Effects of Gastrin Releasing Peptide and its Related Peptides on the Release of Gastrin

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Summary: Gastrin release of porcine gastrin releasing peptide (pGRP) and its related peptides were evaluated. The concentration of serum gastrin after intravenous administration of the peptides was measured by a radioimmunoassay. Among pGRP and its related peptides, pGRP was found to retain full bombesin potency with respect to gastrin release. The carboxy-terminal fragments of pGRP, 14-27, 18-27, and 19-27 were equipotent for gastrin release, though the peak value of serum gastrin caused by each of these three fragments was significantly less than that evoked by pGRP. The pGRP fragment 20-27, despite showing considerably lesser potency, still evoked statistically significant levels of serum gastrin during its infusion. On the other hand, pGRP fragments 21-27 and 1-13 did not exhibit any appreciable activity. It was concluded that the carboxy-terminal octapeptide 20-27 constitutes the vital active core for gastrin releasing activity of the pGRP molecule. The amino-terminal tridecapeptide pGRP (1-13), while itself inactive, appeared to be important for the full active of pGRP.

Key words: gastrin — gastrin releasing peptide (GRP) — bombesin — related peptides — vital active core

Introduction

It is well-known that intravenous administration of bombesin causes release of gastro-entero-pancreatic hormones into the peripheral blood circulation (Bertacceni et al. 1974; Erspamer et al. 1974; Fender et al. 1975, 1976) this activity has been attributed to the carboxy-terminal heptapeptide (Melchiorri et al. 1978).

However, while mammalian gastrin releasing peptide (GRP) isolated from porcine upper gastric tissue shares a common decapetide carboxy-terminus with bombesin except for an 8th amino acid residue (McDonald et al. 1978), the vital active core of porcine gastrin releasing peptide (pGRP) has not yet been established.

To define the core of this vital activity, we carried out experiments with pGRP and its related peptides in dogs; our particular interest was in establishing the minimal length of the amino acid chain required for expressing GRP activity.

Materials and Methods

Five mongrel dogs, each weighing between 10 and 15 kg, were utilized. All the dogs were fasted for 15 hours before the experiments, with free access to water. No premedication was given. Each dog was anesthetized with sodium pentobarbital, and, after endotracheal intubation, ventilation with room air was maintained by a respirator. The room temperature was kept at 26 °C and the rectal temperature...
of each dog was maintained at $37\pm 1.5^\circ C$ with an electric warmer. Polyethylene catheters were inserted into the veins of both forelimbs and into the inferior vena cava through the femoral vein. One forelimb catheter was used for peptide infusion and a second for lactated Ringer infusion at a rate of 10 ml/kg/h for the duration of the experiment. The femoral venous catheter was used for blood sampling.

pGRP and its related peptides, all from Prof. Yanaihara, Shizuoka College of Pharmacy, Japan, were freshly prepared for each experiment and dissolved in 0.9% NaCl containing 1% (wt/vol) bovine serum albumin (Yanaihara et al. 1981). The structures for the peptides are shown in Figs 1 and 2). After a base-line period of 15 min, equimolar doses each of pGRP and its related peptides were infused at a rate of 67 pmol/kg/min over 10 min; the peptides were administered at intervals of 30 min in random order. The infusion rate was 2.0 ml/min. Blood samples were drawn from the femoral venous catheter at times of -15, 0, 5, 10, 20, 30, 60, and 90 min. Blood samples were collected in ice-chilled tubes containing Aprotinin (500 KIU/ml Blood, Trasylol). The samples were centrifuged at 4°C and the sera were stored at -20°C until assayed. The concentration of serum gastrin was measured by radioimmunoassay using antiserum R1303-2 as described in detail by Yanaihara et al. (1978).

Fig. 1. Amino acid sequences of pGRP and bombesin.

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1          13
pGRP :    H-Ala-Pro-Val-Ser-Val-Gly-Gly-Thr-Val-Leu-Ala-Lys-
14            20
- Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$
27
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Fig. 2. Comparison of the structures of pGRP and its related peptides used in the present study.
The results were expressed as mean±SEM; a matched paired t-test was employed for the statistical analysis of differences. P-values of less than 0.05 were considered as significant.

Results

Under our experimental conditions, the basal serum immunoreactive gastrin (IRG) level varied between 10 and 51 pg/ml. Infusions of both pGRP and bombesin elicited increases in the serum IRG. Generally, pGRP and bombesin produced similar, significant increases in gastrin (Table 1 and Fig. 3). The three carboxy-terminal fragments of pGRP, 14-27, 18-27, and 19-27 were equipotent with respect to gastrin release; the release was significant though the peak value of IRG elicited by either of these three fragments was significantly less than that induced by pGRP or bombesin (Table 1 and Fig. 3). pGRP (20-27), despite being considerably less potent than the other pGRP fragments evoked significant elevation of the levels of IRG during the infusion. On the other hand, the pGRP fragments 21-27 and 1-13 did not exhibit any appreciable activity.

Following infusion of either pGRP or bombesin, the serum concentration of IRG remained at high level for 30 min. In contrast, following the infusions of the carboxy-terminal fragments of pGRP, the serum concentrations of IRG fell rapidly to the initial level (Table 1 and Fig. 3).

### TABLE 1

Values of serum immunoreactive gastrin to pGRP, its related peptides and bombesin. The value is expressed as pg/ml.

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Discussion

pGRP exhibits a carboxy-terminal decapeptide which is common with that of bombesin, except for an 8th amino acid residue; pGRP appears to act similarly to mammalian bombesin as its heptapeptide carboxy-terminal contains or is, the region essential for bioactivity.

However, Yanaihara et al. (1981) have reported that widespread distribution of pGRP-like immunoreactivities were observed not only in the porcine alimentary tract, but also in the brain; they reported also that the gel-chromatographic profiles of these tissues showed the presence of two major molecular forms which are necessary for pGRP-like immunoreactivity. These two forms were present in the position where synthetic pGRP and pGRP (14-27) were eluted, respectively. These results indicated the existence of both pGRP and pGRP (14-27) in porcine tissue.

Results reported here demonstrated that pGRP is indeed similar to bombesin not only structurally, but also pharmacologically, and that the removal from the amino-terminal of the thirteenth, the seventeenth, and the eighteenth, amino acid residues did not affect the response of serum IRG during infusion. However, the duration of the effect of pGRP was drastically shortened by the shortening of the peptide chain. Removal of the nineteenth amino acid residue caused significant decrease in the gastrin releasing activity. The next, 20th, residue was found to be essential for the gastrin releasing activity of pGRP as the 21-27 carboxy-terminal hexapeptide of pGRP did not exhibit any appreciable activity.

Thus, the carboxy-terminal octapeptide 20-27 must be the essential core of the pGRP molecule for gastrin releasing activity of the pGRP molecule. Our results indicate also that the carboxy-terminal
tetradecapeptide 14-17 exhibits bioactivity similar to that of the carboxy-terminal decapeptide 18-27 and of the nonapeptide pGRP 19-27. Our results also show that the pGRP fragment 14-19, that is (-Met-Tyr-Pro-Arg-Gly-Asn-), plays no role in the duration of the effect of gastrin release. However, the amino-terminal tridecapeptide 1-13 of pGRP, while not active, appeared to be important for the full activity of pGRP.

Broccardo et al. (1975) described the syntheses and the biological properties such as the effect on smooth muscle preparation, blood pressure, gastric acid secretion, and intestinal myoelectric activity of a large number of bombesin analogues. They found that the minimal length of the amino acid chain required for the first appearance of bombesin-like effects was represented by the carboxy-terminal heptapeptide, while the minimal length of the chain necessary for the maximal effects by the carboxy-terminal nonapeptide. However, River et al. (1978) reported that the carboxy-terminal decapeptide of bombesin was sufficient for maximal thermoregulatory effect, and Tache et al. (1981) found that the carboxy-terminal octapeptide of pGRP retained full effect on gastric secretion upon intracisternal injection. Thus, different carboxy-terminal fragments exhibit different biological effects. We speculated that the blood enzymes hydrolyse pGRP and its related peptides more efficiently than do the enzymes present in the cisterna of the brain.

It appears altogether that only pGRP shows bioactivity with respect to gastrin release which is similar to that of bombesin, while the pGRP fragment 14-27 is less potent than pGRP, and the pGRP fragment 20-27 is essential for the activity of pGRP. However, since both of pGRP and pGRP (14-27) are present in mammalian tissues (Yamashita et al. 1981), it remains unclear which is the true bioactive peptide.

References


