Br. J. Pharmac. (1974), 52, 219-225

GASTRIN RELEASE BY BOMBESIN IN THE DOG

G. BERTACCINI
Institute of Pharmacology, University of Parma, 43100 Parma, Italy

V. ERSPAMER, P. MELCHIORRI & N. SOPRANZI
Institute of Medical Pharmacology I, University of Rome, 00185 Rome, Italy

1 The intravenous infusion of bombesin produced in intact dogs and, more strikingly in dogs provided with gastric fistulae a sharp increase in plasma levels of immunoreactive gastrin and at the same time a stimulation of gastric acid secretion. Gastrin response was correlated with the dose of bombesin from approximately 0.1 $\mu g\ kg^{-1}\ h^{-1}$ (threshold) to 1 $\mu g\ kg^{-1}\ h^{-1}$ (maximum gastrin release).

2 Atropine and metiamide reduced or inhibited gastric acid secretion stimulated by bombesin, but did not affect the rise in gastrin levels.

3 Acidification of the whole stomach or of a perfused antral pouch caused a reduced or delayed response to bombesin. However, the inhibitory effect of acidification could be surmounted by prolonging the duration of bombesin infusion.

4 Antrectomy greatly reduced the rise in gastrin levels and the increase in acid gastric secretion produced by bombesin, but left unaffected the gastric secretagogue effect of pentagastrin.

5 It is concluded that bombesin is a potent releaser of gastrin from the antral mucosa.

6 The possible influence of the renal effects evoked by bombesin in the dog on the gastrin response to the polypeptide is discussed.

Introduction

It was shown in a previous paper (Bertaccini, Erspamer & Impicciatore, 1973) that bombesin (Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly- His-Leu-Met-NH_2), an active tetradecapeptide isolated from methanol extracts of the skin of two European discoglossid frogs *Bombina bombina* and *Bombina variegata variegata* (Anastasi, Erspamer & Bucci, 1972), displayed a potent stimulant action on secretion from the denervated fundic pouch of the dog. There was a conspicuous increase in the volume of gastric juice accompanied by an increase in total acid and pepsin outputs and in hydrochloric acid concentration. The threshold dose of bombesin was 5-10 ng/kg by the subcutaneous route, and 0.05-0.1 $\mu g\ kg^{-1}\ h^{-1}$ by intravenous infusion. In contrast to gastrin and caerulein, bombesin also elicited a moderate secretory response following rapid intravenous injection. The acid secretion provoked by bombesin was almost completely inhibited by atropine.

In this paper strong evidence is presented in favour of the hypothesis that the secretagogue effect of bombesin in the dog stomach is due to its capacity to release gastrin.

Methods

Intact dogs

A limited number of experiments was carried out in 6 intact dogs, before surgery.

Surgical procedures

Six mongrel dogs weighing between 14 and 23 kg were prepared with gastric fistulae drained by a Thomas cannula inserted into the most dependent portion of the stomach. Thirteen dogs weighing from 15 to 25 kg were provided with a gastric fistula and a Heidenhain pouch. The gastric fistula was drained by a Thomas cannula, and the Heidenhain pouch by a Gregory cannula. In 4 dogs of 20 to 25 kg, provided with gastric fistulae and Heidenhain pouches, vagally innervated pouches of antrum were prepared, care being taken to exclude all acid secreting tissue from these pouches. The innervated antral pouch was drained to the exterior by a Gregory cannula at the pyloric end. Finally 7 dogs, weighing from 15 to 27 kg, were antrectomized by dividing the antrum from the fundus and the duodenum, immediately below the pyloric ring. Gastrointestinal continuity was

re-established by means of an end-to-end gastroduodenostomy. Four to five weeks were allowed for recovery from these procedures before experiments were started. The animals were fasted for 18 h before each experiment but permitted access to water throughout. Studies on each dog were performed not more than once weekly.

Gastric juice was collected from the main stomach by gravity drainage from the gastric cannula; secretions from the Heidenhain pouch were obtained by the washout technique (Burstall & Schofield, 1953).

Acid output, expressed in mEq HCl/15 min, was measured by titration to pH 7 with 0.1 N or 0.01 N NaOH using a glass electrode (Radiometer) and an automatic titrating burette. The antral pouch was perfused by the method described by Czendes, Walsh & Grossman (1972).

Radioimmunoassay of gastrin

Blood samples for the determination of plasma gastrin by radioimmunoassay were obtained from superficial leg veins and collected in precooled plastic tubes to which disodium edetate had been added to a final concentration of 1 mg/ml of blood. After centrifugation the plasma samples were stored at -20°C until the immunoassay was performed.

An antibody prepared in rabbits against porcine gastrin was used at a final dilution of 1 : 35,000. Human synthetic gastrin I was used as a standard, and purified, monoiodinated ^{125}I-labelled human gastrin I as a tracer. In absence of cold gastrin, the ratio of bound to total labelled gastrin was 30%, at the final dilution of the antibody.

Gastrin concentrations were determined in plasma diluted 1 : 10 in 0.02 M barbitone buffer, at pH 8.0. All plasma samples were assayed in duplicate. Plasma gastrin concentrations as low as 10 pg/ml could be measured by this assay, with an average difference of 10% between duplicate specimens. With our antibody superimposable dose-response curves could be obtained for standard and diluted dog plasma.

No cross reaction was found between the antibody and bombesin. However, on a molar basis the ratio between the dose producing 50% inhibition (ID_{50}) for synthetic porcine gastrin I and pure porcine cholecystokinin was 0.01.

Drugs

Bombesin was synthesized at the Farmitalia S.p.A. Laboratories for Basic Research, Milan. Pentagastrin was obtained from ICI, England and metiamide (*N*-methyl-*N'*{2[(-methylimidazol-4-yl)methylthio]ethyl}thiourea) from the Smith, Kline & French Lab. Ltd, England. Synthetic human gastrin I (1-17) synthetic human ^{125}I-gastrin I (1-17) and antibody to porcine gastrin were purchased from Sorin, Saluggia, Italy.

Other substances used were as follows: sodium pentobarbitone (Abbott), disodium edetate (Merck, Darmstad), atropine sulphate (British Drug Houses).

Results

Intact dogs

The intravenous infusion of 1 μg kg^{-1} h^{-1} of bombesin, over a 60 min period, elicited a rise in plasma levels of immunoreactive gastrin in the intact dog. However, this rise was never very intense and reached its peak at different times in different dogs. Sometimes maximum levels were observed 7 to 15 min after the start of bombesin infusion while in other cases they occurred at the end of the infusion. When the response was prompt, the peak was followed by a decrease in gastrin levels in spite of continuing the infusion. Basal values of gastrin in the plasma of 6 intact dogs were 51 ± 8 pg/ml, and peak values observed after bombesin administration were 105 ± 22 pg/ml.

Dogs provided with gastric fistulae and Heidenhain pouches

In dogs provided with gastric fistulae alone or with gastric fistulae plus Heidenhain pouches, bombesin produced a considerable increase in gastrin levels in plasma and at the same time a potent stimulation of acid secretion both in the main stomach and in the Heidenhain pouch.

Figure 1 shows the peak increases in plasma gastrin levels above basal values (63 ± 9 pg/ml) observed in 6 dogs with open gastric fistulae after the intravenous infusion of graded doses of bombesin, over a 60 min period. The threshold dose of the polypeptide was of the order of 0.1 μg kg^{-1} h^{-1} and there was an evident dose-response relationship up to 1 μg kg^{-1} h^{-1}. A further increase in the infusion rate did not produce more pronounced effects.

Figure 2 illustrates the effects of an intravenous infusion of 1 μg kg^{-1} h^{-1} of bombesin, over a 60 min period, on plasma gastrin levels and on acid output of the main stomach and of the Heidenhain pouch. Gastrin levels rose from 66 ± 10 pg up to 289 ± 17 pg/ml plasma, acid output of the main stomach rose from 0.095 ± 0.015 to 2.12 ± 0.36 mEq HCl/15 min, and acid output of the

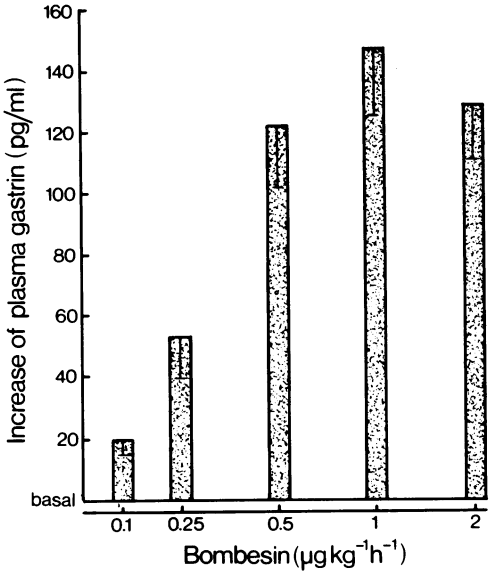

Fig. 1 Gastric fistula dogs. Peak increases of plasma gastrin levels, above basal levels, after intravenous infusion, for 60 min, of graded doses of bombesin. Columns represent the mean of 2 measurements in each of 6 dogs. Vertical bars show s.e. mean.

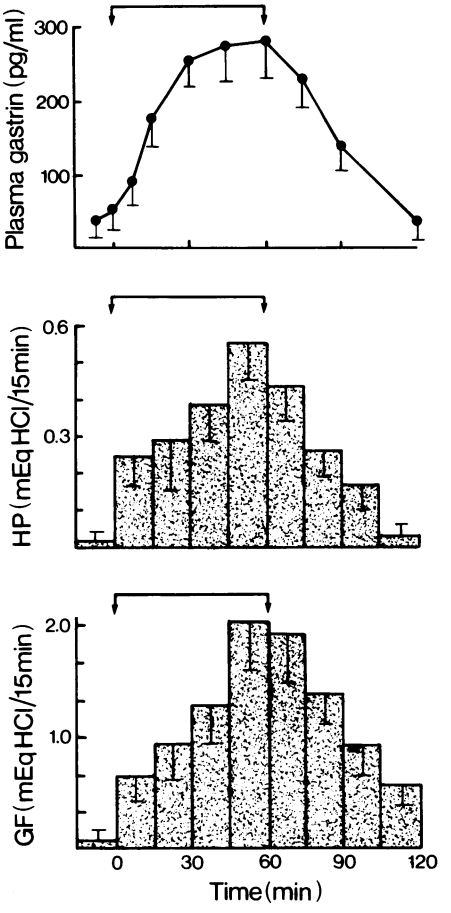

Fig. 2 Gastric fistula dogs provided with Heidenhain pouches. Plasma gastrin levels and acid outputs, in the Heidenhain pouch (HP) and in the main stomach (GF) following intravenous infusion of 1 $\mu g\ kg^{-1}\ h^{-1}$ of bombesin for 60 min (brackets). Columns represent the mean of one measurement in each of 13 dogs. Vertical bars show s.e. mean.

Heidenhain pouch increased from 0.015 ± 0.007 to 0.585 ± 0.095 mEq HCl/15 minutes.

The effect of bombesin on plasma gastrin levels was prompt and increased progressively throughout the infusion period, attaining a maximum at the end of this period. After the bombesin infusion was stopped, gastrin levels began to decrease immediately, reaching basal values within 60 minutes. Gastric acid secretion both of the main stomach and of the Heidenhain pouch was strictly correlated with plasma gastrin levels. The concentration of HCl in bombesin-induced gastric juice was always greater than in control juice.

Pre-medication with atropine gave somewhat erratic results depending mainly on the dose of the alkaloid. However, with a subcutaneous dose of 0.2 mg/kg fairly uniform and reproducible effects were obtained, as shown in Figure 3. The prominent feature of the response to bombesin infusion (2 $\mu g\ kg^{-1}\ h^{-1}$) after atropine was a sharp dissociation between plasma gastrin levels and gastric acid secretion. In fact, whereas rises in gastrin levels were of almost the same magnitude as those observed without atropine treatment, there was a complete or nearly complete inhibition of gastric acid secretion.

Dogs with gastric fistulae differed from intact dogs in that their gastric juice was drained to the exterior during the whole period of bombesin infusion, thus avoiding to a great extent acidification of the antrum. The difference in the response to bombesin of dogs with open and closed gastric fistulae, the latter resembling intact dogs, was studied in 3 dogs with gastric fistulae, each provided with a Heidenhain pouch.

In a first series of experiments the gastric fistula was left open during bombesin infusion and gastric juice was allowed to drain freely to the exterior; in a second series of experiments, on the same dogs, the gastric fistula was kept closed during bombesin infusion after 60 ml 0.1 N HCl had been introduced into the main stomach. It is evident that under these conditions part of the HCl from

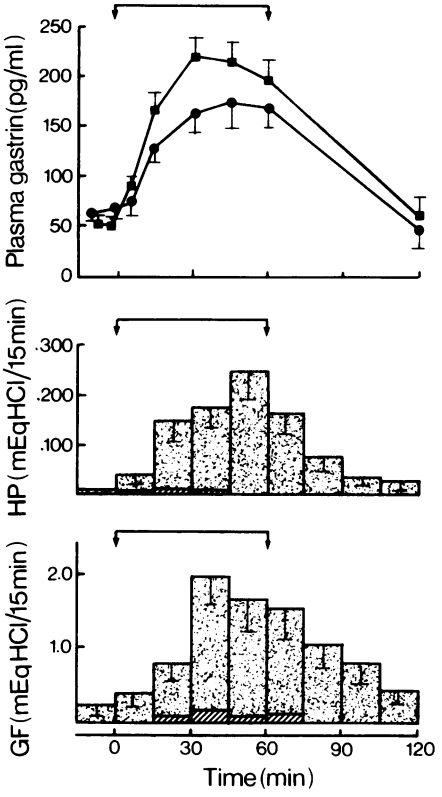

Fig. 3 Gastric fistula dogs provided with Heidenhain pouches. Plasma gastrin levels and acid outputs, in the Heidenhain pouch (HP) and in the main stomach (GF), following intravenous infusion, for 60 min, of 2 μg kg^{-1} h^{-1} of bombesin (brackets) in 5 animals either non-premedicated (▪, stippled areas) or premedicated with atropine sulphate (0.2 mg/kg, s.c., •, hatched areas). The columns represent the mean and the vertical bars the s.e. mean.

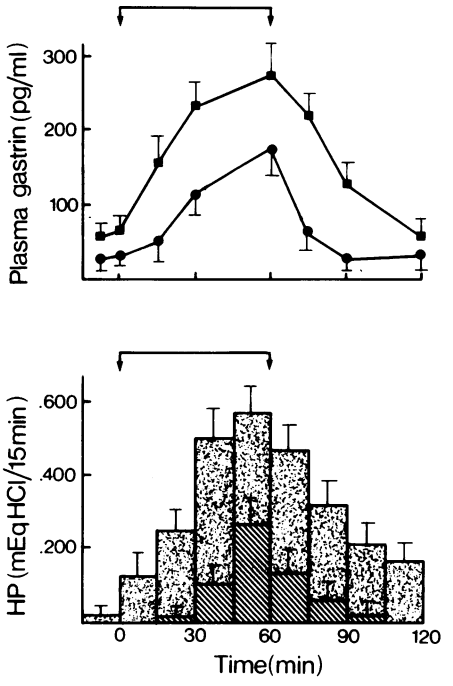

Fig. 4 Gastric fistula dogs provided with Heidenhain pouches. Plasma gastrin levels and acid outputs, in the Heidenhain pouch (HP) following intravenous infusion of 1 μg kg^{-1} h^{-1} of bombesin (brackets), for 60 minutes. The gastric fistula was kept either open (▪, stippled areas) or closed (•, hatched areas) during the bombesin infusion. Columns represent the mean of 3 measurements in each of 3 dogs. Vertical bars show s.e. mean.

the main stomach could pass into the duodenum, with possible liberation of duodenal peptides.

Figure 4 demonstrates that gastric acidification sharply reduced, but did not abolish, the response to bombesin infused over a 60 min period at a rate of 1 μg kg^{-1} h^{-1}. Gastrin levels presented a moderate increase, which closely resembled that observed in intact animals. Total acid output in the Heidenhain pouch was also reduced, attaining its maximum at the end of bombesin infusion, when gastrin levels reached their peak.

Dogs provided with Heidenhain pouches and innervated antral pouches

In a first series of experiments (3 experiments on each of 3 dogs) bombesin was infused for 150 min at the rate of 1 μg kg^{-1} h^{-1} while the antral pouch was left at neutral pH; in a second series of experiments on the same dogs bombesin infusion was repeated while the antral pouch was irrigated with 0.1 N HCl at a rate of 1 ml/minute. It could be observed that whereas rise in plasma gastrin levels was prompt and rapid with the neutral antrum, it was considerably slower after acidification of the antral pouch. For example, after 30 min of bombesin infusion gastrin had risen from a basal level of 61 ± 10 pg/ml to 192 ± 16 pg/ml with the neutral antrum, but only from 28 ± 6 pg/ml to 77 ± 11 pg/ml with the acid antrum. The behaviour of acid secretion in the Heidenhain pouch paralleled changes in gastrin levels, although in these experiments rise of gastrin levels clearly preceded increase in acid secretion.

By continuing bombesin infusion the initial inhibitory effect of antral acidification could be overcome, and after 150 min increases in plasma

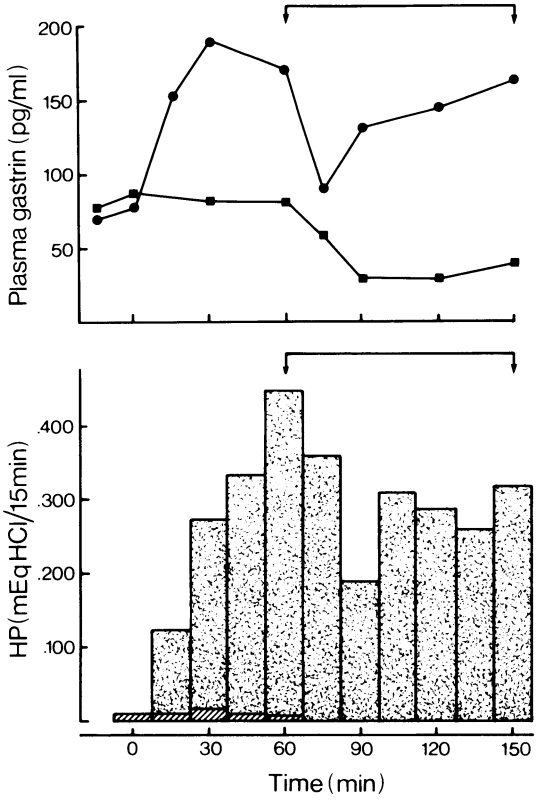

Fig. 5 Dogs provided with innervated antral pouches and Heidenhain pouches. Plasma gastrin levels and acid output in the Heidenhain pouch (HP) following intravenous infusion of 1 μg kg^{-1} h^{-1} of bombesin (●, stippled) or saline (■, hatched), for 150 minutes. During the first 60 min of infusion the antral pouch was neutral, between 60 and 150 min it was irrigated with 0.1 N HCl (brackets). The points represent the mean of two experiments.

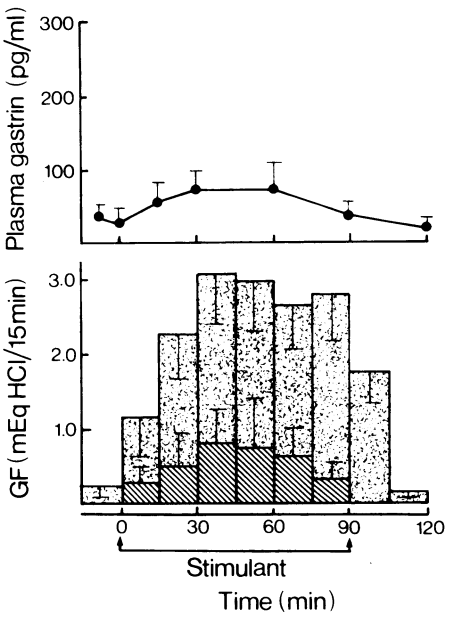

Fig. 6 Antrectomized dogs provided with gastric fistulae. Plasma gastrin levels and acid output in the main stomach (GF) following intravenous infusion of 1 μg kg^{-1} h^{-1} of bombesin (hatched) or 5 μg kg^{-1} h^{-1} of pentagastrin (stippled). Columns represent the mean of one measurement in each of 5 dogs. Vertical bars show s.e. mean.

gastrin levels and acid outputs were approximately of the same magnitude with the neutral and with the acid antrum.

The inhibitory effect of antral irrigation with acid on responses evoked by bombesin was easily demonstrated also during the course of a bombesin infusion with neutral antrum. In 2 experiments the antral pouch was irrigated with 0.1 N HCl between 60 and 120 min of the bombesin infusion. There was an immediate fall in plasma gastrin levels and a more delayed reduction of acid gastric secretion. However, in spite of continuing acid irrigation plasma gastrin levels and acid gastric secretion tended to return rather promptly to pre-acidification values (Figure 5). In control experiments (infusion of 0.9% w/v NaCl solution, saline) HCl irrigation of the antrum caused a decrease in basal gastrin levels and basal acid secretion.

Antrectomized dogs with gastric fistulae

The infusion of bombesin (1 μg kg^{-1} h^{-1} over a 90 min period) into antrectomized dogs produced only a small increase in plasma gastrin levels, accompanied by a similar small increase in gastric acid secretion (Figure 6). However, the fundic mucosa retained its capacity to secrete in response to gastrin, as shown by the conspicuous secretion evoked by the infusion of 5 μg kg^{-1} h^{-1} of pentagastrin.

Preliminary experiments on the interaction between metiamide and bombesin

Metiamide, a potent blocking agent of histamine H_2-receptors, which is known to be an inhibitor of the effects of histamine and of gastrin on gastric secretion (Wyllie, Ealding, Hesselbo & Plack, 1973), was given by rapid intravenous injection into 2 dogs, at a dose of 5 mg/kg, 30 min after an

intravenous infusion of 1 $\mu g\ kg^{-1}\ h^{-1}$ of bombesin was started. Whereas bombesin-induced increase in plasma gastrin levels remained unchanged, gastric acid secretion was reduced by 60%.

Discussion

Previous studies (Bertaccini, Erspamer & Impicciatore, 1973) and the results obtained in this investigation have shown that bombesin is a potent stimulant of acid gastric secretion in dogs provided with gastric fistulae. All available experimental data suggest that bombesin acts on the oxyntic cells not directly, but indirectly through liberation of gastrin. Thus bombesin is a powerful gastrin releaser. The following evidence strongly supports this view:

(a) bombesin produced a prompt rise of plasma levels of immunoreactive gastrin. In gastric fistula dogs the increase in gastrin levels was proportional to the bombesin infusion rate and, after having reached a peak, lasted as long as the infusion was continued. When the infusion of bombesin was stopped plasma gastrin levels fell rapidly to pre-infusion values;

(b) acid outputs in the main stomach and in the Heidenhain pouch were proportional and time-correlated to the gastrin increases produced by bombesin;

(c) pre-medication with atropine greatly reduced or abolished the gastric secretagogue effect of bombesin, like that of gastrin and caerulein (Grossman, 1967; Bertaccini, Endean, Erspamer & Impicciatore, 1968). However, increase in plasma gastrin levels remained unchanged or was affected to a much smaller degree. Similarly, metiamide given during a bombesin infusion did not appreciably affect rise in plasma gastrin, but reduced by 60% acid gastric secretion;

(d) antrectomy, i.e. removal of the main site of production of gastrin, greatly reduced the increase in plasma gastrin levels evoked by bombesin infusion, and also caused a parallel decrease in acid gastric secretion. However, in antrectomized animals pentagastrin retained its secretagogue effect;

(e) acidification of an antral pouch caused a delayed and less intense response to bombesin.

There is no explanation for these results other than the hypothesis that bombesin releases gastrin from its site of production and storage in the antral mucosa.

A minor increase in plasma gastrin levels and in acid output of the main stomach was also seen, following bombesin infusion in antrectomized dogs. It is possible that this is due to release of gastrin(s) of extra-antral origin. However, it is also possible that at least the increase in gastric acid secretion is elicited by cholecystokinin released by bombesin. There is in fact strong evidence that bombesin besides releasing gastrin may also cause a release of cholecystokinin from the duodenal mucosa of the dog (Erspamer, Improta, Melchiorri & Sopranzi, 1974).

It is well known that antral acidification prevents gastrin release by all forms of stimulation, including acetylcholine (Walsh, 1973). Antral acidification also inhibited gastrin release by bombesin. However, if bombesin infusion was prolonged for a sufficiently long time the inhibitory effect of antral acidification was overcome and plasma gastrin rose to levels similar to those seen with the neutral antrum.

It is likely that the less intense gastrin-releasing effect displayed by bombesin in intact fasting dogs is a consequence of antral acidification produced by the acid first secreted under the influence of the polypeptide.

Present experiments do not solve the problem of whether gastrin release produced by bombesin may be reinforced or modulated by a cholinergic mechanism.

It is possible that the high gastrin levels caused by bombesin in gastric fistula dogs may be the result of two different effects of this polypeptide: increased release and delayed inactivation of gastrin. Bombesin at infusion rates similar to those used in the present experiments, produced a strong constriction of the afferent arterioles in the kidney of the dog, with consequent reduction of renal blood flow and impairment of tubular function (Erspamer, Melchiorri & Sopranzi, 1973). Since the kidney is considered one of the major sites in which circulating gastrin is inactivated, especially during periods of stimulated gastrin release (Clendinnen, Reeder & Thompson, 1971; Booth, Reeder, Hjelmquist, Brandt & Thompson, 1973), it is obvious that if the kidney is not fully functional this may contribute to the rise in gastrin levels and/or to the maintenance of these levels above normal for a longer time. However, even in man, where the renal effects of bombesin seem to be small, bombesin acted as a potent gastrin releaser (Erspamer, Melchiorri, Sopranzi, Torsoli, Corazziari & Improta, 1973).

It appears from preliminary experiments (Melchiorri, Erspamer & Sopranzi, unpublished observations) that the entire sequence of 14 amino acid residues present in the bombesin molecule is not necessary for the gastrin-releasing activity of the polypeptide. The C-terminal nonapeptide had approximately 50% of the activity of bombesin,

on a molar basis, and the C-terminal octapeptide 5%.

A fundamental problem, which is now being studied by our group is whether bombesin-like peptides are present in the gastrointestinal mucosa of mammals.

This work was supported by grants from the Consiglio Nazionale delle Richerche, Rome. Gifts of pentagastrin from Dr J.S. Morley, ICI, England and of metiamide from Smith, Kline & French, England, are gratefully acknowledged.

References

ANASTASI, A., ERSPAMER, V. & BUCCI, M. (1972). Isolation and amino acid sequence of alytesin and bombesin, two analogous active tetradecapeptides from the skin of European discoglossid frogs. *Arch. Biochem. Biophys.*, **148**, 443-446.

BERTACCINI, G., ENDEAN, R., ERSPAMER, V. & IMPICCIATORE, M. (1968). The actions of caerulein on gastric secretion of the dog and the rat. *Br. J. Pharmac.*, **34**, 311-329.

BERTACCINI, G., ERSPAMER, V. & IMPICCIATORE, M. (1973). The action of bombesin on gastric secretion of the dog and the rat. *Br. J. Pharmac.*, **47**, 437-444.

BOOTH, R.A.D., REEDER, D.D., HJELMQUIST, B.E., BRANDT, E.N., Jr. & THOMPSON, J.C. (1973). Renal inactivation of endogenous gastrin in dogs. *Arch. Surg.*, **106**, 851-854.

BURSTALL, P.A. & SCHOFIELD, B. (1953). Secretory effects of psychic stimulation and insulin hypoglycemia on Heidenhain gastric pouches in dogs. *J. Physiol., Lond.*, **120**, 383-408.

CLENDINNEN, B.G., REEDER, D.D. & THOMPSON, J.G. (1971). Renal uptake and excretion of gastrin in the dog. *Surg. Gynec. Obstet.*, **132**, 1039-1043.

CZENDES, A., WALSH, J.H. & GROSSMAN, M.I. (1972). Effect of atropine and antral acidification on gastrin release and acid secretion in response to insulin and feeding in dogs. *Gastroenterology*, **63**, 257-263.

ERSPAMER, V., MELCHIORRI, P. & SOPRANZI, N. (1973). The action of bombesin on the kidney of the anaesthetized dog. *Br. J. Pharmac.*, **48**, 438-455.

ERSPAMER, V., MELCHIORRI, P., SOPRANZI, N., TORSOLI, A., CORAZZIARI, E. & IMPROTA, G. (1973). Preliminary data on the action of bombesin on secretion and motility of the gastrointestinal tract in man. *Rendic. Gastroenterol., Roma*, **5**, 16-17.

ERSPAMER, V., IMPROTA, G., MELCHIORRI, P. & SOPRANZI, N. (1974). Evidence of cholecystokinin release by bombesin in the dog. *Br. J. Pharmac.*, **52**, 227-232.

GROSSMAN, M.I. (1967). Some aspects of gastric secretion. *Gastroenterology*, **52**, 882-892.

WALSH, J.H. (1969). Control of gastric secretion. In: *Gastrointestinal Disease: Pathophysiology, Diagnosis, Management*, ed. Sleisenger, M.H. & Fordtran, J.S., pp. 144-162. Philadelphia: Saunders Co.

WYLLIE, J.H., EALDING, W.D.P., HESSELBO, T. & PLACK, J.W. (1973). Inhibition of gastric secretion in man by metiamide: a new, orally active histamine H_2-receptor antagonist. *Gut*, **14**, 424.

(Received January 3, 1974)