The effect of the mammalian neuropeptide, gastrin-releasing peptide (GRP), on gastrointestinal and pancreatic hormone secretion in man

SUSAN M. WOOD, ROLAND T. JUNG, JOAN D. WEBSTER, MOHAMMED A. GHATEI, THOMAS E. ADRIAN, NOBURU YANAIHARA*, CHIZUKO YANAIHARA* AND STEPHEN R. BLOOM

Department of Medicine, Hammersmith Hospital, London. *Laboratory of Bio-organic Chemistry, Shizuoka College of Pharmacy, Shizuoka, Japan, and †National Institute for Physiological Sciences, Okazaki, Aichi, Japan

(Received 29 November 1982/18 March 1983; accepted 8 April 1983)

Summary

1. Gastrin-releasing peptide, a newly isolated mammalian peptide similar in its structure and actions to the amphibian peptide, bombesin, has recently been localized to nerves in the brain, gut and pancreas. The present study investigates its effects on gut and pancreatic peptides in man.

2. Intravenous infusion of 0.7 and 2.9 pmol min⁻¹ kg⁻¹ produced significant elevation of plasma gastrin, cholecystokinin-like immunoreactivity and neurotensin. It was found also to potentiate glucose-dependent insulin secretion.

3. Its specific location in nerve fibres in the proximal gut and pancreas and its selective effect on gastroenteropancreatic peptides may favour its role as a physiological regulatory neuropeptide.

Key words: gastrin-releasing peptide, gastrointestinal hormones, pancreatic hormones.

Abbreviations: CCK, cholecystokinin; GIP, glucose-dependent insulinotropic peptide; GRP, gastrin-releasing peptide.

Introduction

The autonomic nervous system plays a central role in the regulation of the gastrointestinal tract and pancreas [1]. Until recently it has been considered to consist exclusively of cholinergic and adrenergic nerve fibres; it is now evident, however, that a large and complex peptidergic component exists [2, 3]. The ganglia of the intrinsic plexuses of the gut and pancreas are richly supplied by these peptidergic fibres [4, 5]. These appear to be part of the local neural modulation of motility, secretion and blood flow within the gut, being in turn influenced by the extrinsic nerve supply [1]. A variety of putative peptide neurotransmitters have been identified by radioimmunoassay and immunocytochemistry in the gut and pancreas [4, 6, 7]. One of these reacts with antibodies to bombesin, a 14 amino acid peptide originally isolated from the skin of the frog Bombina bombina [8]. This peptide has been shown to release a number of other peptides in the mammal [9-12]. Bombesin-like immunoreactivity has been localized to intrinsic nerves throughout the mammalian gut [13], and may function as a stimulatory neuropeptide in a wide variety of sites. It has been suggested for example that bombesinergic neurons correspond to the postganglionic nerves of the vagal innervation of the endocrine cells in the stomach, where they may regulate the secretion of such peptides as gastrin [14], and so modify gastric acid and exocrine secretion.

More recently a 27 amino acid peptide, gastrin-releasing peptide (GRP), has been isolated from porcine gut [15]. This peptide shows remarkable homology with bombesin at the biologically active C-terminal region of the peptide (Table 1). Furthermore its effects on gastroenteropancreatic hormone secretion are very similar to those of bombesin [16]. Immunostaining with anti-GRP sera has
revealed GRP nerve fibres in the myenteric nerve plexus of the porcine duodenum and intrapancreatic ganglia [17]. As bombesin's mammalian counterpart, GRP is therefore a strong contender for the stimulatory neurotransmitter or neuromodulator of the gut and pancreas.

Its effects on gastrointestinal and pancreatic hormone secretion have not previously been investigated in man. We have therefore studied these effects at basal plasma glucose concentrations, and during glucose infusions adjusted to mimic the postprandial rise in plasma glucose.

**Methods**

Studies were carried out on six healthy volunteers (four females and two males, mean age 24 ± 0.6 years, and mean weight 60.6 ± 3.5 kg). All subjects gave their informed consent to take part in the investigations, which had been approved by the Royal Postgraduate Medical School Ethical Committee.

Each subject was studied on five different mornings after an overnight fast. Abbott butterfly cannulae (Abbott Laboratories, North Chicago, U.S.A.) were inserted into arm veins, one for infusion of peptide and glucose, the other for blood sampling.

Glucose was infused at a rate of 0.3 g/min for 30 min to mimic the normal postprandial plasma glucose profile. On a separate occasion synthetic gastrin-releasing peptide was infused at a rate of 0.7 pmol min⁻¹ kg⁻¹ and on a further occasion at a rate of 2.9 pmol min⁻¹ kg⁻¹ each over 30 min. Simultaneous infusions of glucose (0.5 g/min) and GRP, at each infusion rate, were also performed. The infusions were carried out in random order.

The GRP used in the study was a synthetic preparation of the porcine peptide, which coeluted as single peak with the natural 27 amino acid peptide on column chromatography [18]. For infusion it was dissolved in sterile 0.9% sodium chloride solution (154 mmol/l) containing 0.5% (70 μmol/l) human serum albumin (Lister Institute, Elstree, Herts, U.K.), the latter to reduce adsorption of the peptide to the plastic syringe and tubing of the infusion equipment.

Basal blood samples were taken before and then at 5 min intervals during each 30 min infusion period, and finally at 15 and 30 min after the end of infusion.

For estimation of the gastroenteropancreatic hormones 10 ml of blood was taken into chilled tubes containing 4000 kallikrein inactivator units (KIU) of aprotinin and 100 units of heparin and immediately centrifuged. The plasma was removed and stored at −20°C until the time of assay. Additional samples (2 ml) were collected into sodium fluoride/sodium oxalate tubes for glucose estimation. GRP infusate samples were collected at the beginning and end of each infusion for measurement of GRP to assess losses of peptide and allow estimation of the actual concentration of GRP infused.

The gastroenteropancreatic hormones were measured by previously described radioimmunoassays for insulin [19], pancreatic polypeptide, glucagon, enteroglucagon, glucose-dependent insulinotropic peptide (GIP), neurotensin and gastrin [20]. These assays showed no cross-reactivity between peptides, with the exception of enteroglucagon with glucagon. In the last-named enteroglucagon was calculated by subtraction of pancreatic glucagon (measured using a specific C-terminal directed antiserum, RCSS, which gave zero readings after total pancreatectomy) from total N-terminal glucagon immunoreactivity (measured by antiserum R59, which fully reacted with pure porcine enteroglucagon, glicentin [20]).

A subtraction assay was also used to measure cholecystokinin (CCK)-like immunoreactivity. Rabbit antiserum raised to the sulphated C-terminal octapeptide, CCK-8, cross-reacted with CCK-8, CCK-33 and the gastrins. When used at a final antiserum dilution of 1:160,000 with 125I-labelled unsulphated CCK-8, and sulphated CCK-8 as standard, the assay could measure changes of

---

**TABLE 1. Sequences of porcine GRP and amphibian bombesin**

<table>
<thead>
<tr>
<th>PGRF</th>
<th>Ala</th>
<th>Pro</th>
<th>Val</th>
<th>Ser</th>
<th>Gly</th>
<th>Gly</th>
<th>Thr</th>
<th>Val</th>
<th>Leu</th>
<th>Ala</th>
<th>Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Gly</td>
<td>Asn</td>
<td></td>
<td>His</td>
<td>Trp</td>
<td>Ala</td>
<td>Val</td>
<td>Gly</td>
<td>His</td>
<td>Leu</td>
<td>Met</td>
</tr>
<tr>
<td>25</td>
<td>Gly</td>
<td>Asn</td>
<td></td>
<td>Gln</td>
<td>Trp</td>
<td>Ala</td>
<td>Val</td>
<td>Gly</td>
<td>His</td>
<td>Leu</td>
<td>Met</td>
</tr>
</tbody>
</table>

Amino acid residues present in identical positions in both are enclosed in the continuous line.
Gastrin-releasing peptide and pancreatic and gut hormone secretion

1 pmol of cholecystokinin-like immunoreactivity/l between adjacent tubes with 95% confidence. The gastrin antibody (Gas-179) cross-reacted with all forms of gastrin but showed less than 2% cross-reactivity with the CCK. Therefore plasma concentrations of CCK-like immunoreactivity were obtained by subtracting gastrin, as measured by Gas-179, from the total CCK and gastrin measurements determined by the CCK-like immunoreactivity assay. These subtraction assays were used, as specific single assays for enteroglucagon and CCK are not available.

Antiser to bombesin were raised in rabbits by using a synthetic Lys3-bombesin analog conjugated to bovine serum albumin as antigen. A radiolabelled antigen (125I-labelled bombesin) was prepared from Tyr5-bombesin C-terminal nonapeptide analog by the chloramine-T oxidation technique. The product was purified on a Sephadex G-25 column eluted with formic acid (0.1 mol/l) containing 2.0% human serum albumin and 40 KIU of aprotonin/ml. Synthetic porcine GRP was used as standard. This assay measured bombesin and pure porcine GRP with equal potency, detecting 5 pmol/l with 95% confidence [16]. Plasma glucose was measured by a standard glucose oxidase method.

Results are expressed as means and SEM. Statistical significance was assessed by using Student's paired t-test.

Results

Gastrin-releasing peptide

GRP produced no subjective or objective side effects at either dose. The concentration of GRP measured in the infusates at the end as compared with the start of each infusion demonstrated a variable loss of peptide of between 15 and 20% of the total during any 30 min infusion. The infusion rates were therefore calculated from the mean of the infusate concentrations at the beginning and end of each infusion for each subject and were 0.7 ± 0.02 and 2.9 ± 0.15 pmol min⁻¹ kg⁻¹ for the low and high dose infusions respectively. These resulted in elevation of plasma GRP from 7.5 ± 0.7 pmol/l to 24.2 ± 1.7 pmol/l and 79.8 ± 1.9 pmol/l for the low and high dose respectively. A plateau concentration of plasma GRP was reached at 10 min in either case (Fig. 1). Intravenous glucose had no effect on the GRP rise.

Pancreatic peptides

There was no change in plasma insulin during infusion of GRP at fasting plasma glucose concen-
Gastrointestinal peptides

GRP at both infusion rates produced large rises in plasma gastrin (P < 0.01) and cholecystokinin-like immunoreactivity (P < 0.01) (Figs. 4 and 5), and a smaller rise in plasma neurotensin (P < 0.05) (Fig. 6). There was no significant change in entero-glucagon or GIP.

Discussion

In the present study gastrin-releasing peptide (GRP) was found potently to release a number of gastrointestinal hormones, including neurotensin, gastrin and cholecystokinin-like immunoreactivity, when circulating concentrations of the peptide were as low as 24 pmol/l. The higher infusion rate of GRP resulted in a circulating concentration of 80 pmol/l, but this did not significantly enhance the peptide responses, suggesting that the maximal response had already been reached with the lower dose. The effect of GRP on these gut peptides was more potent than that previously reported for synthetic amphibian bombesin, although circu-

![Fig. 3. Plasma glucose concentrations during 30 min infusions of: 10 g of glucose (●); GRP 0.7 pmol min⁻¹ kg⁻¹ alone (○) and in the presence of the 10 g of glucose infusion (●); GRP 2.9 pmol min⁻¹ kg⁻¹ alone (▲) and with 10 g of glucose (▲). Infusion period is shown by the bar.](image)

![Fig. 4. Plasma gastrin concentrations in response to infusions of GRP: 0.7 pmol min⁻¹ kg⁻¹ (○) and 2.9 pmol min⁻¹ kg⁻¹ (▲).](image)
Gastrin-releasing peptide and pancreatic and gut hormone secretion

25

20

15

10

8

5

4

3

2

1

CCK (pmol/L)

Time (min)

0

30

60

FIG. 5. Plasma cholecystokinin (CCK) concentrations in response to infusions of GRP: 0.7 pmol min⁻¹ kg⁻¹ (○) and 2.9 pmol min⁻¹ kg⁻¹ (△).

Neurotensin (pmol/L)

Time (min)

0

30

60

FIG. 6. Plasma neurotensin concentrations in response to infusions of GRP: 0.7 pmol min⁻¹ kg⁻¹ (○) and 2.9 pmol min⁻¹ kg⁻¹ (△).

lating plasma concentrations of bombesin-like immunoreactivity were virtually identical in the two studies [21]. This is perhaps predictable as GRP is the mammalian form of the peptide. The possible physiological relevance of these actions needs to be considered in relation to the location of GRP in the gut. By using a recently developed specific radioimmunoassay for GRP [17], high concentrations have been detected in the mucosa and muscle of the body of the porcine stomach, in both layers of the antrum where the majority of gastrin cells are localized and in jejunal muscle [17]. The two molecular forms of GRP identified at these sites included one coeluting with synthetic porcine GRP and the other with porcine GRP-(14-27) [17]. Immunohistochemical studies have shown numerous GRP immunoreactive nerve fibres in the myenteric nerve plexus of the porcine duodenum [17]. In the light of this distribution and the action of GRP on gastrin release reported here in man, it is tempting to speculate that GRP plays a part in the neural control of gastrin release and gastric acid secretion. Many studies have shown that amphibian bombesin and more recently porcine GRP potently stimulate gastric acid secretion in animals and man [22, 23, 27], an effect which appears to be dependent on gastrin release [21].

As has been suggested for bombesin, GRP may play a part in the regulation of pancreatic exocrine secretion [22, 25] and gall bladder function via the release of cholecystokinin [16, 25-27]. The recent localization of GRP to nerves in the porcine duodenum and jejunum strengthens such a concept. Bombesin's reported action on gastrointestinal motility [23, 28] may also be shared by GRP, particularly if this effect is found to be mediated by peptides such as CCK or neurotensin.

In the present study no stimulation of enteroglucagon release by GRP was found. This differs from the studies on GRP in the dog [16] (N. Yanaihara, personal communication), which may be explained by species variation in the response.

GRP was found in this study to have no significant effect on basal insulin secretion but to enhance glucose-induced insulin secretion. This agrees with the reported effects of bombesin on isolated perfused rat pancreas [29] and its infusion in the dog [3]. Surprisingly GRP has been reported to stimulate basal insulin secretion in the dog [16]. The possible physiological significance of the action of GRP on glucose-induced insulin secretion gains more relevance in the light of the recent localization of GRP to porcine intrapancreatic neural ganglia [17]. Nerve fibres from these ganglia have been shown to innervate the islets of Langerhans. This is further supported by our recent
finding of GRP like-immunoreactivity in human pancreas (8.2 ± 2.8 pmol/g, n = 4).

There was no significant effect of GRP on the other islet peptides, glucagon and pancreatic polypeptide. This contrasts with the reported action of bombesin on these peptides in the dog and man [16, 21, 30], and of GRP in the dog [16]. In many of these studies there seems to be marked differences in responses between dog and man. For instance, the effect of bombesin on secretion of pancreatic polypeptide in man is small compared with its effect in the dog, and it has been suggested that bombesin might have a dual inhibitory-stimulatory effect on secretion of pancreatic polypeptide in man [31]. This may be a reason for the indefinite pancreatic polypeptide responses in this study.

The localization of GRP to the enteric plexi of the gut and pancreas [17] and its effect on a limited number of peptides, gastrin, CCK, neuropeptide Y and insulin at low circulating concentrations may imply a physiological role for it as a neurotransmitter or neuromodulator. It appears to have a more selective effect on peptide release than its amphibian counterpart, possibly suggesting a greater specificity for receptor sites, perhaps by virtue of its greater molecular size. It is of interest, however, that significant quantities of a smaller polypeptide or neuromodulator could be identified in porcine gut and pancreas [16, 21, 30] and of GRP in the dog [16]. In many cases, GRP is a potent stimulator of the release of active pancreatic enzymes and of gastric motility and these effects are mediated by a specific receptor site for GRP.

References


gastrin release and gastric acid secretion. Regulatory Peptides, 1, 289-296.


