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Dual Effect of Bombesin and Gastrin Releasing Peptide on Gastric Emptying in Conscious Cats

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CHIKH-ISSA, A. R., C. SCARPIGNATO, M. COLLINET, J.-A. CHAYVIALLE AND M. VAGNE. *Dual effect of bombesin and gastrin releasing peptide on gastric emptying in conscious cats.* PEPTIDES **10**(2) 281–287, 1989.—The effect of bombesin (BBS) and gastrin releasing peptide (GRP) on gastric emptying was studied in conscious cats. This effect was measured simultaneously with antral motility. Acid and pepsin secretions as well as blood hormonal peptide release were additionally measured. A dual effect was observed. First, BBS and GRP slowed gastric emptying of liquids, while antral motility was decreased, then after 60 minutes of continuous intravenous infusion, antral motility returned to basal values and gastric emptying effect reversed. The mechanism of this peculiar action is independent of gastrin, pancreatic polypeptide, somatostatin and motilin release and most probably connected with a cholinergic stimulation induced by the peptides, the late predominance of which counterbalances the inhibitory effect of bombesin-like peptides on antral motility.

Bombesin Gastrin releasing peptide Gastric emptying Cats Antral motility

SINCE the isolation of the porcine gastrin releasing peptide (GRP) by McDonald *et al.* (13), several studies have compared the effect of GRP to that of the structurally related peptide, bombesin (BBS), isolated from frog skin (4). The amphibian decapeptide was shown to strongly delay gastric emptying in rats (16) and humans as well (17,27). This inhibitory effect seems to be connected with the marked contracting action of BBS on gastroduodenal region (16), the dependence on gastrin release being still questionable (15, 17, 27).

Previous studies from other (14) and our laboratories (26) have shown that BBS and GRP possess the same spectrum of biological actions although some qualitative differences were reported (6,8). In conscious cats, we have shown that not only the potency but also the efficacy of GRP can be lower than those of BBS, depending on the biological response involved (26). In this study, we wanted to check whether BBS is able to affect gastric emptying *also* in cats and if this effect is shared by GRP.

Antral motility is affected by BBS and GRP in cats: an increase of low amplitude and a decrease of high amplitude contractions was observed during the first 30 minutes of peptide administration (25,26). However, this effect, which is comparable to that of gastrin and cholecystokinin in the cat (2), disappeared progressively with time despite continuing the administration of both BBS and GRP (26). Since the effect on gastric emptying can be regarded as the results of the action(s) on the different regions of the stomach (7, 9, 12), we compared the effect of either BBS and GRP on gastric emptying and antral motility in order to correlate both parameters. Acid and pepsin secretion as well as gastrin, pancreatic polypeptide, somatostatin and motilin release induced by BBS and GRP were additionally studied.

METHOD

Animals

Seven cats, 4 males and 3 females, weighing 3.3 to 3.8 kg, were used. Under pentobarbital anesthesia (Nembutal, Abbott, 25 $mg \cdot kg^{-1}$), they were surgically equipped with both gastric and duodenal cannulae (26).

Experiments started 3 weeks or more after surgery. The conscious animals were fasted 18 hours before each test. Tests were not repeated more than twice a week.

Experimental Design

A continuous intravenous infusion of saline was administered at a rate of 12 ml per hour by means of a catheter placed in a leg vein of the animals and connected to a peristaltic pump (Harvard).

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Two sets of experiments were carried out. In the first one (4 cats), four meals were given in the same day at 10 min intervals and drained either 5, 10, 15, or 20 minutes later. The experiment was repeated on 4 different days in which the duration of the meal was chosen according to a latin square design. Peptides or saline were injected intravenously 5 min before the first meal and continued throughout the experiment. The dose of BBS used (0.6 $\mu g \cdot kg^{-1} \cdot hr^{-1}$, 0.37 nmol) proved to be a submaximal one for antral motor activity (25). Since in some experimental conditions GRP appeared to be 5 times less potent than its amphibian counterpart (26), a dose of 4.8 $\mu g \cdot kg^{-1} \cdot hr^{-1}$ (1.71 nmol) of GRP was employed.

In the second set of experiments, three different test meals were administered to each cat. The first one during saline infusion, and the two others 5 and 60 minutes after the beginning of BBS or GRP administration, respectively. The cannula was opened 10 minutes after instillation of the test meal for recovery of gastric contents. Two doses were used for BBS (0.37 and 0.74 $nmol \cdot kg^{-1} \cdot hr^{-1}$and for GRP (0.86 and 1.71 $nmol \cdot kg^{-1} \cdot hr^{-1}$).

Drugs

Synthetic BBS was a gift from Farmitalia-Carlo Erba Res. Labs. (Dr. R. Castiglione) and synthetic GRP was purchased from Peninsula (Belmont, CA).

Measurement of Gastric Emptying

Measurement of gastric emptying was performed by the method of Cooke (1), with minor modifications. A plastic adapter with a stopcock was connected to gastric cannula through which the test meal was instilled and drained. Prior to each test meal, the stomach was rinsed with 30 ml of warm distilled water and completely drained via the gastric fistula. The test meal consisted of a solution of aqueous methylcellulose (1%) containing phenol red (60 mg/l) as nonabsorbable marker (9). Fifty ml prewarmed (37°C) meal was instilled into the stomach via the gastric cannula in exactly 2 minutes by means of a peristaltic pump (Harvard). The stopcock was then closed and reopened at different time intervals after the test meal to allow the recovery of the gastric contents.

To ensure a complete recovery of the test meal, a wash solution of warm distilled water (30 ml) was instilled through the gastric cannula and quickly drained. Continuous recording of antral motility was performed as described below.

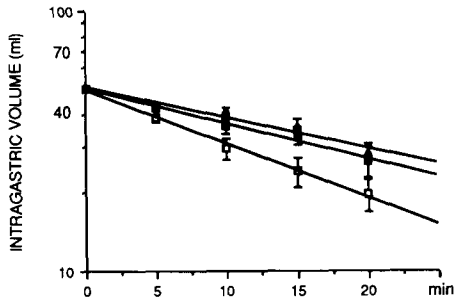

FIG. 1. Semilogarithmic plot of volume remaining in the stomach versus time during saline, bombesin (0.37 $nmol \cdot kg^{-1} \cdot hr^{-1}$) and GRP (1.71 $nmol \cdot kg^{-1} \cdot hr^{-1}$) infusion in conscious cats. Each point is the mean of 16 experiments in 4 cats and the vertical bars are the SEM. The lines are the least-square calculated regression lines. □ Saline, ■ bombesin, ▲ GRP.

Recording of Gastric Motility

An open-tip polyethylene tube (1 mm i.d.) was introduced through the gastric cannula, fixed 1 cm above the pylorus, perfused with water at a rate of 6 ml/hr and connected to a pressure transducer (Statham SC 1001) and to a recorder (Servotrace) as described previously (2).

Laboratory Analyses

The volume of gastric contents was measured to the nearest 1 ml and in each sample phenol red concentration was measured spectrophotometrically at 560 nm, after alkalinization with 0.1 N

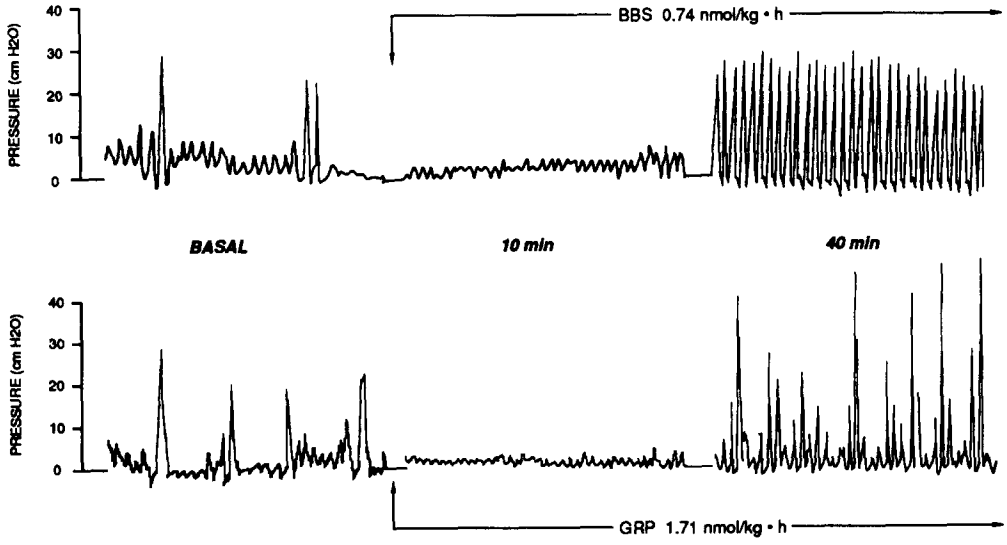

FIG. 2. Representative recording of antral motility in one cat given bombesin (BBS) or gastrin releasing peptide (GRP) infusion. Note the strong difference in tracings obtained 10 and 40 min after the beginning of the infusion.

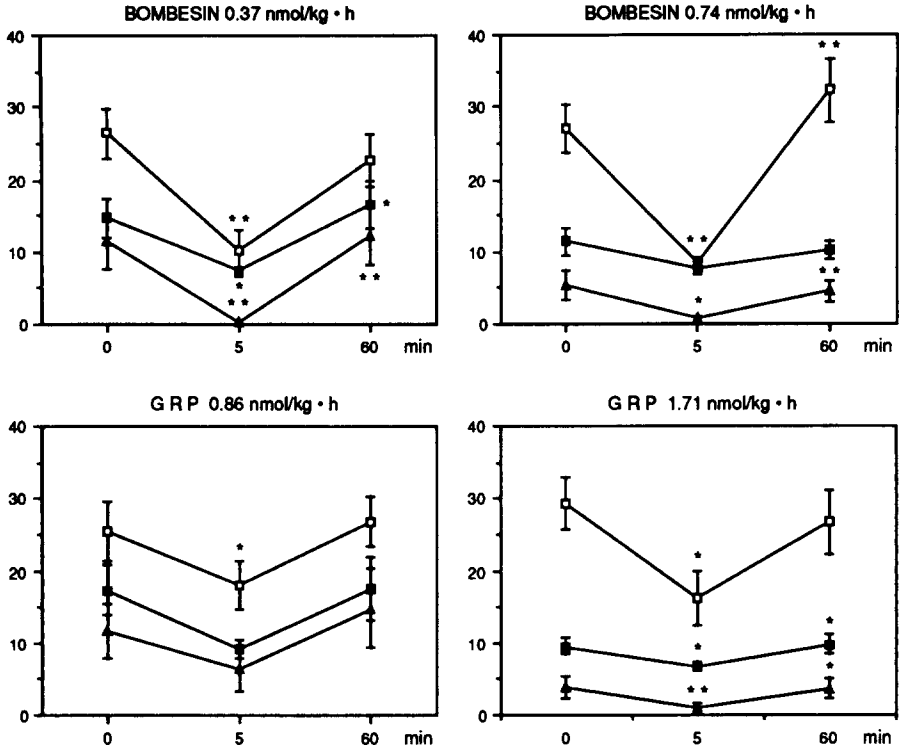

FIG. 3. Gastric emptying (expressed as the volume passing the pylorus) after a 10 min meal, before or 5 and 60 minutes after the beginning of intravenous infusion of two doses of BBS and GRP, measured simultaneously with antral motility (mean amplitude and number of peaks >20 cm H_2O pressure). Each point is the mean of 12 experiments in 4 cats. The asterisks indicate a statistically significant difference between basal and 5 min values or 5 and 60 min values (*$p<0.05$, **$p<0.01$, Mann-Whitney U-test). □ Volume passing the pylorus (ml/10 min), ■ amplitude (cm water), ▲ peaks greater than 20 cm water (n/10 min).

NaOH. Acid concentration was determined in each sample by titrating 1 ml of gastric contents with 0.1 N NaOH to pH 7, using a glass electrode and an automated titrator (Radiometer).

Pepsin concentration was measured by using a method previously described (23) and validated (2,26).

Measurement of Peptides

The blood samples were obtained in separate experiments but in the same animals to avoid interference with antral motility. Two ml samples were collected just before the beginning of the peptide infusion, at 15, 30 and 60 minutes after the beginning of the infusion and 15 and 30 minutes postinfusion. About 12 ml of blood were collected per experiment and per cat. The cats were fed with fresh liver diet between the experiments and were in good health during the experiment period with normal weight curves.

Gastrin, pancreatic polypeptide (PP), somatostatin and motilin were determined according to the radioimmunoassays which have been fully described and previously validated (26).

Calculations

For each cat and for each test, the volume of the original meal remaining in the stomach (V_r), taken as an index of gastric emptying, was calculated according to the following formula:

$$V_r = V_n + (V_w P_w / P_n) \times P_n / P_o$$

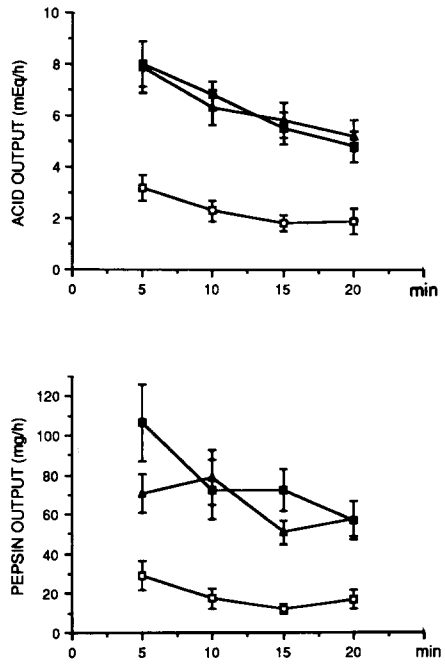

FIG. 4. Acid and pepsin output during saline, bombesin (0.37 nmol·kg^{-1}·hr^{-1}) and GRP (1.71 nmol·kg^{-1}·hr^{-1}) infusion in conscious cats. Each point refers to the mean of the values obtained from 16 experiments in 4 cats. Vertical bars are SEM. □ Saline, ■ bombesin, ▲ GRP.

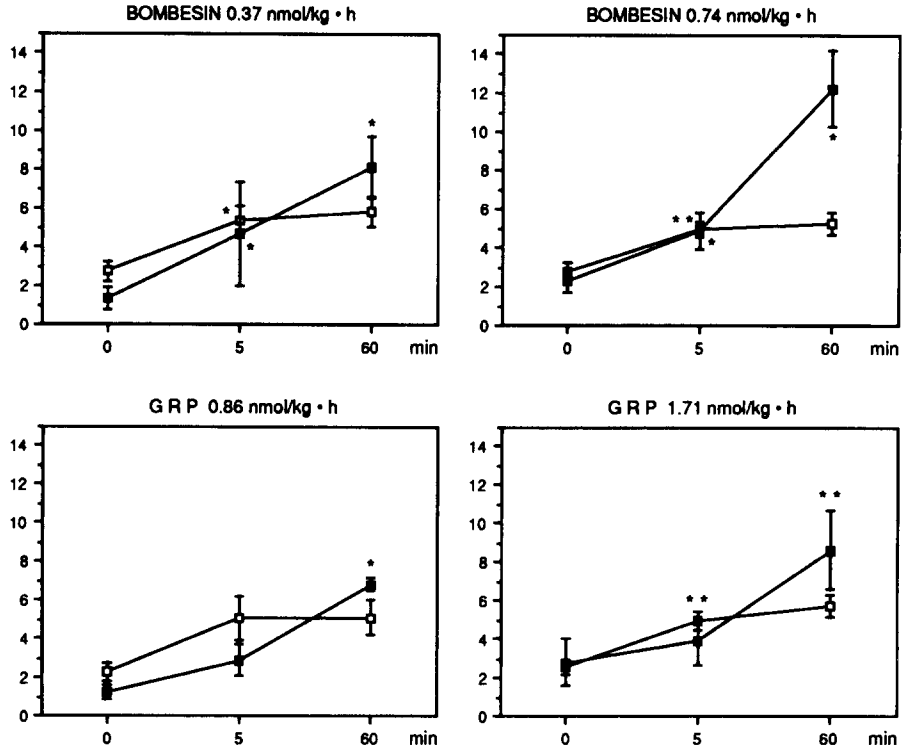

FIG. 5. Acid and pepsin outputs after a 10 min meal, before or 5 and 60 min after the beginning of intravenous infusion of two doses of BBS and GRP. Each point is the mean of 12 experiments in 4 cats. The asterisks indicate a statistically significant difference between basal and 5 min values or 5 and 60 min values (*$p<0.05$, **$p<0.01$, Mann-Whitney U-test). □ Acid output (mEq/hr), ■ pepsin output (mg·10/hr).

where V_n is the volume of gastric contents collected at completion of the test period and V_w is the volume of the wash solution. The concentration of phenol red in the initial meal is P_o, in the gastric contents is P_n and in the wash solution is P_w.

The total volume passing the pylorus (in ml), the acid and pepsin output (in $\mu Eq \cdot hr^{-1}$ and $mg \cdot hr^{-1}$ respectively) were also computed as previously described (26). All these values were corrected for the recovery of wash solution.

Statistical Analysis

All data are presented as mean ± SEM. The Mann-Whitney U-test was employed to check statistical significance.

RESULTS

Early Emptying and Motility Responses to BBS and GRP

Bombesin delayed gastric emptying of liquids in conscious cats. Compared with saline, the volume of the test meal remaining in the stomach was statistically higher at each time interval. Figure 1, which depicts a semilogarithmic plot of gastric retention versus time, clearly indicates that gastric emptying during saline infusion proceeds by apparent first order kinetics. In our experimental conditions, the emptying half-time was 12 min during saline and 18 min during BBS. The slopes of the two regression lines were not significantly different from each other ($b = -0.024 \pm 0.005$ and $b = -0.018 \pm 0.004$ for saline and BBS, respectively). The peptide therefore delayed emptying rate without modifying the first order kinetics. As expected, GRP slowed emptying rate too, its effect being not dissimilar to that observed with BBS. Its potency was, however, significantly lower (about 5 times on a molar basis) than that of the amphibian counterpart. The calculated emptying half-life during GRP infusion was estimated to be 24 min, a value significantly higher than that observed during either saline or BBS.

Figure 2 shows the effect of BBS-like peptides on antral motility. It is evident that during the first 30 minutes of infusion of BBS and GRP, high amplitude contractions almost completely disappeared and were replaced by low amplitude contractions, so that the mean amplitude was lower than that observed during saline. However, although the increase in motility observed later after BBS or GRP was always present when compared with the early tracing, the same was not true when the late motor response was compared to basal motility. Accordingly, mean changes reached statistical significance only between 60 and 5 min.

Late Emptying and Motility Responses to BBS and GRP

Figure 2 shows that, while the infusion of peptides was continued, the effect of both peptides on antral motility strongly reversed with an increase of the number of high amplitude peaks and a decrease of low amplitude peaks to a frequency reaching, at least, the basal level.

The comparison of the effects of BBS and GRP on gastric emptying of the meal (i.e., volume passing the pylorus), given either 5 or 60 minutes after the beginning of the peptide infusion, clearly shows an inversion of these effects with a return to basal emptying for both BBS and GRP, in spite of the continuous

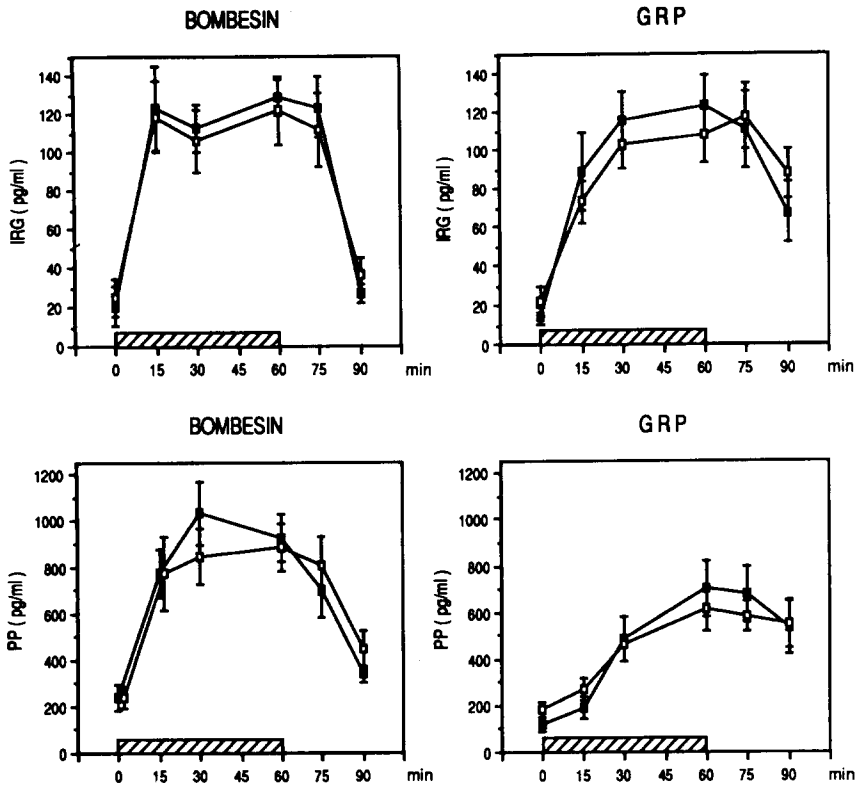

FIG. 6. Gastrin (IRG) and pancreatic polypeptide (PP), during saline administration and in response to two doses of BBS and GRP. □ Bombesin: 0.37 nmole/kg·hr, GRP: 0.86 nmole/kg·hr; ■ bombesin: 0.74 nmole/kg·hr, GRP: 1.71 nmole/kg·hr.

intravenous infusion (Fig. 3). The return to values not dissimilar to those observed during basal period was synchronous for the gastric emptying and antral motility. Their changes were strictly correlated with each other for each cat.

Acid and Pepsin Responses to BBS and GRP

Results concerning acid and pepsin secretion are shown in Fig. 4. As previously reported (26), BBS and GRP are equally effective in increasing acid secretion. The shorter was the residence time of the meal in the stomach, the higher was the acid response to the peptides. The same is true for the acid response to meal during saline. These data are consistent with the idea that gastric distention, which was larger after shorter time intervals, adds to the stimulatory effect of both peptides.

BBS and GRP (in the ratio of 1:5 on a molar basis) were equally effective in stimulating pepsin secretion. As for acid, the pepsin responses decreased with the increase of the meal residence time.

The early (5 min) and late (60 min) acid responses to meal during infusion of either BBS or GRP were not significantly different from each other (Fig. 5). On the contrary, late pepsin response was significantly higher than that observed earlier for both peptides (Fig. 5).

Simultaneous Hormonal Peptide Release

Gastrin (IRG), PP, somatostatin (IRS) and motilin (IRM) responses to BBS and GRP infusion are depicted in Figs. 6 and 7. It is evident that gastrin and PP concentrations, which were strongly increased by BBS and GRP, were still at their maximum level 60 minutes after the beginning of peptide infusion. Thirty minutes after stopping the infusion, blood peptide concentrations returned to basal levels, for BBS but not for GRP. Changes in blood somatostatin were small and not statistically significant. The release of motilin, recognised by the antibody directed against the C terminal peptide, was inhibited by BBS and GRP.

DISCUSSION

Results of the present investigation show that BBS and GRP, which are known to slow gastric emptying in some animal species (16, 17, 27), have the same effect in the cat. BBS is 5 times more potent than GRP on a molar basis. This effect is concomitant with a decrease of antral motility and is associated with a stimulation of gastric acid and pepsin secretion. As previously described (25,26), the decrease of antral motility consists of a decrease of high amplitude contractions in spite of an increase of low amplitude (<20 cm H^2O) contractions. These changes are quite similar to those observed—in the same experimental conditions—with pentagastrin (2) and cholecystokinin (2). This work also confirmed that there is a similar trend of the emptying of a liquid meal and the motility of the antropyloric region. These data are in agreement with previous results showing a relationship between emptying of liquids and antral motility (3, 7, 9, 19, 20, 28).

The most interesting finding obtained in the present experiments is represented by the dual effect of both BBS and GRP on gastric emptying, i.e., an inhibition of emptying rate followed by a return to baseline despite continuous peptide administration. The mechanism of this peculiar effect is difficult to explain. The

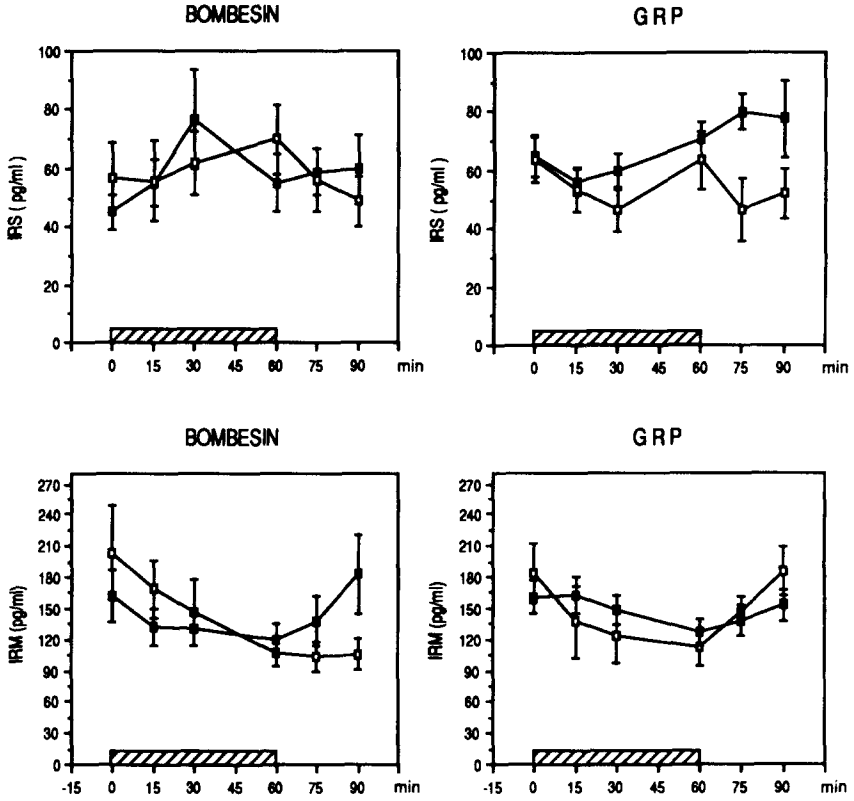

FIG. 7. Somatostatin (IRS) and motilin (IRM) during saline administration and in response to two doses of BBS and GRP. □ Bombesin: 0.37 nmole/kg·hr; GRP: 0.86 nmole/kg·hr; ■ bombesin: 0.74 nmole/kg·hr; GRP: 1.71 nmole/kg·hr.

concurrent behaviour of antral motility and gastric emptying strongly suggests that changes in emptying rates are consequent to motility changes. It is, indeed, well known that gastric emptying represents the net effect of the actions of a compound on different regions of the stomach (12).

Plasma hormone concentrations clearly indicate that IRG and PP levels reached a plateau which was maintained at all times (i.e., 5 and 60 min) of peptide administration. These data seem to exclude an interference of gastrin and PP in the inversion of BBS effect on emptying. This applies also to somatostatin, whose plasma levels were not significantly modified by the peptides.

Motilin is not a better candidate since plasma motilin (recognised in the cat only by antibodies directed against C-terminal motilin) (26) was decreased by peptide administration and motilin fall was even more pronounced at the end of peptide administration than in the early stage. However, a "paracrine" influence of the G.I. peptides released by both BBS and GRP cannot be discarded on the bases of our experiments.

Conversely from acid secretion, pepsin response to meal 60 min after either BBS or GRP infusion was significantly higher than that observed earlier (5 min), despite a reversing of the motor effects of both peptides (Figs. 3 and 5). Cholinergics are well known stimulants of pepsin secretion both in vitro (5) and in vivo (24), particularly in the cat. The additional increase in pepsin secretion observed after 60 min of peptide infusion could reflect an increased vagal drive, which might have counterbalanced the inhibitory effect of bombesin-like peptides on gastric motility.

The increased vagal activity is also reflected by the late increase in plasma concentrations of PP, under GRP administration, the release of which is dependent upon cholinergic pathway (18). On the other hand, bethanechol inhibits (21) and atropine increases (11) bombesin-induced gastrin release, thus suggesting an involvement of the cholinergic system in the action of the peptide.

Furthermore, the in vitro stimulatory action of bombesin on longitudinal smooth muscle from dog antrum seems to be mediated by acetylcholine release (15).

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