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Short communication

Distinct receptors mediate gastrin-releasing peptide and neuromedin B-induced delay of gastric emptying of liquids in rats

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Abstract

The present study was carried out to define which bombesin receptors are involved in the delay of gastric emptying induced by bombesin-like peptides. Adult male rats were fitted with gastric and jugular vein cannulas. Gastric emptying was determined 5 min after a 3-ml intragastric load of 0.9 M NaCl using phenol red as a marker. Mammalian bombesin-like peptides gastrin-releasing peptide-10 and neuromedin B both induced a delay of gastric emptying. When [Phe^6]bombesin-(6–13)-methyl ester, a selective antagonist of the gastrin-releasing peptide-preferring subtype of bombesin receptors, was injected 5 min before the agonists, the effect of gastrin-releasing peptide-10 was competitively inhibited, whereas that of neuromedin B remained unaffected. Our results indicate that gastrin-releasing peptide-10 and neuromedin B delay gastric emptying by acting on distinct receptors in rats, in vivo.

Keywords: Bombesin; Bombesin receptor subtype; Bombesin receptor antagonist; Gastric emptying

1. Introduction

Bombesin, an amphibian skin tetradecapeptide, has a wide range of biological effects (Erspamer and Melchiorri, 1980) that includes release of hormones, stimulation of pancreatic enzyme secretion, inhibition of gastric emptying, and modulation of gastric acid secretion. However, knowledge about the physiological role of bombesin-like peptides is limited. At present, three mammalian bombesin-like peptides have been identified: gastrin-releasing peptide, C-terminal decapeptide of gastrin-releasing peptide (also known as GRP-10 or neuromedin C), and neuromedin B. Gastrin-releasing peptide and gastrin-releasing peptide-10, like bombesin, have leucine in the penultimate position from their C-terminus, while neuromedin B, like ranatensin, another structurally related amphibian peptide, has phenylalanine as its penultimate residue (Jensen and Coy, 1991).

Recent studies led to the discovery of two different subtypes of bombesin receptors (Von Schrenck et al., 1990) as defined with various classes of bombesin receptor antagonists. One was described in pancreatic acinar cells and was called gastrin-releasing peptide-preferring bombesin receptor because of its higher affinity for gastrin-releasing peptide-10 than for neuromedin B. D-[Phe^6]Bombesin-(6–13)-methyl ester is a selective antagonist for gastrin-releasing peptide-preferring receptors (Coy et al., 1992; Jensen and Coy, 1991; Varga et al., 1991). With an affinity constant of 1.29 nM for its receptor (Varga et al., 1991), D-[Phe^6]bombesin-(6–13)-methyl ester is one of the most potent bombesin receptor antagonists known. The other bombesin receptor subtype was first identified in esophageal smooth muscle cells, and was named neuromedin B-preferring bombesin receptor because of its preferential affinity for neuromedin B (Von Schrenck

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et al., 1990). Most recently a new class of antagonists for the neuromedin B-preferring receptors has been characterized (Orbuch et al., 1993). Unfortunately, the relatively low potency of these antagonists makes them suitable for in vitro but not for in vivo experiments.

Bombesin-like immunoreactivity has been described in the entire gastrointestinal tract, and is localized mainly in fibers innervating both mucosa and muscle layers (Bunnett, 1994). Specific bombesin binding sites have been demonstrated in fibers innervating both circular and longitudinal muscle tissue (Moran et al., 1988). Moreover, bombesin, gastrin-releasing peptide and neuromedin B have been shown to stimulate contractions of various smooth muscle preparations from isolated gut (Bunnett, 1994). The potency of bombesin-like peptides depends on the origin of the smooth muscle. In some preparations bombesin, gastrin-releasing peptide and neuromedin B are equipotent to elicit contractions, while in others their potency is markedly different (Kortezova et al., 1994; Severi et al., 1991; Von Schrenck et al., 1990). These observations indicate that different subtypes of bombesin receptors may mediate the action of bombesin-like peptides.

In vivo, bombesin was shown to be a potent inhibitor of gastric emptying in rats (Scarpignato and Bertaccini, 1981). The physiological significance and the receptor background of this effect have not been clarified. In the present study we used selective bombesin receptor agonists, as well as an antagonist to determine the receptor subtype(s) mediating the effect of bombesin-like peptides on gastric emptying.

2. Materials and methods

2.1. Materials

Male, 250–300 g Sprague-Dawley rats were housed in individual cages at constant room temperature (22°C) and 12–12 h light cycle. The animals were fed standard rat chow ad libitum. The bombesin receptor antagonist D-[Phe^6]bombesin-(6–13)-methyl ester was synthesized according to methods described previously (Jensen and Coy, 1991). D-[Phe^6]Bombesin-(6–13)-methyl ester, synthetic gastrin-releasing peptide-10 and neuromedin B (Peninsula Laboratories, Belmont, CA, USA) were dissolved in 0.2% (w/v) bovine serum albumin (Sigma, St. Louis, MO, USA) in saline, and stored frozen at −70°C in multiple portions. Further dilutions from stock solutions were made for each experimental day using 0.2% bovine serum albumin in saline. The cholecystokinin (CCK) receptor antagonists, devazepide and L365,260 (Merck Sharp & Dohme, West Point, PA, USA), were dissolved in dimethyl sulfoxide/saline (3:1).

2.2. Surgical procedures and experiments

The rats were operated on under pentobarbital anesthesia (40 mg/kg intraperitoneally). A stainless steel gastric cannula was implanted in the forestomach and an indwelling catheter was inserted into the jugular vein and tunnelled to the neck under the skin. The experiments were started after at least one week's recovery, during which the animals were allowed to adapt to restraint for some hours twice a week in Bollman-type cages.

Before experiments, the animals were fasted overnight and placed in Bollman-type cages. After opening of the gastric cannula, the stomach was rinsed with warmed saline and the experiments were started after at least 30 min. NaCl 0.9% solution labelled with phenol red (0.6 g/l) was used as non-caloric liquid test meal; 3 ml of pre-warmed (37°C) test meal was slowly (20 s) instilled into the stomach via a plastic catheter passed through a rubber plug fixed to the gastric cannula. The cannula was opened 5 min later by removing the plug and the remaining gastric contents were collected by gravity in graduated tubes. The stomach was then rinsed with 3 ml saline and the washing solution was added to the recovered gastric content. The phenol red concentration in the mixture was then measured spectrophotometrically at 560 nm by adding 0.1 N NaOH and the total amount of marker recovered from the stomach was calculated.

Bombesin receptor agonists, gastrin-releasing peptide-10 and neuromedin B were injected in different doses intravenously 5 min before instillation of the meal. Antagonists were injected 5 min before the agonists.

2.3. Evaluation of data

All data are presented as means ± S.E.M. ($n = 6$–12). Dose-response curves for both gastrin-releasing peptide-10 and neuromedin B, in the presence or absence of antagonists, were fitted to a sigmoid curve by using a non-linear regression program providing an estimate of log ID_{50} and maximal response values. One-way analysis of variance (ANOVA) was used to evaluate statistical significance. Differences are considered significant when $P < 0.05$. Computation of the data and statistical analyses were done with the INPLOT and INSTAT statistical program packages (GRAPHPAD, San Diego, CA, USA).

3. Results

Under control conditions, 5 min after loading, 2.49 ± 0.11 ml saline emptied from the stomach of the rats. Gastrin-releasing peptide-10 dose dependently delayed

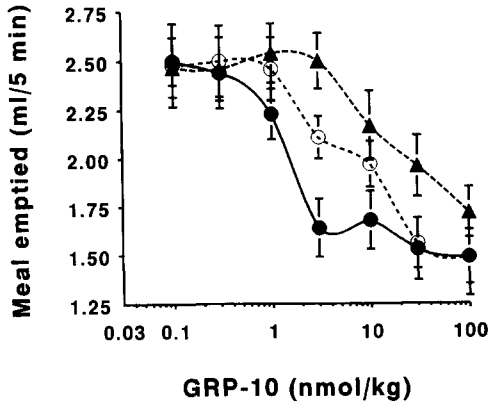

Fig. 1. Effect of D-[Phe^6]bombesin-(6–13)-methyl ester on gastrin-releasing peptide (GRP-10) induced delay of gastric emptying of a saline load in unanesthetized rats. Values are means ± S.E.M.; n = 8–12. D-[Phe^6]Bombesin-(6–13)-methyl ester dose (in nmol/kg): 0 (•), 10 (○), 100 (▲).

gastric emptying of this non-caloric liquid meal (Fig. 1), with an ID_{50} of 1.36 nmol/kg (log $ID_{50} = 0.13 \pm 0.07$). The gastrin-releasing peptide-10-induced delay in gastric emptying was reduced by D-[Phe^6]bombesin-(6–13)-methyl ester in a dose-dependent fashion (Fig. 1). Sigmoid curve fitting was performed to calculate log ID_{50} and maximal response for each curve. The log ID_{50} values in response to gastrin-releasing peptide-10 were significantly higher when 10 or 100 nmol/kg D-[Phe^6]bombesin-(6–13)-methyl ester was applied concomitantly (0.91 ± 0.17 and 1.24 ± 0.14, respectively; $P < 0.01$ in both cases). The maximal response to gastrin-releasing peptide-10 was not affected by application of D-[Phe^6]bombesin-(6–13)-methyl ester (values for 0, 10 and 100 nmol D-[Phe^6]bombesin-(6–13)-methyl ester-treated groups were 1.56 ± 0.05, 1.40 ± 0.14, and 1.64 ± 0.12 ml, respectively), thus suggesting competitive antagonism.

Increasing doses of neuromedin B also delayed gastric emptying in a dose-related manner (Fig. 2) with an

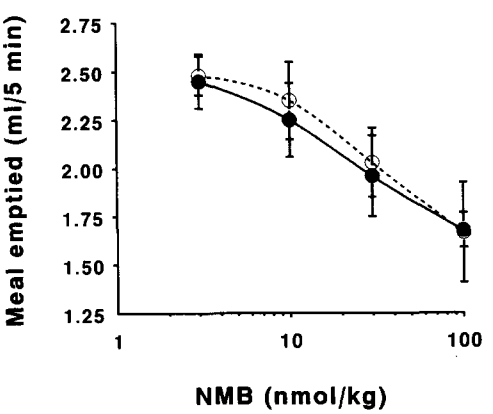

Fig. 2. Effect of D-[Phe^6]bombesin-(6–13)-methyl ester on neuromedin B (NMB) induced delay of gastric emptying of a saline load in unanesthetized rats. Values are means ± S.E.M.; n = 6–10. D-[Phe^6]Bombesin-(6–13)-methyl ester dose (in nmol/kg): 0 (•), 100 (○).

ID_{50} of 23.9 nmol/kg and maximal response at 1.55 ± 0.07 ml fluid. The log ID_{50} value (1.37 ± 0.07) was significantly higher than that of gastrin-releasing peptide-10 ($P < 0.01$). D-[Phe^6]Bombesin-(6–13)-methyl ester (100 nmol/kg) did not affect these actions of neuromedin B (log $ID_{50} = 1.49 \pm 0.04$; maximal response = 1.54 ± 0.04 ml).

In order to check whether the action of gastrin-releasing peptide-10 is mediated by CCK-like peptides, the selective CCK_A receptor antagonist, devazepide (1 mg/kg), and the CCK_B/gastrin receptor antagonist, L365,260 (3 mg/kg), were used to block their receptors. No significant change in the gastric motor effect of gastrin-releasing peptide-10 was observed when these antagonists were applied (data not shown).

4. Discussion

In the present study an attempt was made to characterize the activity of two bombesin-like peptides, gastrin-releasing peptide-10 and neuromedin B, on gastric emptying in rats. It was found that the delay of gastric emptying induced by gastrin-releasing peptide and neuromedin B was mediated via different bombesin receptors.

The bombesin receptor agonists, gastrin-releasing peptide-10 and neuromedin B, both inhibited the emptying of non-caloric liquid from the stomach. There was, however, a significant difference in the potency of these peptides, with the potency of gastrin-releasing peptide-10 much higher than that of neuromedin B. Different smooth muscle preparations do not uniformly respond to bombesin-like peptides. Bombesin, gastrin-releasing peptide and neuromedin B were shown to be equipotent to elicit contractions of some preparations, while their potency is markedly different with others (Kortezova et al., 1994; Severi et al., 1991; Von Schrenck et al., 1990).

Difference in the order of potency of agonists is only one of the criteria required when distinguishing receptor subtypes. The most reliable benchmark is the blockade of receptor subtypes by a selective competitive antagonist. In the present study, D-[Phe^6]bombesin-(6–13)-methyl ester, an antagonist known to selectively block gastrin-releasing peptide-preferring bombesin receptors (Jensen and Coy, 1991; Varga et al., 1991), behaved as a competitive receptor antagonist against gastrin-releasing peptide-10 but not against neuromedin B. This finding suggests that bombesin receptors located on gastric smooth muscle are heterogeneous in nature, and that they represent not only the gastrin-releasing peptide-preferring subtype of bombesin receptors, but also another subtype. Our results are in line with the previous observations of Severi et al. (1991) who demonstrated in vitro that gastric smooth

muscle cells possess two different subtypes of bombesin receptors in guinea pigs (Severi et al., 1991). To provide direct evidence that the effect of neuromedin B on emptying is actually a consequence of an interaction with neuromedin B-preferring bombesin receptors will depend on the availability of a potent, selective antagonist for this type of receptor. That work is in progress (Orbuch et al., 1993).

Bombesin-like peptides have been shown to release numerous hormones including CCK and gastrin (Bunnett, 1994), an effect found to be mediated by gastrin-releasing peptide-preferring receptors (Varga et al., 1994). Therefore it was of interest to check whether the in vivo motor activity of gastrin-releasing peptide is mediated through the release of these peptides. The intravenous injection of devazepide, a CCK_A receptor antagonist, at a dose (1 mg/kg) previously found capable of counterbalancing the maximal stimulation of pancreatic enzyme secretion (O'Rourke et al., 1990) and delay of gastric emptying (Scarpignato et al., 1993) induced by exogenous or endogenous CCK, was unable to reverse the effect of GRP-10 on gastric emptying. Similarly, L365,260, a selective antagonist for CCK_B/gastrin receptors, at a dose (3 mg/kg) we (Varga et al., 1993) previously found to be able to completely inhibit the gastrin-17-I-induced maximal stimulation of acid secretion, was unable to modify the gastrin-releasing peptide-induced delay in emptying. These observations indicate that CCK-like peptides are not involved in the gastrin-releasing peptide-induced delay of gastric emptying in rats. In summary and conclusion, the results of the present investigation showed that gastrin-releasing peptide delays gastric emptying through activation of specific gastrin-releasing peptide receptors without the involvement of CCK or gastrin release.

Acknowledgements

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