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EFFECT OF BOMBESIN AND RELATED PEPTIDES ON THE RELEASE AND ACTION OF INTESTINAL HORMONES ON PANCREATIC SECRETION

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SUMMARY

1. Pancreatic volume flow as well as bicarbonate and protein secretion from pancreatic fistulas have been measured in response to I.V. infusion of graded doses of bombesin and related peptides containing the COOH-terminal fragment of the bombesin molecule in conscious dogs with intact antrum and in anaesthetized animals with antrectomy, or antrectomy and enterectomy.

2. Bombesin and related peptides given to conscious dogs produced a potent and dose-dependent increase in pancreatic protein output reaching a maximum equal to that induced by the octapeptide of cholecystokinin (OP-CCK) as well as a small rise in bicarbonate output attaining a peak amounting to about 10% of that evoked by secretin. The serum gastrin level rose progressively during the infusion of bombesin to reach a peak with the highest dose of the peptide.

3. Bombesin infused I.V. in anaesthetized animals with resected antrum also evoked a marked increase in pancreatic protein secretion without significant change in the serum gastrin level. Following the removal of the antrum and small intestine, bombesin failed to show any stimulation of the pancreatic secretion or any change in the serum gastrin level. It is concluded that the strong stimulatory action of bombesin and related peptides on pancreatic secretion cannot be entirely ascribed to the release of gastrin but might be attributed at least in part to the release of intestinal hormones, particularly CCK.

4. Atropine and the growth hormone-release inhibiting hormone (GH–RIH), which were shown to inhibit the release of CCK induced by duodenal perfusion of an amino acid mixture, also caused the inhibition of pancreatic protein secretion by bombesin but failed to affect the pancreatic response to OP–CCK. The results indicate that bombesin releases,

in addition to gastrin, CCK from the gut by a mechanism largely dependent upon cholinergic innervation.

INTRODUCTION

Bombesin, a tetradecapeptide recently isolated from the skin of certain frogs of the genus *Bombina* (Anastasi, Erspamer & Bucci, 1971) was shown to release the antral hormone by a mechanism sensitive to pH and atropine (Basso, Improta, Melchiorri & Sopranzi, 1974). No study has as yet been undertaken to examine the influence of bombesin on the release and action of intestinal hormones affecting pancreatic secretion.

This investigation was carried out to assess the effects of bombesin and related peptides on pancreatic secretion in conscious dogs with intact antrum and in anaesthetized animals with the antrum and small intestine resected.

METHODS

Three groups (*A*, *B*, *C*) of adult mongrel dogs, weighing 12–18 kg each, were used for studies of pancreatic secretion under normal conditions (*A*) and after antrectomy (*B*) or after antrectomy plus enterectomy (*C*).

The dogs of group *A* (four dogs) were prepared with a gastric fistula drained by a Thomas cannula (Thomas, 1941) and a pancreatic fistula constructed according to the modified method of Herrera, Kemp, Tsukamoto, Woodward & Dragstedt (1968). Secretory studies were started about 2 months after surgery, the dogs were deprived of food but not water for at least 18 hr before each test. The stomach was rinsed by irrigation through the gastric fistula and the collection of basal secretion from the pancreatic fistula was started. Test experiments were not begun until two consecutive 15 min basal collections from the pancreatic fistula were 1·5 ml./15 min or less. If basal secretion remained higher the study was cancelled for the day. Throughout the test, the gastric fistula was left open to allow drainage of gastric juice to the outside and to prevent the gastric acid from entering the duodenum and releasing endogenous hormones. Pancreatic secretion was collected continuously and separated into 15 min samples for volume, bicarbonate and protein determinations according to the methods described previously (Konturek, Demitrescu & Radecki, 1974).

Several series of tests were performed on the animals of group *A*. The peptides used in the test were natural bombesin, the synthetic COOH-terminal nonapeptide of bombesin (NP-bombesin) and natural litorin, a new peptide similar to bombesin, isolated from the skin of *Litoria aurea*. The pancreatic dose–response curves to bombesin and related peptides were determined by I.V. infusion of each peptide separately in graded doses ranging from 0·06 to 1·0 μg/kg.hr. The dose level was changed every 60 min and differed by a factor of 2. For comparison, the maximal response to I.V. secretin (8 u./kg.hr) or I.V. OP-CCK (0·5 μg/kg.hr) was obtained in these animals.

From the dose–response test, a dose of bombesin (0·5 μg/kg–hr) producing maximal pancreatic protein output was selected and used in a constant I.V. infusion for 210 min period. After allowing 90 min for pancreatic secretion to reach a steady state, atropine (100 μg/kg.hr) or growth hormone-release inhibiting .hormone

(GH-RIH, 2·5 μg/kg.hr) was added to I.V. infusion for a period of 60 min. Finally, bombesin alone, was continued for an additional 60 min. Similar experiments with atropine or GH-RIH were performed using known stimulants for the release of endogenous CCK (L-tryptophane plus L-phenylalanine mixture) or for the direct stimulation of the exocrine pancreas (OP-CCK). Amino acid mixture was instilled intraduodenally at a constant rate through the hollow obturator of the pancreatic cannula and OP-CCK was infused I.V. at a constant dose. With both stimulants pancreatic protein secretion was maintained at a level equal to that attained with bombesin (0·5 μg/kg.hr). Atropine or GH-RIH was given in similar way as in tests with bombesin. In control tests, bombesin, duodenal amino acids or OP-CCK alone was administered for the time of experiment.

The study on the influence of the antrum and small intestine on the action of bombesin on the pancreatic secretion was performed in fasted animals anaesthetized with Nembutal. The abdomen was opened with mid-line incision. In animals of group *B* (thirteen dogs) the antral portion of the stomach was divided from the main stomach, then divided from the duodenum immediately below the pyloric ring and finally removed. The completeness of antrectomy was verified by histological examination of the excised tissue. In animals of group *C* (thirteen dogs) antrectomy and total enterectomy was performed. In both groups of animals (*B* and *C*) the minor pancreatic duct was ligated and the major pancreatic duct was tied and cannulated at the opening in the duodenum with a polyethylene tube, 1·0 mm in diameter, which was brought outside through a stab wound in the abdominal wall. A rubber tube, 20 mm in diameter, was inserted into the distal portion of the stomach by means of a gastrostomy to drain the gastric juice to the outside. In both groups of animals the choledochus was tied and cannulated at the opening in the duodenum with a polyethylene tube, 2·0 mm in diameter. Bile was diverted to the outside by this tube.

Pancreatic secretion was collected continuously and divided in 15 min aliquots for volume, bicarbonate and protein determination as in chronic experiments. Bombesin was infused I.V. in graded doses ranging from 0·06 to 1·0 μg/kg.hr. Since the pancreatic flow rate with bombesin alone was negligible, a constant dose of natural secretin (GIH Lab., Karolinska Institutet, Stockholm, Sweden) was infused I.V. in a dose of 0·4 u./kg.hr throughout the period of bombesin administration. In separate control animals of group *A* (five dogs) and *B* (three dogs), OP-CCK was given I.V. in a dose of 0·5 μg/kg.hr for a 2 hr period together with a constant of secretin (0·4 u./kg.hr) before and after antrectomy and antrectomy combined with total enterectomy, respectively. The secretory responses to OP-CCK in these tests were used as an index of the sensitivity of the pancreas to direct hormonal stimulation.

In tests with bombesin, gastrin concentration in serum was measured by radioimmunoassay (Yalow & Berson, 1970). The routine detection limit of the assay, as employed in the present study, was 5 pg equivalent synthetic human gastrin/ml. serum. A serum gastrin concentration of 10 pg/ml. or more could be measured.

The difference in pancreatic volume flow, bicarbonate and protein output in response to bombesin, OP-CCK or duodenal amino acids with or without GH-RIH or atropine determined by *t* test for paired values (Siegel, 1956). As used in the test, significance $P < 0.05$.

RESULTS

The effects of bombesin, NP-bombesin or litorin on pancreatic bicarbonate and protein outputs are presented in Fig. 1. Graded doses of these peptides evoked graded increases in pancreatic secretion, attaining the

highest level at the dose of 0·5 μg/kg.hr. Further increase in the dosage of peptides did not result in any additional increase in the volume, bicarbonate and protein output. The mean highest bicarbonate output was about 10% of the maximal response to secretin (μ./kg.hr) whereas the mean highest protein output was not significantly different from the maximal response to OP-CCK (0·5 μg/kg.hr).

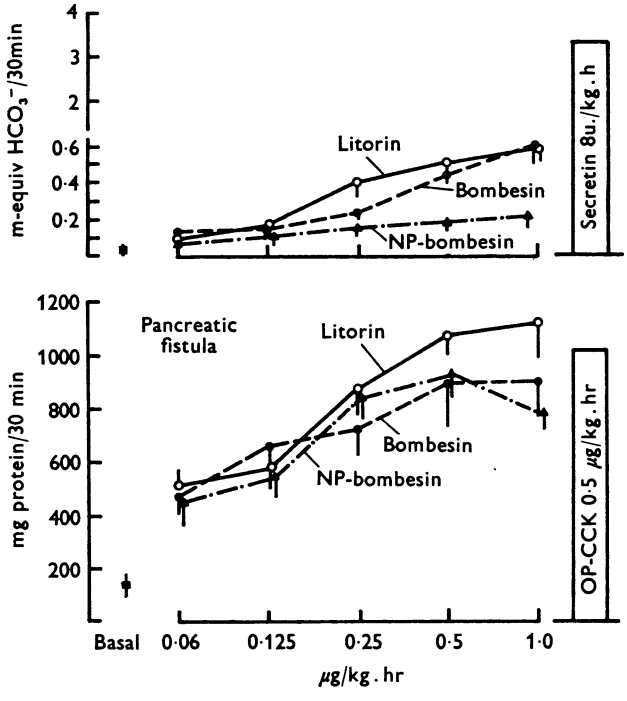

Fig. 1. Effect of bombesin, NP-bombesin and litorin given I.V. in graded doses on pancreatic bicarbonate and protein output in conscious dogs. The columns represent mean peak 30 min outputs in tests with secretin (8 u./kg.hr) or OP-CCK (0·5 μg/kg.hr). In this and subsequent Figures each line in a mean of three tests on each of three dogs. Vertical bars indicate the S.E. of the mean.

Basal concentration of immunoassayable serum gastrin was 65 ± pg/ml. Bombesin given I.V. in graded doses of 0·06, 0·12, 0·25, 0·50, and 1·0 μg/kg.-hr produced a progressive rise in serum gastrin level amounting to 82 ± 8, 112 ± 13, 152 ± 16, 180 ± 28 and 210 ± 38 pg/ml., respectively.

Bombesin infused I.V. in a constant dose of 0·5 μg/kg.hr produced a negligible bicarbonate secretion but a potent protein secretion which reached a peak in the first hour of bombesin infusion and then showed a tendency to decline, falling at the end of experiment to about 63% of the

initial peak level (Fig. 2). GH-RIH given in a standard dose of 2·5 μg/-kg.hr caused a profound inhibition of protein output amounting to about 30 % of the control level. Pancreatic bicarbonate output was also significantly reduced by GH-RIH.

Serum gastrin level during bombesin infusion was about three times as high (184 ± 25 pg/ml.) as that under basal conditions. GH-RIH resulted in a significant decrease in serum gastrin level to the basal level. Upon the withdrawal of GH-RIH infusion, serum gastrin showed a tendency to return toward the control value.

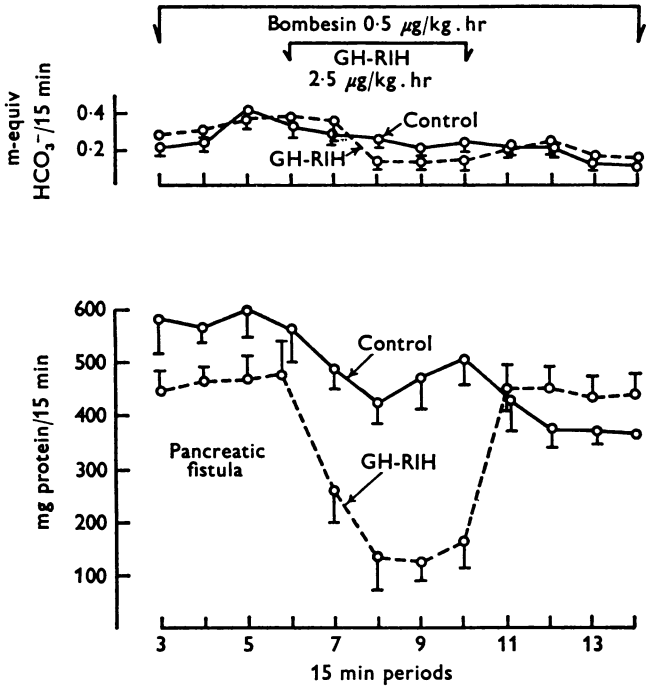

Fig. 2. Effect of GH-RIH on pancreatic bicarbonate and protein responses to bombesin.

Pancreatic protein response to L-tryptophane and L-phenylalanine mixture instilled into the duodenum at a rate of 8·0 m-mole/hr was almost as high as that attained with bombesin. GH-RIH in a standard dose (2·5 μg/kg.hr) resulted in a strong inhibition of this response (Fig. 3). Pancreatic response to OP-CCK was characterized by a high protein response relatively well sustained throughout the experiment. GH-RIH given during a stable rate of pancreatic response to OP-CCK had no effect on protein secretion.

Pancreatic bicarbonate secretion in response to duodenal amino acids or I.V. OP-CCK was negligible and not affected significantly by GH-RIH. These results are omitted for the clarity of presentation.

Administration of atropine in a dose of 100 μg/kg.hr strongly inhibited pancreatic protein secretion induced by bombesin (Fig. 4) and duodenal perfusion of amino acid mixture. The pancreatic response to OP-CCK,

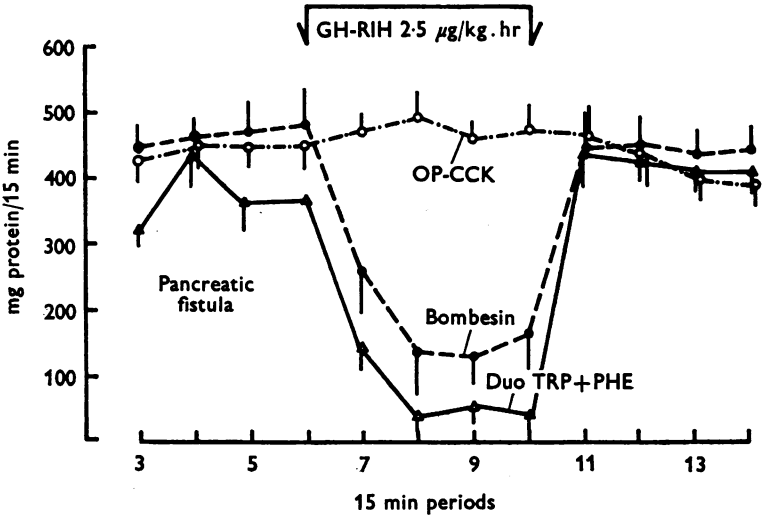

Fig. 3. Effect of GH-RIH on pancreatic protein secretion in response to bombesin, duodenal perfusion of amino acid mixture or OP-CCK.

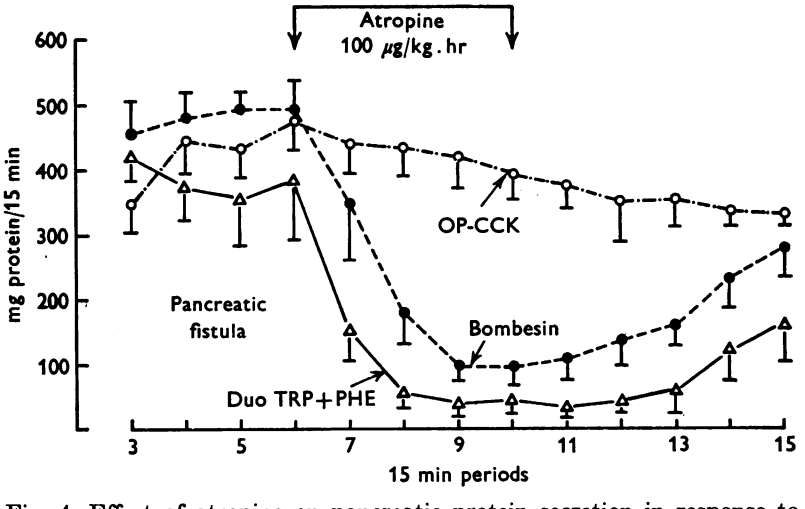

Fig. 4. Effect of atropine on pancreatic protein secretion in response to bombesin, duodenal perfusion of amino acid mixture or OP-CCK.

producing similar rate of protein secretion to that induced by bombesin or duodenal amino acids, remained unaffected by atropine.

Atropine caused a significant decrease in serum gastrin level, dropping from the mean of 192 ± 35 pg/ml. with bombesin alone to the lowest of 110 ± 26 pg/ml. with the combination of bombesin plus atropine.

In anaesthetized dogs with antrum removed, bombesin alone infused I.V. in graded doses produced a negligible pancreatic flow rate. To increase the flow rate to the measurable level, secretin in a constant dose of 0·4 μg/kg.hr was added to I.V. infusion. Protein outputs in the test with bombesin infusion was significantly above the basal level but was not dose-dependent (Fig. 5).

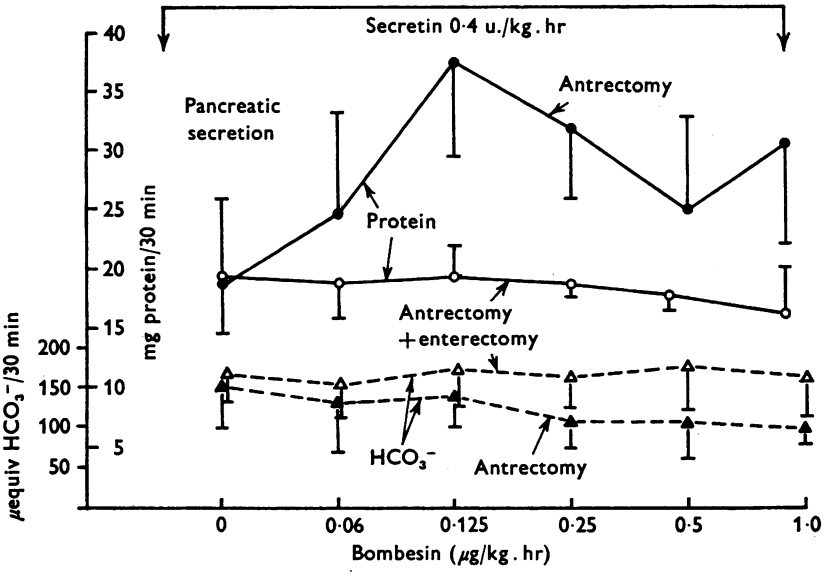

Fig. 5. Pancreatic protein and bicarbonate secretion stimulated by graded doses of bombesin in anaesthetized dogs with antrectomy, or antrectomy plus enterectomy.

Protein output in control animals of group *B* in response to OP-CCK reached a mean peak value of 72 ± 12 mg/30 min before antrectomy and was not significantly changed after the removal of the antrum (67 ± 8 mg/30 min).

Following antrectomy plus enterectomy, bombesin failed to cause any change in pancreatic secretion.

Protein output in control animals of group *C* in response OP-CCK before antrectomy and enterectomy reached a mean peak value of 89 ± 14 mg/

30 min and it was not significantly different after these procedures (74 ± 17 mg/30 min).

Serum gastrin level in dogs with resected antrum or resected antrum and small intestine did not show any significant change from the basal level and these data are not presented.

DISCUSSION

The results of the present study indicate that bombesin and related peptides have a marked effect on the release of both gastrin and CCK from the gastro-intestinal tract. Gastrin release by bombesin has been proved directly by radioimmunoassay in conscious animals with intact antra, thus confirming a previous report (Basso *et al.* 1974).

Following antrectomy bombesin failed to raise the serum gastrin level but retained its pancreozymin-like properties, suggesting that the major source of bombesin-induced gastrinaemia is the gastric antrum and that the stimulation of protein secretion cannot be ascribed solely to the release of gastrin. The resection of the antrum and small intestine resulted in the disappearance of both the gastrin-releasing and pancreozymin-like actions of bombesin but did not affect the pancreatic response to OP-CCK. This may be taken as evidence that the pancreozymin-like action of bombesin is not due to the direct influence of this peptide on the exocrine pancreas but is mediated by the release from the gut of the hormone stimulating pancreatic protein secretion, presumably CCK. This assumption can be proved only by the accurate radio-immunoassay of CCK and other intestinal hormones.

The pancreozymin-like action of bombesin is also shared by its fragment containing the COOH-terminal nonapeptide, which has been previously found to be the shortest amino acid sequence of the bombesin molecule still able to induce myoelectrical changes (Caprilli, Melchiorri, Improta, Vernia & Frieri, 1975). The finding that litorin, a natural analogue of bombesin in which leucine of the C-terminal active fragment is substituted by phenylalanine, shows similar secretory activity to bombesin indicates that the change in the amino acid sequence of the active fragments may not result in alteration of its biological activity. Further studies on the structure–function relationship are needed to establish which of the amino acid residues of the active fragment are dispensable and what is the functional or binding group of the bombesin molecule.

The comparison of the pancreatic dose–response curves to bombesin and related peptides shows that they exhibit similar potency in stimulating pancreatic protein secretion reaching a maximum not significantly different from that of OP-CCK. Bombesin-like peptides have also a small but

significant bicarbonate stimulating potency attaining about 10% of the maximal response to secretin. Since neither gastrin nor CCK, released by bombesin, is a stimulant of bicarbonate secretion, it may therefore be accepted that bombesin also releases the stimulant of bicarbonate secretion. The simplest assumption is that the additional stimulus is secretin released in small amounts of vasoactive intestinal peptide (VIP) which has recently been shown (Konturek, Thor, Dembiński & Król, 1975) to be a secretin-like partial agonist of pancreatic secretion in the dog. It is impossible at this time to decide whether the weak secretin-like action of bombesin is due to secretin, VIP or some other factor not yet isolated.

The most striking observation of this study is the marked inhibition of bombesin-induced pancreatic secretion by atropine and GH-RIH. Atropine has previously been shown to inhibit the release of CCK from the intestinal mucosa without affecting significantly the response of the pancreas to CCK itself (Konturek, Tasler & Obtułowicz, 1972). This observation was fully confirmed by the present study, which also showed that bombesin-induced pancreatic protein secretion equivalent to that evoked by OP-CCK can be inhibited by atropine to a similar degree to that induced by CCK released endogenously by duodenal perfusion with amino acids. The most probable explanation of this finding would be that atropine removes the cholinergic facilitation of CCK-release by bombesin but does not affect the action of CCK on the pancreas. This assumption, however, requires direct proof because atropine also caused a suppression of the bombesin-induced release of gastrin, which in the dog exhibits a pancrozymin-like activity almost as strong as CCK itself (Stening & Grossman, 1969).

The experiments showing that GH-RIH possesses a potent inhibitory action on bombesin-induced pancreatic secretion, and on that evoked by endogenous CCK but not on that stimulated by the exogenous hormone itself (OP-CCK), also favour the concept that bombesin releases CCK. GH-RIH is known to suppress the release of gastrin, and because duodenal perfusion of amino-acids has been shown to release substantial amounts of gastrin from the gut (Konturek, Becker & Thompson, 1974) it is difficult to assess whether the suppression of gastrin release by GH-RIH participates in the observed inhibition of pancreatic secretion.

The physiological role of bombesin and related peptides has not been established either in the frog or in mammals. It is very interesting that other biologically active peptides from amphibian skin, such as caerulein (Erspamer, Bertaccini, Cheli, de Caro, Endean, Impicciatore & Roseghini, 1967) have mammalian analogues. It is likely that bombesin-like material exists in the mucosa of the digestive tract and plays an important role as a local releaser of gastro-intestinal hormones, particularly of gastrin

and CCK (Erspamer & Melchiorri, 1975). These effects are perhaps counteracted by locally released GH-RIH, which has been found in large quantities in the intestinal mucosa (Arimura, Sato, DuPont, Nishi, & Schally, 1975) and shown to suppress the release of gastrin and CCK.

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