per min) stimulated the secretion of HCl but not of pepsin and did not induce pancreatic secretion. In the second half of the experiment when pentagastrin was given simultaneously with secretin, not only did the output of HCl increase as previously, but peptic secretion increased sharply also, and a small increase in the volume of pancreatic juice was apparent (Fig. 3). The output of bicarbonate and of lipase increased minimally.

The patterns of the gastric and pancreatic responses to an infusion of gastrin II (0·2 μm/kg per min) given with and without secretin were similar to those evoked by the same dose of pentagastrin. When a smaller dose of gastrin II (0·02 μg/kg per min) was given with secretin in Expt. G_1 (Fig. 4) the output of pepsin increased but no increase in pancreatic secretion was detected.

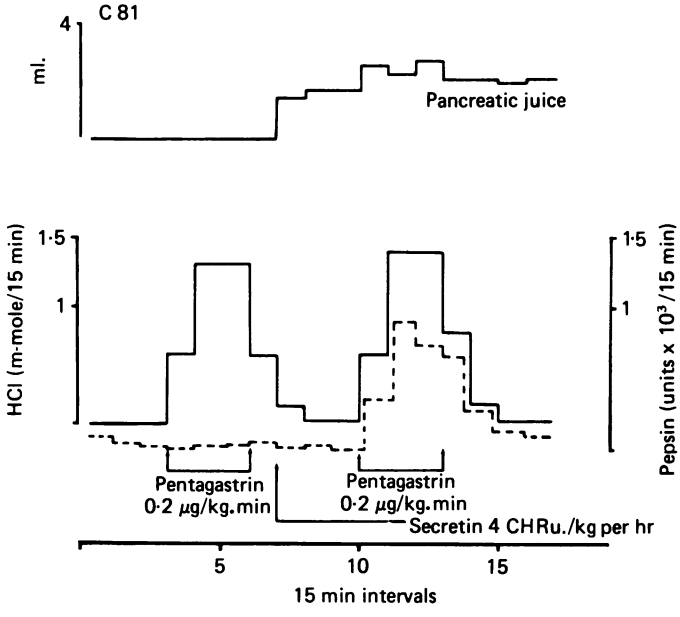

Fig. 3. Gastric and pancreatic responses of cat C81 to an infusion of pentagastrin 0·2 μg/kg per min given singly, and simultaneously with 4 CHR u./kg per hr of Boots' secretin.

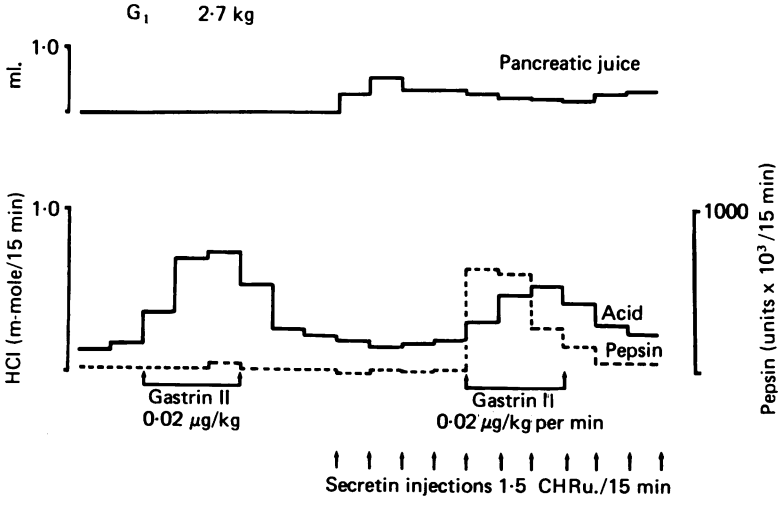

Fig. 4. Gastric and pancreatic responses of cat G_1 to an infusion of gastrin II 0·02 μg/kg pepsin given singly, and simultaneously with Boots' secretin. Observations recorded at 15 min intervals.

Experiments with secretin (Fig. 5)

The pattern of acid response to pentagastrin or gastrin II given in the presence of secretin was similar to the pattern observed when either acid stimulant was given alone. The total output of HCl after pentagastrin was 1·81 ± 0·14 m-mole and after gastrin II 2·23 ± 0·43 m-mole (mean ± S.E. of mean of five experiments in each group; 0·3 < P < 0·4). These values did not differ from the corresponding acid outputs when the acid stimulants were given in the absence of secretin.

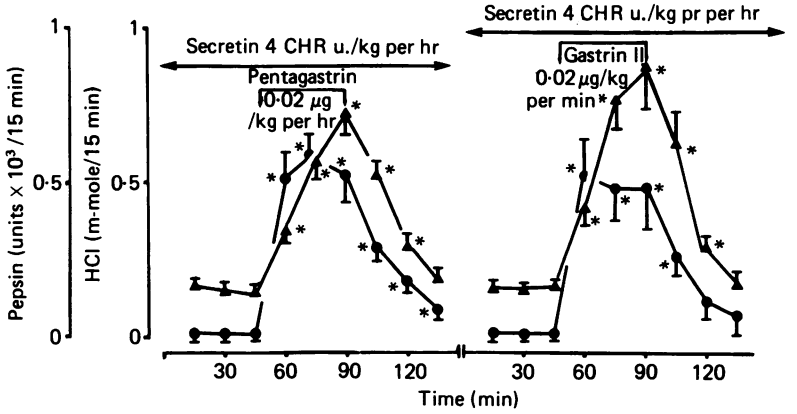

Fig. 5. Acid and peptic responses to infusions of pentagastrin 0·02 μg/kg per min (means ± S.E. of mean in five cats) and gastrin II 0·02 μg/kg per min (means ± S.E. of mean in five cats) given simultaneously with an infusion of Boots' secretin. ▲, HCl; ●, pepsin; ★, P < 0·05.

The secretion of pepsin in the control periods was similar in experiments with and without secretin (Figs. 1–5). However in the presence of secretin the output of pepsin increased sharply within 15 min of infusing the acid stimulant, to attain peak values earlier than did the acid output, and remained significantly elevated for 45 min after the test infusions ended (Fig. 4 and Table 2).

DISCUSSION

While gastrin is considered to be a most potent stimulus of gastric acid secretion, its status as a peptic stimulant remains controversial. The conflicting reports have been ascribed to differences in the preparation tested (whether crude gastrin extract, synthetic gastrin or peptides of gastrin), dissimilar doses of the stimulants, variations in innervation of the stomach and species differences. Discrepancies are apparent however even when the discussion is restricted to cats and dogs with intact vagal innervation and the use of comparable doses of the stimulants.

It is generally accepted that crude gastrin extract stimulates peptic secretion, but there is less agreement regarding the relative pepsin-stimulating activity of purified gastrin and the synthetic gastrin-related peptides. Infusion of synthetic hog gastrin II produced dose-related increases in peptic secretion in conscious gastric-fistula cats (Way, 1971), as did synthetic human gastrin I and hog gastrin II (Cooke,

1967) and hog gastrins I and II (Stening & Grossman, 1969) in conscious gastric-fistula dogs. A dose-response relationship was less apparent after pentagastrin: the highest peptic outputs in gastric-fistula dogs often occurred in response to the smallest doses of pentapeptide (Cooke, 1967; Emås, Billings & Grossman, 1968). Nevertheless the peak output of pepsin after pentagastrin was twice as high as the peak output after crude gastrin extract, synthetic human gastrin I and hog gastrin II (Cooke, 1967), while gastrin tetrapeptide proved to be the most powerful stimulant of all, twice as potent as the pentapeptide. By contrast Sewing (1967) was unable to detect any increase in peptic output using comparable doses of tetragastrin in anaesthetized gastric-fistula cats, while Reed & Sanders (1971) found no change in pepsin secretion when an infusion of 0·1 μg pentagastrin per min was given to anaesthetized cats in which the pylorus was occluded.

Our experiments are particularly suitable for the study of peptic secretion since occlusion of the pylorus and bile duct prevents the release by HCl or bile of a peptic stimulant on contact with the duodenal mucosa. In reported experiments in which a gastric fistula drained the stomach it is still possible that, following the infusion of acid stimulants which usually also increase gastric motility, some HCl escapes into the duodenum and thereby stimulates pepsin by a secondary mechanism. This factor would be more likely to operate in conscious rather than anaesthetized animals. The gastric wash-out technique using 0·005 N-HCl facilitates the recovery of viscid secretions and preserves peptic activity.

In this study I.V. infusions of pentagastrin and gastrin II given alone in doses which stimulated gastric acid secretion submaximally and maximally did not increase peptic secretion (Figs. 2 and 3). Therefore we interpret the transient peptic response to pentagastrin injections as a wash-out phenomenon, but, if this is the sole explanation, it is perhaps surprising that a similar peptic response did not occur in the first 15 min after infusion of the stimulants (Fig. 2) when the outputs of HCl were as large as that after pentagastrin injection. This apparent anomaly may relate to differences in the kinetics of drug metabolism, and differences in the rate of increment in drug concentration within the gastric mucosa when the stimulant is given by bolus injection compared to I.V. infusion.

When secretin (4 CHR u./kg per hr) was infused simultaneously with each acid stimulant (Figs. 3 and 5) the output of pepsin increased significantly and the output of acid increased minimally. This pattern of events is identical to that which we have reported when caerulein, histamine and N-methyl histamine are given with a dose of secretin subthreshold for peptic stimulation (Braganza, Gibbs & Howat, 1975, 1976). We have previously argued that the likeliest explanation for the potentiation by acid stimulants of the peptic response to secretin is the increase in gastric mucosal blood flow which accompanies acid secretion in response to these acid stimulants of different chemical structure (Jacobson, Eisenberg & Swan, 1967; Harper, Reed & Smy, 1968). The mucosal hyperaemia, although not directly linked to peptic secretion (Reed & Sanders, 1971), would effectively increase the concentration of secretin or a peptic stimulant contained in impure secretin (Braganza & Howat, 1976; Braganza, Howat & Kay, 1976; Vagne, Mutt, Perret & Lemaitre, 1976) at target sites within the peptic cell, and thus indirectly enhance the production of pepsin.

Tracy & Gregory (1964) stated that the C-terminal tetrapeptide of the gastrin

molecule possesses the entire range of physiological activities found in the natural gastrin. Since in the cat gastrin II, pentagastrin, and gastrin tetrapeptide (Sewing, 1967) do not stimulate peptic secretion (provided no HCl enters the duodenum) while crude gastrin extracts do (Uvnas, 1948; Blair, Harper & Lake, 1953), the existence in antral extracts of another substance with specific peptic-stimulating activity seems likely. Whether it is the same as the peptic stimulant in crude secretin Braganza & Howat, 1976; Vagne *et al.* 1976) remains to be elucidated.

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