Br. J. Pharmac. (1974), 52, 227-232

EVIDENCE OF CHOLECYSTOKININ RELEASE BY BOMBESIN IN THE DOG

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1 The intravenous infusion of bombesin elicited in the dog a contraction of the gall bladder with decreased opening pressure of the choledocho-duodenal junction and stimulation of pancreatic secretion.

2 The pancreatic juice produced under the influence of bombesin was poor in bicarbonate and rich in protein. Threshold doses of the peptide were of the order of 0.25 $\mu g\ kg^{-1}\ h^{-1}$ and maximum protein output was obtained with 1 $\mu g\ kg^{-1}\ h^{-1}$. The pancreatic protein response to bombesin was very similar, in its onset and duration, to that elicited by intraduodenal infusion of L-tryptophan. Infusions of bombesin repeated at short intervals produced tachyphylaxis.

3 Antrectomy did not affect the stimulant action of bombesin on the pancreas. Atropine however, reduced the pancreatic protein response to bombesin.

4 It is suggested that bombesin acts on the gall bladder and the exocrine pancreas through release of cholecystokinin from the duodenal mucosa. No release of secretin could be demonstrated. It is likely that the releasing activity of bombesin is limited, in the field of gastrointestinal peptides, to those belonging to the gastrin-cholecystokinin family.

Introduction

It has been shown that the potent effect on gastric acid secretion displayed in the dog by bombesin, a tetradecapeptide isolated from the skin of some European discoglossid frogs, is due to its capacity to release gastrin from the antral mucosa (Bertaccini, Erspamer, Melchiorri & Sopranzi, 1974).

In this study the effects produced by bombesin on the motility of the gall bladder and on the pancreatic secretion of the dog have been investigated. It will be seen that these effects may be ascribed to a release of cholecystokinin from the duodenal mucosa.

Methods

Anaesthetized dogs

Ten adult mongrel dogs of either sex, weighing between 10 and 20 kg, were fasted for approximately 20 hours. Animals were anaesthetized with 30 mg/kg of sodium pentobarbitone, injected intravenously. A tube was placed in the trachea and connected to a Palmer respirator throughout the experiment. The abdomen was opened through a midline incision. A simple gastric fistula was constructed and a rubber ligature was placed around the pylorus to prevent the entry of acid into the duodenum.

A total pancreatic fistula was prepared as described by Nakajima & Magee (1970). Pancreatic juice drops were counted by a photoelectric drop counter. The juice was collected continuously and divided into 15 min samples.

In 4 dogs, after double ligature of the cystic duct, an open tip catheter was introduced into the fundus of the gall bladder, fixed with a tight ligature and connected to a saline-filled pressure transducer to record gall bladder motility (pressure activity). In the same dogs a polyethylene tube (PE 190) was inserted 3-5 cm into the common bile duct to collect hepatic bile. A continuous intravenous infusion of 0.15 M NaCl was delivered into a femoral vein throughout each experiment at a rate of 60 ml/h by a motor-driven syringe. Drugs were added to the infusion fluid to give the desired dosage.

In 2 dogs the superior mesenteric artery was catheterized under fluoroscopic control by introducing a precurved catheter into a femoral artery. The catheter was kept patent by a continuous infusion of saline throughout the experiment. Drugs were dissolved in the infusion solution, which was delivered at a rate of 50 ml/hour.

Measurements of opening pressures of the sphincter of Oddi (choledocho-duodenal junction) were performed in 3 dogs by the technique of Wyatt (1967).

Conscious dogs

Chronic pancreatic and gastric fistulae were constructed in 7 mongrel dogs weighing between 15 and 20 kg. The pancreatic fistula was constructed as described by Herrera, Kemp, Tsukamoto, Woodward & Dragstedt (1968). Studies started three weeks after surgery. Food was withheld for 18 h before each test. The interval between tests was at least 48 hours.

A continuous intravenous infusion of a 0.15 M NaCl solution was given throughout each experiment at a rate of 60 ml/hour. Drugs were added to the saline infusion and administered for 60 minutes. While doses of secretin and cholecystokinin were infused sequentially in ascending order during single experiments, only one dose of bombesin was infused in each experiment, to avoid tachyphylaxis. The interval between two infusions of bombesin was at least 48 hours. During the experiments the gastric fistula was kept open. Pancreatic juice was collected continuously and separated into 15 min samples.

Bicarbonate concentration was measured by addition of 1 ml 0.1 N HCl to 0.5-2 ml samples of pancreatic juice; the mixture was heated until it was just boiling and, when cooled, the residual acid was back titrated to pH 7.0 with 0.1 N NaOH. Total protein concentration was used as an index of pancreatic enzyme secretion. It was estimated by measuring absorbance at 280 nm, using bovine serum albumin as standard.

L-Tryptophan was used to release endogenous cholecystokinin. It was infused into the duodenum through the intestinal limb of the pancreatic cannula at a rate of 2-20 mmol/h dissolved in 200 ml of saline at pH 7.0. Meyer & Grossman (unpublished observations) found that L-tryptophan was about equal to L-phenylalanine as a cholecystokinin releaser in the dog.

Two dogs provided with chronic pancreatic and gastric fistulae were subjected to antrectomy, after having first been treated with intravenous infusions of polypeptides and intraduodenal infusions of L-tryptophan.

Isolated gall bladder preparations

Longitudinal strips of the dog gall bladder were suspended in a 10 ml bath of Krebs solution, at 37° C, gassed with air. The motility of the preparation was recorded on a smoked drum by means of an isometric microdynamometer (7001, U. Basile, Milan) using a DY 2 strain-gauge transducer (force up to 10 g).

Drugs

Bombesin and caerulein were synthesized at the Farmitalia Research Laboratories, Milan; natural porcine cholecystokinin and secretin were a gift from Dr V. Mutt, Karolinska Institutet, Stockholm, Sweden. Other substances used were: sodium pentobarbitone (Abbott), L-tryptophan (Merck, Darmstadt), atropine sulphate (British Drug Houses), and bovine serum albumin (Calbiochem, Los Angeles).

Results

Gall bladder

The intravenous infusion of bombesin (0.5-1 $\mu g\ kg^{-1}\ h^{-1}$) always produced, after a latency of 5-10 min, a progressively increasing contraction of the dog gall bladder *in situ*. Maximum contraction was reached towards the end of the 30 min infusion period. Discontinuing the infusion was followed by slow relaxation of the organ, which took a considerable time, in some experiments up to 1-2 h, to be complete (Figure 1).

Infusion of bombesin into the superior mesenteric artery produced similar results, at much lower infusion rates.

Bombesin had little activity on the dog isolated gall bladder. In one experiment the peptide possessed less than 0.25% of the activity of caerulein.

Following infusion of bombesin (1 $\mu g\ kg^{-1}\ h^{-1}$) for 30 min the opening pressure of the choledocho-duodenal junction was reduced, in 3 dogs, from 18 ± 7 to 12 ± 4 cm water ($P < 0.05$).

Pancreatic secretion

Bombesin infusion produced a stimulation of pancreatic secretion at the same time as the gall bladder contracted (Figure 1). Stimulation of secretion was not necessarily proportional to the intensity of gall bladder contraction. The latency between start of bombesin infusion and start of pancreatic response was of the same order as that observed for gall bladder response. The duration of pancreatic stimulation after the bombesin infusion had ceased appeared to be somewhat shorter.

The pancreatic juice secreted under the influence of bombesin was poor in bicarbonate, but rich in protein. Figure 2 shows bicarbonate and protein outputs in 5 dogs produced by graded doses of bombesin, secretin and cholecystokinin, given by intravenous infusion. Bombesin, like cholecystokinin, caused little increase in bicarbonate output whereas it caused a sharp rise in protein output, the threshold dose being 0.2 $\mu g\ kg^{-1}\ h^{-1}$. Maximum response was elicited by 1 $\mu g\ kg^{-1}\ h^{-1}$. When the rate of bombesin infusion was increased above this level, up to 4 $\mu g\ kg^{-1}\ h^{-1}$, no further increase in pancreatic response

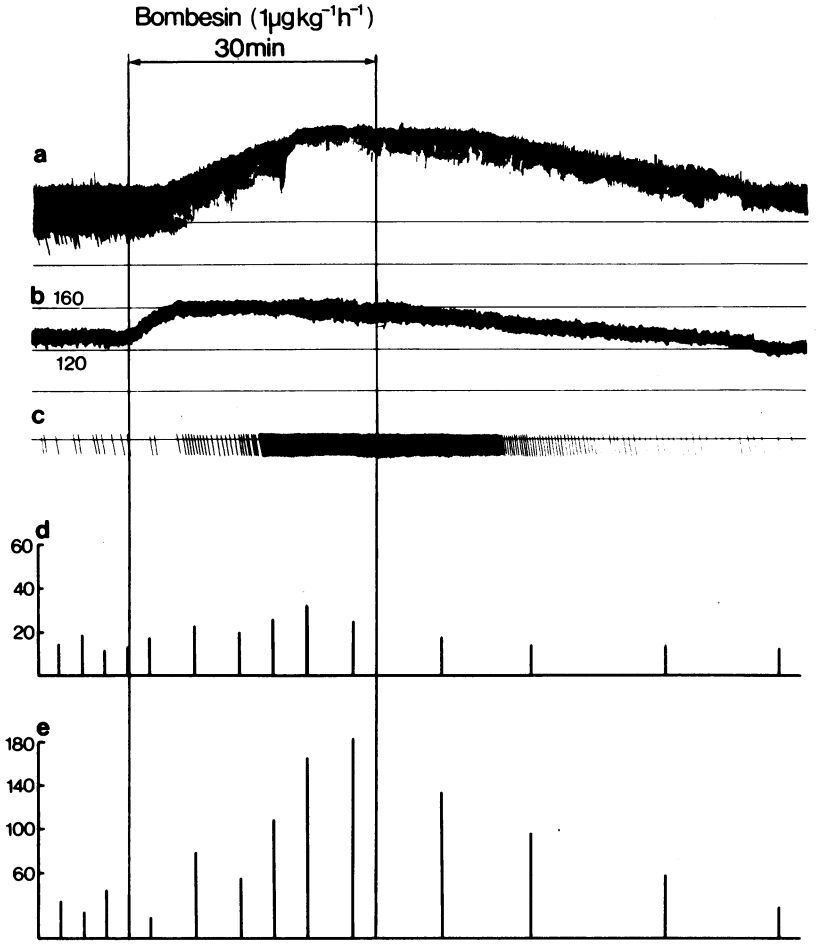

Fig. 1 Dog anaesthetized with sodium pentobarbitone. Responses elicited by the intravenous infusion, over a 30 min period, of bombesin (1 μg kg^{-1} h^{-1}). Figure shows from top to bottom: (a) gall bladder motility, (b) systemic blood pressure; (c) flow of pancreatic juice, in drops; (d) pancreatic bicarbonate output (μEq/15 min) and (e) pancreatic protein output (mg/15 minutes).

occurred. Maximum protein output obtained with bombesin was similar to that produced by 0.7-0.8 μg kg^{-1} h^{-1} cholecystokinin, but less than half that evoked by optimum infusion rates of cholecystokinin (2-4 μg kg^{-1} h^{-1}).

If the ratio between bicarbonate and protein output is plotted against the flow rate of pancreatic juice (Fig. 3), it is evident that after infusion of secretin this ratio increases sharply with the increase in flow of juice, while this did not occur either after infusion of cholecystokinin or bombesin or after intraduodenal administration of L-tryptophan.

A more detailed comparison of the effects of several pancreatic stimulants is presented in Figure 4. Apart from confirming the small effect of secretin, the figure shows that exogenous cholecystokinin produced an immediate rise in protein output which lasted unchanged as long as the infusion was continued, whilst bombesin infusion and intraduodenal administration of L-tryptophan caused an increase in protein output which was delayed and could not be maintained at a constant level throughout the infusion period. Exogenous cholecystokinin was the most effective stimulant, and L-tryptophan the least effective.

Antrectomy did not affect the pancreatic protein response to bombesin. In fact, protein output in the same 2 dogs, before and after antrectomy (3 experiments in each of the 2 dogs)

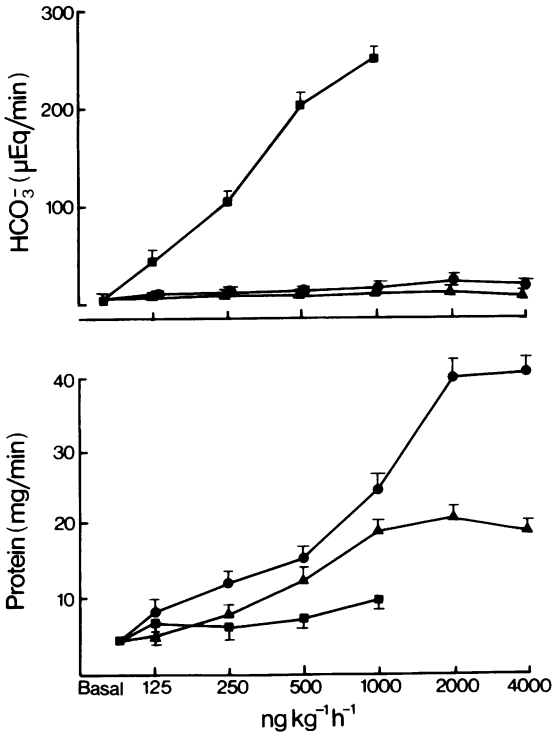

Fig. 2 Conscious dogs with chronic pancreatic fistulae. Pancreatic bicarbonate and protein outputs after intravenous infusions, over a 60 min period, of graded doses of secretin (■), bombesin (▲) and cholecystokinin (●). The points show the mean of 2 measurements in each of 5 dogs. The vertical bars show the s.e. mean.

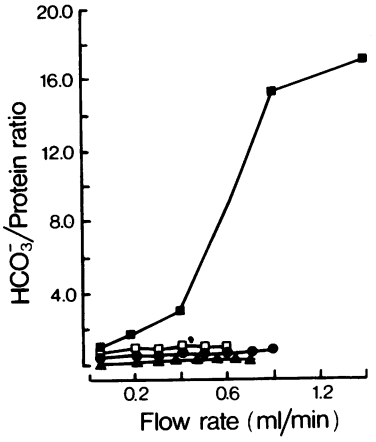

Fig. 3 Conscious dogs provided with chronic pancreatic fistulae. Ratios between pancreatic bicarbonate and pancreatic protein outputs are plotted against flow rates of pancreatic juice produced by different doses of stimulants given either by intravenous infusion (secretin (■), bombesin (▲) and cholcystokinin (●)) or by intraduodenal infusion (L-tryptophan (□)). The points represent the mean of 2 measurements in each of 4 dogs.

was as follows: before antrectomy–basal protein output, 4.2 ± 0.7 mg/min; protein output following infusion of 1 μg kg^{-1} h^{-1} of bombesin, 17.3 ± 1.6 mg/min; after antrectomy–basal protein output was 3.6 ± 0.6 mg/min; protein output following infusion of 1 μg kg^{-1} h^{-1} of bombesin was 18.8 ± 1.7 mg/minute.

However, premedication with atropine (0.1 mg/kg, s.c.; 3 experiments in each of 2 dogs) reduced basal protein output from 4.8 ± 0.8 to 1.2 ± 0.3 mg/min and similarly decreased protein output following infusion of 1 μg kg^{-1} h^{-1} of bombesin from 16.5 ± 1.2 to 6.3 ± 0.8 mg/minute.

The effects of bombesin administration repeated at short intervals were studied in 4 experiments in which 2 infusions of bombesin (1 μg kg^{-1} h^{-1}), each lasting 30 min, were given 60 min apart. A clear-cut reduction in the pancreatic protein response to the second infusion was evident, as shown by the following values: basal protein output, 4.8 ± 0.9 mg/min; peak during the first infusion, 18 ± 0.9 mg/min; peak during the second infusion, 6.2 ± 0.7 mg/minute.

Bile flow

Bombesin infusion produced, after a latency period, an evident increase in flow of hepatic bile, collected from the common bile duct after ligature of the cystic duct. The effects of bombesin on flow and composition of the bile will be described in detail elsewhere.

Systemic blood pressure

The intravenous infusion of bombesin at rates above 0.5 μg kg^{-1} h^{-1} nearly always produced a prompt rise of blood pressure, never exceeding 20-30 mmHg. No hypertensive effect was observed after infusion of bombesin into the superior mesenteric artery, at rates capable of stimulation of the gall bladder and the pancreas.

Discussion

The infusion of bombesin in the dog produced not only a release of gastrin from the antral mucosa (thus stimulating gastric acid secretion) but also

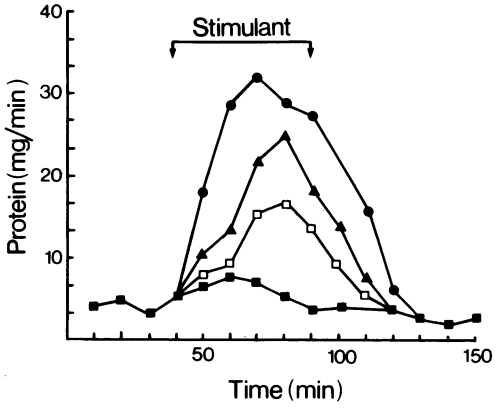

Fig. 4 Conscious dogs provided with chronic pancreatic fistulae. Pancreatic protein output after intravenous infusion, over a 50 min period, of secretin (■, 0.6 μg kg^{-1} h^{-1}), bombesin (▲, 1 μg kg^{-1} h^{-1}), and cholecystokinin (●, 2 μg kg^{-1} h^{-1}), and after intraduodenal infusion of L-tryptophan (□, 11 mmol/hour). Each point represents the mean of two experiments.

contraction of the gall bladder accompanied by a reduction of the opening pressure of the sphincter of Oddi and stimulation of pancreatic secretion, with production of a juice rich in protein and poor in bicarbonate.

These effects were produced after a latent period of variable duration. They reached a maximum during the bombesin infusion and disappeared when the administration of the polypeptide stopped. When introduced by close infusion into the superior mesenteric artery bombesin was active at dose levels which were without effect when given by systemic infusion. Bombesin was virtually ineffective on isolated strips of the dog gall bladder.

The actions of bombesin were indistinguishable, apart from the latency period, from those elicited by either exogenous cholecystokinin or caerulein. The pancreatic response was very similar to that caused by endogenous cholecystokinin released following intraduodenal infusion of L-tryptophan.

There is only one acceptable explanation for the effects of bombesin on the gall bladder and the pancreas, namely, that bombesin acts through release of cholecystokinin from the duodenal mucosa. In keeping with this hypothesis are the following facts:

(a) the virtual ineffectiveness of bombesin on the isolated gall bladder. This demonstrates that it is not bombesin itself which contracts the gall bladder. Caerulein and cholecystokinin acting directly on the bladder smooth muscle were equally effective on isolated and on *in situ* preparations;

(b) the latency period before the action of bombesin starts which is similar to that observed (so far as pancreatic secretion is concerned) after intraduodenal infusion of L-tryptophan. There was virtually no latency in the onset of the effects produced by caerulein or exogenous cholecystokinin;

(c) in the case of prolonged infusion of bombesin the decrease of the response during the infusion period itself, and reduced effectiveness of a second polypeptide infusion given shortly after the first one;

(d) the relationship between dose and pancreatic protein response, appreciable only up to a certain infusion rate of bombesin, with a maximum response which was decidedly smaller than that obtainable with exogenous cholecystokinin.

Points (c) and (d) seem to demonstrate that the amount of endogenous cholecystokinin which may be released by bombesin from the duodenal mucosa is limited, and that a prolonged bombesin infusion may exhaust the deposits of bombesin-releasable cholecystokinin, which then will take some time to be restored. At no dose level did bombesin appear capable of producing a maximal stimulation of the pancreas, i.e. of liberating an amount of endogenous cholecystokinin comparable to that of exogenous peptide required to elicit maximal secretory effects.

Atropine reduced the pancreatic protein response to bombesin in our experiments. On the other hand, it has been observed that vagotomy or administration of atropine does not affect the pancreatic protein response to exogenous cholecystokinin (Henriksen, 1969), but reduces pancreatic enzyme secretion in response to peptone in the intestine (Crider & Thomas, 1944; Thomas, 1964). These findings have been interpreted as indicating that the action of cholecystokinin on the pancreas is not cholinergically dependent, but that the release of cholecystokinin from the intestinal mucosa is (Grossman, 1973).

Without excluding alternative explanations for the results of our experiments with atropine, it appears possible that cholecystokinin release produced by bombesin is also modulated by cholinergic impulses.

There is no evidence of release of secretin after bombesin infusion, as shown by the scanty increase in bicarbonate output. Similarly a release of antral gastrin intervening in the production of the effects of bombesin may be excluded because

this peptide was equally active before and after antrectomy.

As far as extra-antral gastrin is concerned, which could also be released by bombesin in antrectomized dogs, it is difficult to conceive that it contributes substantially to the effects of bombesin on the pancreas and in releasing cholecystokinin. In fact, on the *in situ* gall bladder of conscious dogs both gastrin I and gastrin II showed barely 5% of the activity of cholecystokinin (Vagne & Grossman, 1968). Assuming that all of the small amount of extra-antral gastrin available in a dog (Nilson, Yalow & Berson, 1973; Jorpes & Mutt, 1973) is released during a 30 min bombesin infusion, it could not produce any appreciable gall bladder stimulation.

On the other hand, the effects of bombesin described here cannot be attributed to release of duodenal hormones produced either by the gastric juice or the bile arriving into the duodenum. In fact, entry into the duodenum of acid juice stimulated by bombesin was prevented in anaesthetized dogs by ligation of the pylorus, and minimized in conscious dogs by drainage of the gastric juice to the exterior through a gastric fistula. In anaesthetized dogs bile was similarly drained to the exterior through a cannula inserted into the common bile duct.

In spite of lack of direct evidence, by radioimmunoassay, of increases of cholecystokinin levels in plasma of dogs infused with bombesin, we believe that data so far collected are sufficient to permit the conclusion that bombesin produces its effects on the gall bladder and the pancreas through release of cholecystokinin. If so, bombesin would be a releaser of both the gastrointestinal hormones belonging to the gastrin-cholecystokinin family, having in common a peculiar sequence of amino acid residues in the C-terminal hexa- or heptapeptide of their molecule:

-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
cholecystokinin heptapeptide

-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH_2
gastrin II hexapeptide

The amino acid sequence of bombesin, in which the C-terminal nonapeptide seems crucial for full activity does not offer a key to interpret the peculiar properties of the polypeptides:

-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
bombesin nonapeptide

It is hoped that the availability of bombesin and related peptides will help in solving many problems connected with gastrin and cholecystokinin release and turnover in the different animal species, with possible diagnostic and therapeutic implications in man.

This work was supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

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(Received January 3, 1974)