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The actions of bombesin on gastric secretion of the dog and the rat

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Summary

1. Bombesin stimulated acid secretion from the denervated fundic pouch of the dog. Whereas the concentration of hydrochloric acid in bombesin-produced juice was always higher than in control juice this did not occur for pepsin, the concentration of which remained below the basal values. The threshold dose of bombesin was 5–30 ng/kg by the subcutaneous route and 0·05–0·2 (μg/kg)/h by intravenous infusion. At low doses bombesin was more active than caerulein, even on a molar basis, and at high dose levels was as active as caerulein. In contrast to gastrin and caerulein, bombesin elicited a moderate secretory response also following rapid intravenous injection.

2. The acid secretion provoked by bombesin was almost completely inhibited by atropine and reduced by approximately 50% by hexamethonium.

3. Bombesin did not stimulate acid secretion in the lumen-perfused preparation of the rat stomach when administered by subcutaneous injection (up to 10 μg/kg) or by intravenous infusion (up to 10 (μg/kg)/hour). An irregular increase in acid output was observed only following rapid intravenous injection and this was of doubtful significance.

4. The mechanism of the secretagogue action of bombesin on the dog stomach is discussed.

Introduction

Bombesin, the tetradecapeptide occurring in the skin of the European discoglossid frogs *Bombina bombina* and *Bombina variegata variegata* has been shown to act on several isolated and *in situ* smooth muscle preparations, on the systemic blood pressure of some common laboratory animals and on the kidney of the anaesthetized dog (Erspamer, Falconieri Erspamer, Negri & Inselvini, 1972 ; Erspamer, Melchiorri & Sopranzi, 1972, 1973).

This paper describes the effects of bombesin on the secretory activity of the stomach of the dog and rat. The effects of bombesin have been compared with those of caerulein (Bertaccini, Endean, Erspamer & Impicciatore, 1968).

Methods

Dogs with gastric fistulas and Heidenhain pouches

Twelve dogs of either sex weighing 10–14 kg were used. They were provided with gastric fistulas and Heidenhain pouches. Gastric fistulas were placed close to the antral-corpus junction, along the greater curvature, and drained with a metal

cannula brought to the surface through the right mammary line. Between tests, the fistulas were closed with a plug. During the tests gastric juice was removed to avoid the interference of the excessive acidification of the duodenum. Heidenhain pouches were drained through the left mammary line by a metal cannula which remained constantly open.

Secretory tests were not started until at least twenty days after the surgical operations were performed. The animals were fasted for 18 h before tests were started and were used twice a week. They maintained their body weight during the period of observation.

Gastric juice was collected and measured every 15 min when the drugs were administered by continuous intravenous infusion and every 30 min after single intravenous or subcutaneous injections. Hydrochloric acid was titrated with 0·1 N NaOH using phenolphthalein as indicator. Pepsin was titrated by the method of Hunt (1948).

Perfused rat stomach preparation

For continuous recording of gastric acid secretion the procedure described by Mantegazza & Piccinini (1962) was followed, slightly modified as described by Bertaccini *et al.* (1968); female rats of the Wistar strain were used.

Drugs were administered by different routes. Intravenous injections or infusions were made via a needle in the vein of the tail. The average values obtained during three 8-min periods following administration of 0·9% w/v NaCl solution (saline) were taken as basal values.

Drugs

Synthetic caerulein (molecular weight 1,352) and synthetic bombesin (molecular weight 1,621), prepared at the Farmitalia Laboratories for Basic Research, Milan, were used, together with atropine sulphate and hexamethonium bromide.

Results

Dog

Effects of subcutaneous injection

Single subcutaneous injections of bombesin produced in conscious dogs with denervated fundic pouches a highly significant increase in both the volume and acidity of gastric juice. The total output of pepsin was also increased, but the concentration of the enzyme in gastric juice was decreased.

Figure 1 shows the total acid output produced by graded subcutaneous doses of bombesin as compared to the acid output produced by similar doses of caerulein. The threshold dose of bombesin was very low, 5–30 ng/kg, ten times smaller than the threshold dose of caerulein. When the dose was increased the acid secretion did not appreciably change up to 50 ng/kg but thereafter a clear dose-response relationship could be seen up to an optimum of 2 μg/kg, where both volume and acid outputs attained peak values which were 6 to 20 times the basal values.

The threshold dose for caerulein in the present experiments was 0·05–0·1 μg/kg and the magnitude of the response was proportional to the dose up to 2 μg/kg.

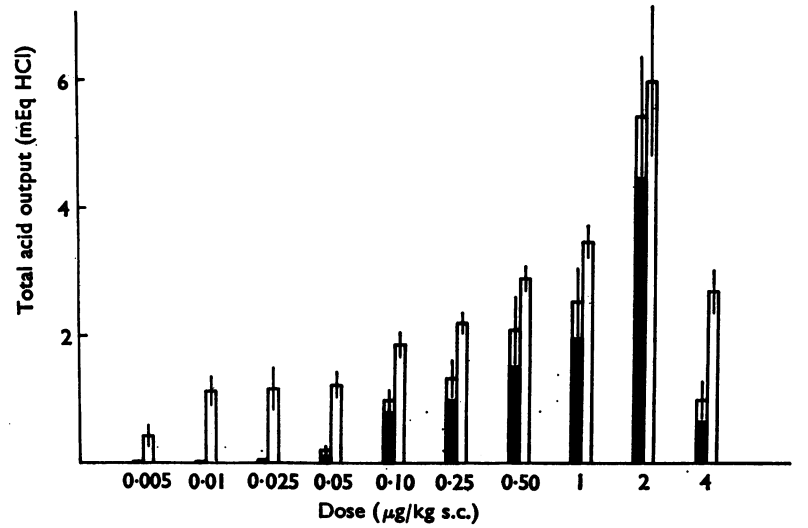

FIG. 1. Denervated gastric pouches of eight dogs. Total acid output (in mEq HCl) produced, over a period of 3·5 h, by increasing subcutaneous doses of bombesin (white columns) and caerulein (black columns). Vertical bars represent standard errors of the mean.

Thereafter the response to caerulein, like that to bombesin, decreased. Thus, at low doses bombesin was more potent than caerulein even on a molar basis ; at doses of 0·5–2 μg/kg the two peptides were approximately equiactive.

The response to a subcutaneous injection of bombesin began after 10–15 min, reached a maximum at 90–120 min and then slowly declined. For large doses the effect of the polypeptide lasted 4–5 hours.

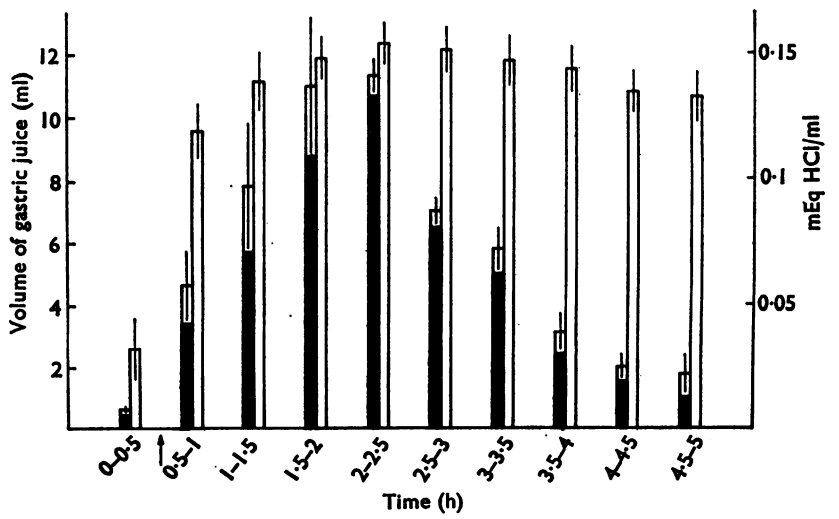

FIG. 2. Denervated gastric pouches of four dogs. Volume of gastric juice in ml (black columns) and concentration of hydrochloric acid, in mEq/ml (white columns) following subcutaneous injection of 2 μg/kg bombesin, at arrow. Gastric juice was collected every 30 minutes. Vertical bars are standard errors.

Figure 2 shows the effect of a subcutaneous injection of 2 μg/kg of bombesin, followed over a 4·5 h period. The outstanding feature was that the gastric juice secreted under the influence of the polypeptide was very acid: from a value of 0·03 mEq HCl/ml in control juice, acidity rose rapidly to values of 0·12–0·15 mEq HCl/ml, which were then maintained throughout the duration of an experiment in spite of the sharp reduction in the volume of gastric juice.

The behaviour of pepsin secretion under the influence of bombesin (2 μg/kg) is shown in Table 1. Total pepsin output increased concomitantly with the increase in volume but pepsin concentrations never exceeded basal values. During the period of maximum acid secretion (60–150 min) pepsin concentrations attained only 20–40% of the basal values. This pattern was different from that seen after administration of caerulein which was a potent stimulant of pepsin secretion.

TABLE 1. *Effects of subcutaneous injection of bombesin (2 μg/kg, four dogs) and caerulein (2 μg/kg, two dogs) on volume of gastric juice and pepsin secretion of the denervated fundic pouch of the dog.*

Observation periods	Volume of gastric juice (ml)	Total pepsin outputs (units)	Pepsin concentration (units/ml)
Pre-injection period			
30– 0 min	0·98±0·19 [1·0]	67±25 [40]	61±0·91 [40]
Post-injection periods			
0– 30 min	4·7 ±0·75 [3·0]	287·5±28 (180]	61±7·5 [60]
30– 60 min	6·0 ±0·56 [12]	195±44 [684]	32±4·0 [57]
60– 90 min	6·9 ±0·56 [10]	124±31 [540]	18±3·7 [54]
90–120 min	6·8 ±0·95 [8·0]	92·5±21 [480]	13±2·3 [60]
120–150 min	6·4 ±1·15 [7·0]	141±46 [406]	24±7·0 [58]
150–180 min	5·3 ±1·05 [3·0]	156±40 [120]	33±7·5 [40]
180–210 min	4·7 ±0·29	176±12	44±10·7

Values obtained with caerulein are in square parentheses.
The values given are the means and, for bombesin, ±S.E.M.

Effects of intravenous infusion

The threshold dose of bombesin capable of producing, by intravenous infusion, an appreciable increase in gastric acid secretion was 0·05–0·2 (μg/kg)/hour. This threshold rate was at least three times less than that of caerulein (Figure 3). The effect was dose-dependent up to 1 (μg/kg)/h; thereafter intensity of response declined. At infusion rates of 0·5–1 (μg/kg)/h bombesin was as active as caerulein and at the infusion rate of 2 (μg/kg)/h it was less active.

Effects of rapid intravenous injection

In contrast to gastrin and caerulein, bombesin stimulated the acid secretion by the fundic pouch of the dog stomach even when given by rapid intravenous injection (Figure 4). However, given this way the polypeptide was less active than by either subcutaneous injection or intravenous infusion. The total acid output produced, over a 2·5 h period, by 1 μg/kg bombesin did not exceed 1·4 mEq HCl, a very low value if compared to the 14 mEq HCl secreted by the gastric pouch, over a 3·5 h period, following intravenous infusion of 1 (μg/kg)/h of the polypeptide (cf. Figure

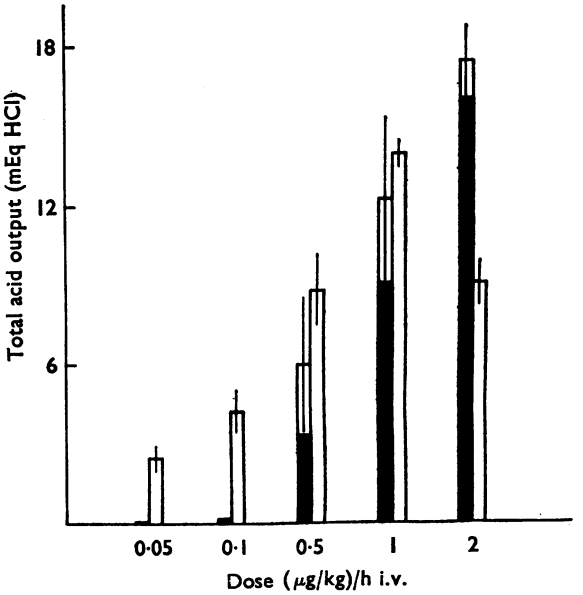

FIG. 3. Denervated gastric pouches of five dogs. Total acid output (in mEq HCl) produced, over a period of 3·5 h, by continuous i.v. infusion of graded doses of bombesin (white columns) and caerulein (black columns). Vertical bars represent standard errors.

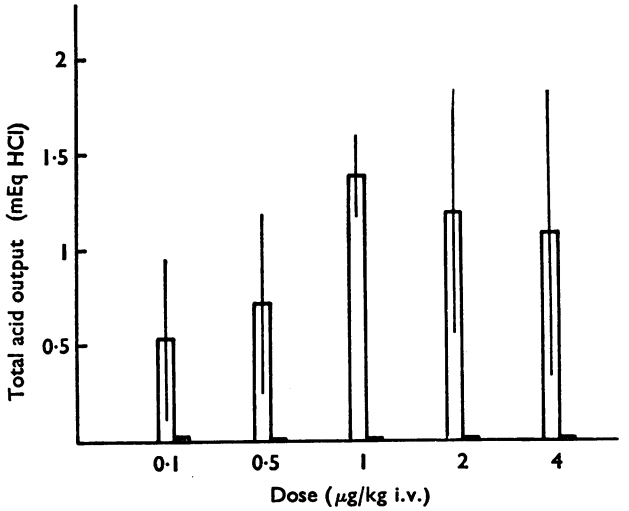

FIG. 4. Denervated gastric pouches of five dogs. Total acid output (in mEq HCl) produced, over a 2·5 h period, by rapid i.v. injection of graded doses of bombesin (white columns) and caerulein (black columns). Vertical bars represent standard errors.

3). The effect of a single intravenous injection of bombesin lasted more than 150 min and again there was an increase in the concentration of hydrochloric acid. No vomiting was observed following intravenous doses of bombesin up to 4 μg/kg.

Effects of atropine and hexamethonium

Atropine, given subcutaneously at the dose of 0·2 mg/kg prior to bombesin administration (1–2 μg/kg, subcutaneously), inhibited almost completely the acid secretion produced by the polypeptide; given at the peak of the acid secretory response elicited by subcutaneous bombesin (2 μg/kg) atropine caused an abrupt drop of acid output nearly to zero (Figure 5). The intramuscular injection of 5 mg/kg hexamethonium reduced by approximately 50% the acid secretion produced by 2 μg/kg of bombesin given subcutaneously.

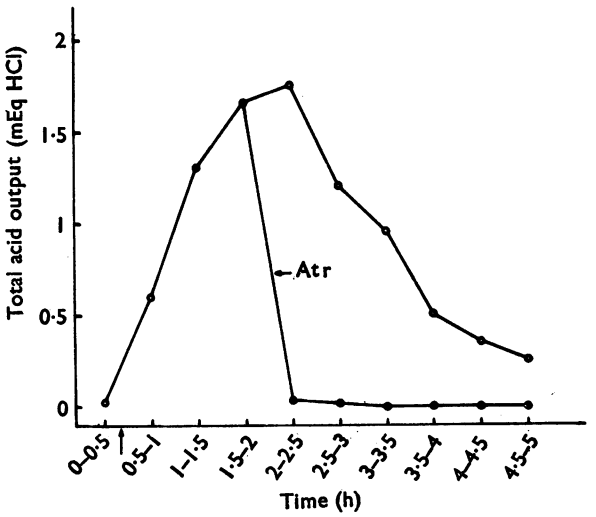

FIG. 5. Denervated gastric pouches of four dogs. Total acid output, in mEq HCl, after subcutaneous injection of 2 μg/kg of bombesin, at arrow, Gastric juice was collected every 30 minutes. In two dogs out of the four subjected to experiment a subcutaneous injection of atropine sulphate (Atr, 0·2 mg/kg) was given 90 min after the administration of bombesin.

Rat

The intravenous infusion of bombesin at rates of 0·5, 2 and 10 (μg/kg)/h had no effect on acid secretion in the perfused stomach preparation. Similarly ineffective was the subcutaneous administration of the polypeptide at doses of 0·5, 2 and 10 μg/kg. In comparison, the threshold dose of caerulein was 0·25 (μg/kg)/h by intravenous infusion and 0·25–0·5 μg/kg by subcutaneous injection.

TABLE 2. *Acid output of the perfused stomach preparation of the rat after intravenous injection of different doses of bombesin and caerulein*

Dose (ng/kg)	Mean increase (%) after bombesin	Mean increase (%) after caerulein
25	0	24·7± 7·2
50	0	50·0± 6·8
100	11·2± 4·0	97·4± 7·9
250	48·2±17·7	119·5±21
500	42·8±19·9	168·0±26
1000	6·0± 3·5	170·0±31

Mean increase is the mean of percent increases over and above the basal level (approximately 3 (μEq HCl/kg)/min) during four consecutive post-injection periods of 8 min each. The values given are the means ±S.E.M.

In apparent contrast with the above results are the puzzling effects produced by rapid intravenous injections of bombesin. Results summarized in Table 2 show that the polypeptide caused some increase in the acid secretion of the perfused stomach preparation, up to 42–48%, following intravenous doses of 100–500 ng/kg. However, 1 μg/kg produced virtually no effect. Bombesin-induced increase in acid output was of short duration (8 min) and was sometimes followed by a secretion actually lower than the basal one. In view of the ineffectiveness of subcutaneous injection and intravenous infusion, the irregular results obtained following quick intravenous injection appear ambiguous. Since bombesin possesses a spasmogenic effect on the smooth muscle of the rat stomach it may be that the recorded increase in acid output is simply due to the contraction of the stomach causing a transitory increase in the volume of the effluent. Similar behaviour has been seen with other spasmogenic drugs like physalaemin (Bertaccini, De Caro & Impicciatore, 1967).

Discussion

Bombesin, which is known to possess a stimulant action on vascular and extravascular smooth muscle and to display in the dog kidney striking effects on the afferent glomerular vessels and on the renin-angiotensin system, also produced a potent stimulant effect on volume and acid outputs of the denervated fundic pouch of the dog. Both on a weight and on a molar basis bombesin appeared to be more effective than caerulein which, in its turn, was found to be more potent than human gastrin I (Bertaccini *et al.*, 1968).

The effect of bombesin on the acid secretion of the dog stomach was inhibited by atropine, like that of gastrin and caerulein. This fact must be borne in mind when the mechanism of action of bombesin is considered. At present three hypotheses may be advanced: (a) bombesin acts directly on the oxyntic cells, occupying their cholinoceptive sites; (b) bombesin acts in the gastric wall on the same atropine-sensitive receptor sites as gastrin and caerulein, (c) bombesin acts indirectly through release of gastrin.

The amino acid sequences shown below indicate that bombesin has apparently little in common with either caerulein or gastrin, while the last two peptides have many features in common:

Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 Bombesin
Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 Caerulein
Pyr-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2 Human gastrin I

This makes it difficult to conceive that bombesin and gastrin may act on the same receptor sites in the gastric wall.

Preliminary experiments have shown that both in dogs (Bertaccini, Erspamer, Impicciatore, Melchiorri & Sopranzi, unpublished) and man (Erspamer, Melchiorri, Sopranzi & Torsoli, unpublished) gastric acid secretion elicited by bombesin was always accompanied by a conspicuous increase of immunoreactive gastrin activity in plasma. This would point to the possibility that bombesin is a gastrin-releasing factor. Our present experiments are directed to checking the validity of this hypothesis.

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