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Characterization of bombesin binding sites in the rat stomach

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Abstract

We characterized the bombesin receptor population in the rat stomach and determined the receptor subtype mediating the contractile effect of bombesin in the gastric fundus. Using in vitro receptor autoradiography, we evaluated the ability of the specific gastrin-releasing peptide-preferring receptor antagonist [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester to inhibit binding of ^{125}I-[Tyr^4]bombesin to the gastric fundus, corpus and antrum. Binding to these regions was completely inhibited by [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester suggesting that these receptors are the gastrin-releasing peptide-preferring subtype. We found that the rank order of potency for the contractile effect of bombesin, and the related mammalian peptides neuromedin C and neuromedin B, was bombesin > neuromedin C > neuromedin B. [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester was equipotent in antagonizing contractions produced by all three peptides. Furthermore, receptor tachyphylaxis to either neuromedin C or neuromedin B abolished the subsequent contractile response elicited by neuromedin C and neuromedin B, suggesting that one bombesin receptor subtype mediates rat gastric fundal contractions. Together, these results demonstrate that the bombesin receptor subtype in the rat stomach is gastrin-releasing peptide-preferring subtype and that this subtype is responsible for the effects of bombesin-like peptides on fundal smooth muscle contraction.

Keywords: Gastrin-releasing peptide; Neuromedin B; Smooth muscle contraction

1. Introduction

Bombesin-like peptides encompass a large family of structurally related mammalian and amphibian peptides. At present, there are three mammalian bombesin-like peptides that have been identified. These are gastrin-releasing peptide (McDonald et al., 1979) and its decapeptide fragment neuromedin C (GRP18-27) (Minamino et al., 1984) which both share the identical heptapeptide amino acid sequence with that of bombesin, and neuromedin B (Minamino et al., 1983) which differs from these by one amino acid substitution. These structural differences have led to the classification of bombesin-like peptides into subfamilies and subsequently to the characterization of two distinct bombesin receptor subtypes (Von Schrenck et al., 1989). The 'gastrin-releasing peptide-preferring' receptor subtype was first described in pancreatic acinar cells and is characterized by having a high affinity for gastrin-releasing peptide, neuromedin C and bombesin and a low affinity for neuromedin B. The 'neuromedin B-preferring' receptor subtype was characterized in esophageal muscularis mucosa and has a high affinity for neuromedin B and bombesin and a low affinity for gastrin-releasing peptide and neuromedin C. Antagonists with different affinities for these receptor subtypes have been developed (Jensen and Coy, 1991) and used to differentiate receptor subtypes in the central nervous system and determine the subtype responsible for mediating some of bombesin's behavioral and pharmacological effects (Flynn, 1992; Ladenheim et al., 1993; Varga et al., 1991, 1995; Von Schrenck et al., 1990).

Bombesin-like immunoreactivity (Costa et al., 1984, Namba et al., 1985) and high affinity binding sites for bombesin (Moran et al., 1987) have been identified throughout the mammalian gastrointestinal tract where

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bombesin-like peptides elicit potent pharmacological actions including contraction of smooth muscle, release of several gastrointestinal peptides and stimulation of pancreatic enzyme secretion (Erspamer et al., 1988; Jensen et al., 1988; Kaneto et al., 1978).

Earlier work using in vitro smooth muscle preparations described differences in the potency of various bombesin-like peptides to induce contractions depending upon the segment of the gastrointestinal tract being tested (Erspamer et al., 1988; Rouissi et al., 1991). In some preparations, the contractile response to bombesin, neuromedin B and gastrin-releasing peptide was equivalent, whereas in others bombesin and gastrin-releasing peptide were much more potent than neuromedin B. More recent work has shown that incubation of colonic smooth muscle cells with gastrin-releasing peptide and neuromedin B receptor antisense oligonucleotides eliminated the contractile response produced by bombesin, neuromedin B and gastrin-releasing peptide (Bitar and Zhu, 1993). Also, Varga et al. (1995) recently reported that the delay in gastric emptying produced by neuromedin C and neuromedin B was mediated by distinct bombesin receptor subtypes. Together, these findings demonstrate that multiple receptor subtypes are likely to be present in the gastrointestinal tract and these receptor subtypes differentially mediate the various actions of bombesin-like peptides.

Despite a variety of functional results supporting the existence of multiple bombesin receptor subtypes in the mammalian gastrointestinal tract, there have been no studies characterizing binding to these subtypes or examining their relative distribution in various gastrointestinal segments or tissue layers. The purpose of this study was to characterize the bombesin receptor subtype(s) found in the rat stomach, the gastrointestinal region possessing the highest density of bombesin binding sites. We used in vitro receptor autoradiography to examine the competitive inhibition of binding of ^{125}I-[Tyr^4]bombesin by the specific gastrin-releasing peptide-preferring receptor antagonist, [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester. This antagonist can distinguish between the two receptor subtypes because of its high affinity for the gastrin-releasing peptide-preferring receptor subtype and low affinity for the neuromedin B-preferring subtype (Coy et al., 1992; Ladenheim et al., 1993). We also characterized the receptor subtype(s) mediating the contractile response produced by bombesin, neuromedin C and neuromedin B in the rat gastric fundus using an in vitro tissue bath preparation.

2. Materials and methods

2.1. Receptor autoradiography

Adult male Sprague-Dawley rats were used for all experiments. Animals were lightly anesthetized with ether and killed by decapitation. Gastrointestinal segments from the esophagus, gastric fundus, corpus and antrum were removed, rinsed and rapidly frozen in isopentane (2-methylbutane) which had been cooled on dry ice. Tissue segments were cut on a cryostat at −20°C at a thickness of 20 μm and thaw-mounted onto gelatin-coated slides. They were then stored in a freezer at −70°C for later use.

For receptor binding studies, tissue sections were preincubated at room temperature in 50 mM 2-[*N*-morpholino]ethane-sulfonic acid buffer containing 0.5% bovine serum albumin at pH 6.5 for 20 min. They were then incubated at room temperature for 300 min in 50 mM 2-[*N*-morpholino]ethane-sulfonic acid buffer containing 0.5% bovine serum albumin, 130 mM NaCl, 7.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.025% bacitracin, 4 mg/ml leupeptin, 2 mg/ml chymostatin and 40 pM ^{125}I-[Tyr^4]bombesin in the presence or absence of unlabeled 100 nM [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester. This concentration was chosen based on results from previous studies showing it to be optimal for inhibiting binding to gastrin-releasing peptide-preferring receptors while not affecting binding to neuromedin B-preferring receptors (Ladenheim et al., 1993). Following incubation, tissue sections were thoroughly rinsed (4 × 5 min) in ice cold 50 mM 2-[*N*-morpholino]ethane-sulfonic acid buffer containing 0.5% bovine serum albumin, dried under a stream of warm air and placed overnight in a dessicator under partial vacuum pressure. The slides were placed in an X-ray cassette with a commercially prepared ^{125}I standard (Amersham, Arlington Heights, IL, USA) and apposed to Hyperfilm-^{3}H (Amersham) for 5 days.

After completion of the autoradiographic procedure, the sections were stained with toluidine blue to allow for histological comparison with the autoradiographs. Localization of binding sites was accomplished by superimposing the autoradiographic image onto the stained tissue section and viewing it under light microscopy. Binding density was evaluated on the autoradiographs with a computerized microdensitometry system (Ip Lab System Analytics) and compared to a standard curve generated from the radioactive ^{125}I standards. Binding density was obtained from 3 different sections of each gastric segment from 5 animals. The esophageal segment was evaluated as a control for the specificity of the antagonist because it contains only neuromedin B-preferring bombesin receptors (Von Schrenck et al., 1989) and thus bombesin binding to the esophagus should not be inhibited by the antagonist. Differentiation of gastrin-releasing peptide-preferring and neuromedin B-preferring receptors was determined by calculating the relative ability of the antagonist to inhibit bombesin binding. The percentage of total binding that was inhibited by [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester was ascribed to binding to GRP preferring sites whereas bombesin binding that was unaffected was attributed to neuromedin B preferring sites. This method of differentiation has been used in both the brain and in cultured cells expressing one or both receptor subtypes (Coy et al., 1992; Ladenheim et al., 1993).

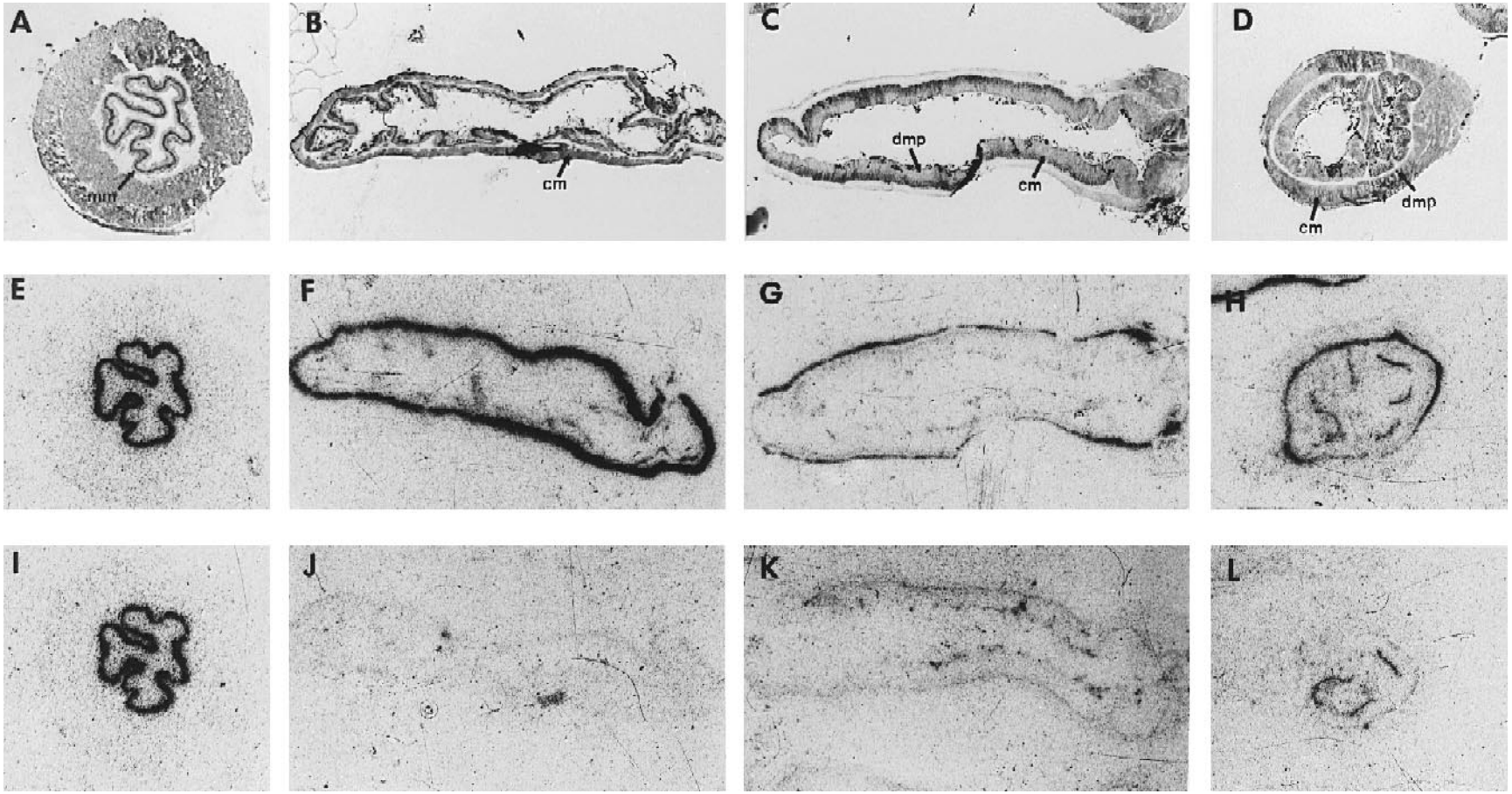


Fig. 1. Stained tissue sections of the esophagus (A), fundus (B), corpus (C) and antrum (D) and corresponding autoradiographs showing total binding of ^{125}I-[Tyr^4]bombesin (E,F,G,H) and binding in the presence of 100 nM [D-F$_5$,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester (I,J,K,L). Binding in the esophagus (E) which contains only neuromedin B-preferring receptors was not inhibited by the addition of the specific gastrin-releasing peptide-preferring receptor antagonist [D-F$_5$,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester (I). In contrast, binding in the fundus (F), corpus (G) and antrum (H) was completely inhibited by [D-F$_5$,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester (I,J,K). Abbreviations: mm, muscularis mucosa; cm, circular muscle; dmp, deep muscular plexus.

2.2. Contraction studies

2.2.1. Antagonist experiment

The gastric fundus was excised and cut longitudinally into strips. It was immediately immersed in Tyrode buffer consisting of 140 mM NaCl, 5 mM KCl. 1 mM $MgSO_4$, 0.7 mM NaH_2PO_4, 12 mM $NaHCO_3$, 1.8 mM $CaCl_2$ and 0.1% glucose at pH 6.5 and rinsed of its luminal contents. Tissue samples were connected to an isometric force transducer at a baseline tension of 1.0 g and placed in an organ bath containing Tyrode buffer at 37°C. The tissue was allowed to equilibrate for 20 min before testing with peptides.

For our initial experiment, we measured the tension after the addition of a range of concentrations of bombesin (0.1 nM to 2.0 μM), neuromedin C (0.32 nM to 2.0 μM) and neuromedin B (0.32 nM to 2.0 μM) (peptides obtained from Bachem, Torrance, CA, USA). This was performed in order to determine the EC_{50} concentration used for the subsequent experiment with the gastrin-releasing peptide receptor antagonist.

The approximate EC_{50} concentrations of bombesin (3.2 nM), neuromedin C (32 nM) and neuromedin B (320 nM) were then used to examine the ability of a dose range of the specific gastrin-releasing peptide preferring receptor antagonist [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester to block the response stimulated by bombesin, neuromedin C and neuromedin B.

2.2.2. Tachyphylaxis experiment

In a second series of experiments also aimed at determining the receptor subtype(s) mediating the contractile actions of bombesin-like peptides in the gastric fundus, we examined the effect of tachyphylaxis to one bombesin-like peptide on the ability of the other to produce a contractile response. The logic of this experiment was that if both receptor subtypes were involved in producing the contraction, then production of tachphylaxis at one receptor subtype should not affect the contractile response elicited by the other. Whereas, if only one receptor subtype was involved, tachyphlaxis to one agonist should prevent a response to the other. This method has been successfully employed in previous studies to determine the presence of multiple bombesin receptor subtypes in guinea pig gallbladder smooth muscle (Parkman et al., 1994).

The tension produced by separate application of neuromedin C (32 nM) and neuromedin B (320 nM) was used as a control. A supramaximal dose of either neuromedin C (3.2 μM) or neuromedin B (10 μM) was given to produce receptor desensitization (tachyphylaxis). This was followed by a repeat application of the control dose of neuromedin C and neuromedin B. At the end of the experiment we administered a dose of 1 μM carbachol to determine whether a contractile response could still be achieved with a non-bombesin related agent.

3. Results

3.1. Receptor autoradiography

The results from the binding experiment are shown in Fig. 1 and Fig. 2. A high density of binding of ^{125}I-[Tyr^4]bombesin was observed in the muscularis mucosa of the esophagus (Fig. 1E). These binding sites have been characterized in previous studies as neuromedin B-preferring. Consistent with this designation, the specific gastrin-releasing peptide receptor antagonist [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester did not inhibit binding of ^{125}I-[Tyr^4]bombesin in this region (Fig. 1I and Fig. 2).

In gastric tissue, the gastric fundus exhibited the highest density of bombesin binding sites which were localized to the circular muscle layer Fig. 1F. In contrast to the binding pattern observed in the esophagus, binding in this region was completely inhibited by [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester (Fig. 1J and Fig. 2).

Bombesin binding sites were also present in the circular muscle layer of the gastric corpus (Fig. 1G) and antrum (Fig. 1H), primarily localized to the deep muscular plexus. As in the fundus, ^{125}I-[Tyr^4]bombesin binding in the corpus and antrum was completely inhibited by 100 nM [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester (Fig. 1K,L and Fig. 2).

3.2. Contraction studies

As shown in Fig. 3 bombesin, neuromedin C and neuromedin B produced dose-related increases in baseline tension. The rank order of potency was bombesin >

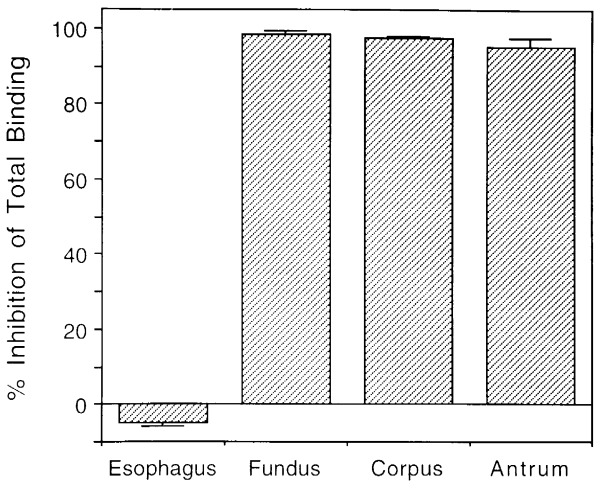


Fig. 2. Percentage inhibition of total binding of ^{125}I-[Tyr^4]bombesin in the presence of unlabeled 100 nM [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester. [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester completely inhibited binding in the gastric fundus, corpus and antrum, whereas binding in the esophagus was not inhibited.

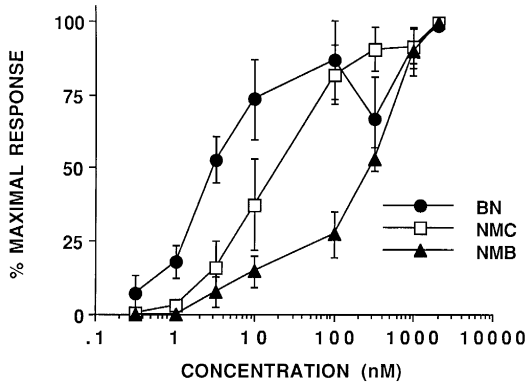


Fig. 3. Dose response curves showing the contractile response of the rat gastric fundus to increasing concentrations of bombesin, neuromedin C and neuromedin B. Changes in baseline tension are expressed as the percentage of maximal tissue response for each peptide. Data are expressed as means ± S.E.M. from 4–5 trials.

neuromedin C > neuromedin B. Bombesin was 10 times more potent than neuromedin C and 100 times more potent than neuromedin B, producing half-maximal increases in tension at 3.2 nM compared to 32 nM for neuromedin C and 320 nM for neuromedin B.

The ability of [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester to block the contractile response produced by 3.2 nM bombesin, 32 nM neuromedin C and 320 nM neuromedin B is shown in Fig. 4. [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester was equally effective in completely antagonizing the increase in tension produced by bombesin, neuromedin C and neuromedin B. A complete blockade by the antagonist occurred for all peptides at a concentration of 1 μM with half maximal inhibition at 10 nM.

Fig. 5 shows the effect of receptor tachyphylaxis produced by 3.2 μM neuromedin C (A) or 10 μM neuromedin B (B) on the subsequent contractile response elicited by 32 nM neuromedin C and 320 nM neuromedin B. Whether tachyphylaxis was produced by a supramaximal dose of neuromedin C or by neuromedin B, the subsequent contraction produced by the addition of either 32 nM neuromedin C or 320 nM neuromedin B was abolished. In both cases, carbachol responses remained intact (data not shown).

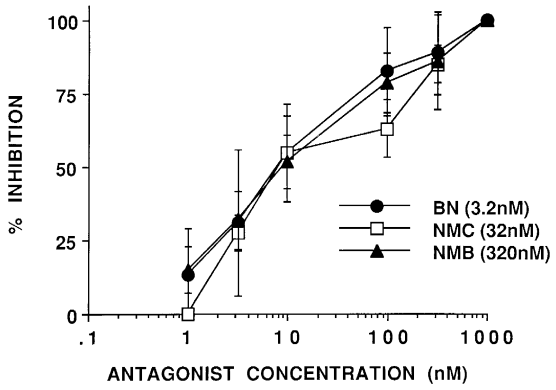
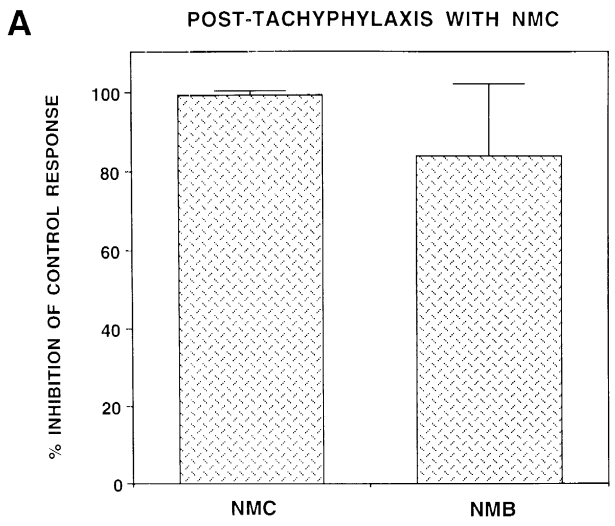


Fig. 4. Ability of increasing concentrations of the [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester to block the contractile reponse produced by 3.2 nM bombesin, 32 nM neuromedin C and 320 nM neuromedin B. Data are expressed as means ± S.E.M. from 3–5 trials.



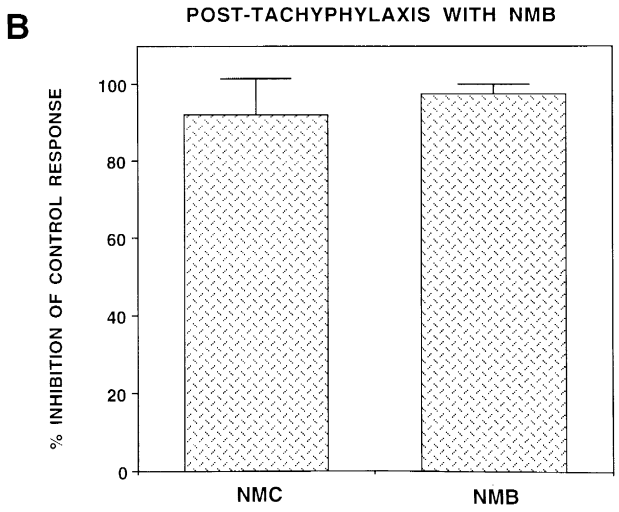


Fig. 5. Effect of tachyphlaxis produced by 3.2 μM neuromedin C (A) or 10 μM neuromedin B (B) on subsequent contractile response to 32 nM or 320 μM neuromedin B. Post-tachyphylaxis data represent the percentage inhibition of the contractile response elicited by neuromedin C and neuromedin B (control response). Data are shown as means ± S.E.M. from 4 trials.

4. Discussion

The present study was designed to characterize the gastric bombesin receptor population in the rat and determine which receptor subtype mediates the contractile response produced by bombesin-like peptides in the rat gastric fundus.

The localization and density of bombesin binding sites in the rat esophagus, gastric fundus, corpus and antrum corresponded with results reported previously (Moran et al., 1987). In the esophagus, high densities of bombesin binding sites were evident in the muscularis mucosa. In gastric regions, bombesin binding sites were localized to the circular muscle layer with the highest density in the gastric fundus. Our finding that 100 nM [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester completely inhibited binding of ^{125}I-[Tyr^4]bombesin to the fundus, corpus and antrum, but not to the esophagus, demonstrates that gastric bombesin receptors in the rat are of the gastrin-releasing peptide-preferring subtype whereas esophageal bombesin receptors are the neuromedin B-preferring subtype. Receptor binding studies have demonstrated that, at this concentration, [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester is a highly selective antagonist for the gastrin-releasing peptide-preferring subtype over the neuromedin B-preferring subtype (Coy et al., 1992; Ladenheim et al., 1993).

As shown in studies elsewhere, bombesin, neuromedin C and neuromedin B dose dependently stimulated smooth muscle contraction in the rat gastric fundus (Girard et al., 1984; Rouissi et al., 1991). Although neuromedin B produced a contractile response, its potency was greatly reduced compared to bombesin and neuromedin C. The relative lack of potency of neuromedin B to stimulate fundal contractions compared to bombesin and neuromedin C is in agreement with previous reports (Rouissi et al., 1991). These results are consistent with our characterization of bombesin receptors in the rat gastric fundus and suggest that neuromedin B is acting at gastrin-releasing peptide-preferring receptors to elicit its contractile response. This conclusion is further supported by studies showing that at well-characterized gastrin-releasing peptide-preferring receptor populations in the rat pancreas, neuromedin B had 25-fold less potency than bombesin in stimulating pancreatic amylase secretion.

Additional evidence for this conclusion was provided by the study examining the ability of [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester to antagonize the contractile response produced by bombesin, neuromedin C and neuromedin B. We found that [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester was equipotent in antagonizing the contractile response produced by all three peptides. It was recently suggested in a study by Varga et al. (1995) that the rat gastrointestinal tract possesses a heterogenous population of bombesin receptors. This conclusion was based on the finding that [D-F_5,Phe^6,D-Ala^{11}]bombesin-(6–13) methyl ester blocked the delay in gastric emptying produced by neuromedin C but failed to block the suppression of emptying produced by neuromedin B. While these data are supportive of a role for both bombesin receptor subtypes mediating the effects of bombesin-like peptides on gastric emptying, the results from the present study do not support the interpretation that it reflects an interaction with both receptor subtypes on gastric smooth muscle.

The finding that neuromedin C or neuromedin B tachyphylaxis abolished the subsequent contractile response to neuromedin C or neuromedin B further suggests that only one bombesin receptor subtype mediates smooth muscle contractions in the rat gastric fundus. These data, in combination with our previous results, support the notion that this receptor subtype is gastrin-releasing peptide-preferring. If both bombesin receptor subtypes mediated fundal contractions, we would have expected to see a profile similar to that reported by Parkman et al. (1994) in the guinea pig gall bladder. They found that tachyphylaxis produced by bombesin eliminated the subsequent contractile response produced by bombesin, gastrin-releasing peptide and neuromedin C but only partially blocked the response to neuromedin B. Conversely, neuromedin B-induced tachyphylaxis greatly inhibited the response to neuromedin B but only partially antagonized the response to bombesin, neuromedin C, neuromedin B. This profile is consistent with the peptides acting at two different bombesin receptor subtypes.

In summary, the results from this study show that rat gastric bombesin receptors are gastrin-releasing peptide-preferring and this receptor subtype is responsible for mediating the contractile response elicited by bombesin-like peptides in the rat gastric fundus.

Acknowledgements

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References

Bitar, K.N. and X.-X. Zhu, 1993, Expression of bombesin-receptor subtypes and their differential regulation of colonic smooth muscle contraction, Gastroenterology 105, 1672.

Costa, M., J.B. Furness, N. Yanihara and T.W. Moody, 1984, Distribution of projections of neurons with immunoreactivity for both gastrin-releasing peptide and bombesin in the guinea pig small intestine, Cell Tissue Res. 235, 285.

Coy, D.H., Z. Mungan, W.J. Rossowski, B.L. Cheng, J.-T. Lin, J.E. Mrozinski, Jr. and R.T. Jensen, 1992, Development of a potent bombesin receptor antagonist with prolonged in vivo inhibitory activity on bombesin-stimulated amylase and protein release in the rat, Peptides 13, 775.

Erspamer, G.F., C. Severini, B. Erspamer, P. Melchiorri, G. Delle Fave and T. Nakajima, 1988, Parallel bioassay of 27 bombesin-like peptides on 9 smooth muscle preparations. Structure-activity relationships and bombesin receptor subtypes, Regul. Pept. 21, 1.

Flynn, F.W., 1992, Fourth ventricular injection of selective bombesin receptor antagonists facilitates feeding in rats, Am. J. Physiol. 264, R218.

Girard, F., H. Bachelard, S. St-Pierre and F. Rioux, 1984, The contractile effect of bombesin, gastrin releasing peptide and various fragments in the rat stomach strip, Eur. J. Pharmacol. 102, 489.

Jensen, R.T. and D.H. Coy, 1991, Progress in the development of potent bombesin receptor antagonists, Trends Pharmacol. Sci. 12, 13.

Jensen, R.T., D.H. Coy, Z.A. Saeed, P. Heinz-Erian, S. Mantey and J.D.

Gardner, 1988, Interaction of bombesin and related peptides with receptors on pancreatic acinar cells, Ann. NY Acad. Sci. 547, 138.

Kaneto, A., T. Kaneto, S. Nakaya, H. Kajinuma and K. Kosaka, 1978, Effect of bombesin infused intrapancreatically on glucagon and insulin secretion, Metabolism 27, 549.

Ladenheim, E.E., R.T. Jensen, S.A. Mantey, J.E. Taylor, D.H. Coy and T.H. Moran, 1993, Bombesin receptor antagonists differentiate receptor subtypes in rat brain, Eur. J. Pharmacol. 235, 121.

McDonald, T.J., H. Jornvall, G. Nilsson, M. Bagne, M. Ghatei, S.R. Bloom and B. Mutt, 1979, Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue, Biochem. Biophys. Res. Commun. 90, 227-233.

Minamino, N., K. Kangawa and H. Matsuo, 1983, Neuromedin B: a novel bombesin-like peptide identified in porcine spinal cord, Biochem. Biophys. Res. Commun. 114, 541.

Minamino, N., K. Kangawa and M. Matsuo, 1984, Neuromedin C: a bombesin-like peptide identified in porcine spinal cord, Biochem. Biophys. Res. Commun. 119, 14.

Moran, T.H., T.W. Moody, A.M. Hostetler, P.H. Robinson, M. Goldrich and P.R. McHugh, 1987, Distribution of bombesin binding sites in the rat gastrointestinal tract, Peptides 9, 643.

Namba, M., M.A. Ghatei, A.E. Bishop, S.J. Gibson, D.J. Mann, J.M. Polak and S.R. Bloom, 1985, Presence of neuromedin B-like immunoreactivity in the brain and gut of rat and guinea-pig, Peptides 6, 257.

Parkman, H.P., M.A. Vozzelli, A.P. Pagano and A. Cowan, 1994, Pharmacological analysis of receptors for bombesin-related peptides on guinea pig gallbladder smooth muscle, Regul. Pept. 52, 173.

Rouissi, N., N.-E. Rhaleb, F. Nantel, S. Dion, G. Drapeau and D. Regoli, 1991, Characterization of bombesin receptors in peripheral contractile organs, Br. J. Pharmacol. 103, 1141.

Varga, G., R.D. Reidelberger, R.-M. Liehr, L.J. Bussjaeger, D.H. Coy and T.E. Solomon, 1991, Effects of potent bombesin antagonists on exocrine pancreatic secretion in rats, Peptides 12, 493.

Varga, G., R.-M. Liehr, C. Scarpignato and D.H. Coy, 1995, Distinct receptors mediate gastrin-releasing peptide and neuromedin B-induced delay of gastric emptying of liquids in rats, Eur. J. Pharmacol. 286, 109.

Von Schrenck, T., P. Heinz-Erian, T.H. Moran, S.A. Mantey, J.D. Gardner, R.T. Jensen, 1989, Neuromedin B receptor in esophagus: evidence for subtypes of bombesin receptors, Am. J. Physiol. 256, G747.

Von Schrenck, T., L.H. Wang, D.H. Coy, M.L Villaneuva, S. Mantey and R.T. Jensen, 1990, Potent bombesin receptor antagonists distinguish receptor subtypes, Am. J. Physiol. 259, G468.