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Receptor Subtype Mediation of Feeding Suppression by Bombesin-Like Peptides

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LADENHEIM, E. E., K. E. WIRTH AND T. H. MORAN. *Receptor subtype mediation of feeding suppression by bombesin-like peptides.* PHARMACOL BIOCHEM BEHAV **54**(4) 705–711, 1996.—Bombesin (BN) and the related mammalian peptides gastrin-releasing peptide (GRP), neuromedin C (NMC), and neuromedin B (NMB) suppress food intake in rats. Recent studies show two distinct receptor subtypes, GRP-preferring and NMB-preferring. BN interacts equally with both subtypes raising the possibility that one or both subtypes mediate the reduction of feeding by BN. To examine this issue, we compared suppression of intake produced by dose ranges (0–100 nmol/kg) of BN, GRP, NMC, and NMB and acetylated NMC and NMB. We found that all peptides elicited dose-dependent reductions of intake with overall differences in potency and efficacy. At intermediate doses, the rank order of potency for suppression was BN = AcNMC > NMC = GRP> NMB = AcNMB; however BN, GRP, and NMC were equipotent at the lowest and highest doses. Coadministration of NMC or GRP and NMB produced suppressions above that of either peptide alone and equivalent to BN. Taken together, these data support a role for both receptor subtypes in the suppression of food intake by BN and BN-like peptides.

Bombesin Gastrin-releasing peptide Neuromedin C Neuromedin B Feeding

BOMBESIN is an amphibian-derived peptide that is biologically active in mammals (1). Since its discovery, several structurally related peptides have been isolated and characterized from mammalian tissue. These peptides include gastrin-releasing peptide (GRP) (16), its carboxyl terminal decapeptide, GRP18-27 or neuromedin C (NMC) (18), and neuromedin B (NMB) (17).

The large family of BN-like peptides has been divided into subfamilies based on their carboxyl terminal amino acid sequences and pharmacological activity (5,19). BN and the mammalian peptides GRP and NMC belong to the same subfamily characterized by the amino acid Leu in the penultimate position from the carboxyl terminal. The other mammalian representative of this family, NMB, belongs to the litorin/ranatensin subfamily differing from those in the BN subfamily by the substitution of a Phe in place of Leu at position 2 from the carboxyl terminal.

The structural differences between members of this family determine their biological activity and receptor interaction. Von Schrenck et al. (23) have shown that the actions of BN are mediated by two distinct subtypes of BN receptors. One subtype characterized in pancreatic acinar cells, has a high affinity for BN and GRP and a 20-fold lower affinity for NMB. This subtype has been termed GRP-preferring. The other BN receptor subtype, characterized in esophageal muscularis mucosa, has a high affinity for BN and NMB but a 100-fold lower affinity for GRP and NMC. This subtype is referred to as NMB-preferring. In each case, biological activity as measured by pancreatic amylase secretion and esophageal muscle contraction, complemented the results obtained in binding studies. These two receptor subtypes have subsequently been cloned (2,20,24) and found to be differentially distributed throughout the rat central nervous system (14,24).

BN-like peptides have diverse behavioral and physiological effects when administered to mammals (3,25). Among these actions is the inhibition of food intake after both central and peripheral administration (8,9,13). Because BN binds with equal and high affinity to both GRP- and NMB-preferring receptors (23), it is possible that BN's interaction with one or both receptor subtypes may mediate the suppression of food intake by BN.

Previous studies have documented the feeding inhibitory effects of a variety of mammalian and amphibian BN-related peptides (4,6,7–10,12,21). Thus GRP, NMC, NMB, ranatensin, and litorin have all been demonstrated to inhibit food intake following peripheral administration in a variety of experimen-

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tal paradigms. However, no systematic comparisons have been performed examining the potencies and efficacies of all mammalian BN-like peptides under the same experimental conditions.

The objective of the present study was to provide such a systematic characterization to identify the receptor subtype(s) responsible for the suppression of food intake by peripherally administered BN-like peptides.

GENERAL EXPERIMENTAL METHODS

Adult male Sprague–Dawley rats (Charles River, Kingston, NY), weighing between 225–250 g at the start of testing, were used for all experiments. Rats were individually housed in wire mesh cages in a temperature-controlled room with a 12 L:12 D cycle. Rat chow pellets and tap water were provided ad lib unless otherwise indicated. All experiments were conducted in the light phase of the light:dark cycle in the rats home cage.

Prior to the initiation of behavioral testing, rats were trained to consume a glucose solution (0.5 kcal/ml) during a 60 min period. Rats were food deprived for 5 h before access to the glucose solution. Five training sessions were conducted to establish a stable baseline.

Each peptide or 0.9% saline (control injection) was administered intraperitoneally 5 min prior to the presentation of a 0.5 kcal/ml glucose solution and glucose intake was monitored at 15- and 30-min time points. Percent suppression of glucose intake was calculated by comparing the amount of glucose consumed after each peptide dose with that consumed following the saline injection. Statistical analyses were performed using between-within ANOVA with peptide as the between subjects factor and dose and time as the repeated factors. Individual dose comparisons were made using planned two-tailed t comparisons.

EXPERIMENT 1

The purpose of Experiment 1 was to systematically compare the suppression of intake produced by peripherally administered BN with that of the related mammalian peptides GRP, the biologically active portion of GRP, NMC, and NMB.

Four groups consisting of six rats per group were the subjects of this experiment. Each animal in each group received the full range of doses for one peptide (BN, GRP, NMC, and NMB; Bachem, CA). Peptide doses of 0, 0.32, 1.0, 3.2, 10.0, 32, and 100 nmol/kg were administered in randomized order. At least 48 h separated the administration of any two peptide doses. All peptides were dissolved in a vehicle of 0.9% saline. As described above, 5 min after peptide administration, rats were given access to 0.5 kcal/ml glucose and intakes were recorded at 15 and 30 min time points.

Results

The relative abilities of BN, GRP, NMC, and NMB to suppress glucose intake are shown in Fig. 1. The overall analysis indicated a significant effect of group (i.e., peptide), $F(3, 20) = 4.2$, $p < 0.02$, and dose, $F(5, 100) = 11.5$, $p < 0.0001$.

All peptides dose dependently reduced glucose intake when compared to intake following saline administration ($p < 0.05$). The minimum effective dose tested that elicited a significant suppression of intake for BN, GRP, and NMB was 1 nmol/kg. A significant suppression of intake was not observed with NMB until the 10 nmol/kg dose ($p < 0.01$).

Planned comparisons revealed differences in the magnitude of suppression between BN and the mammalian peptides

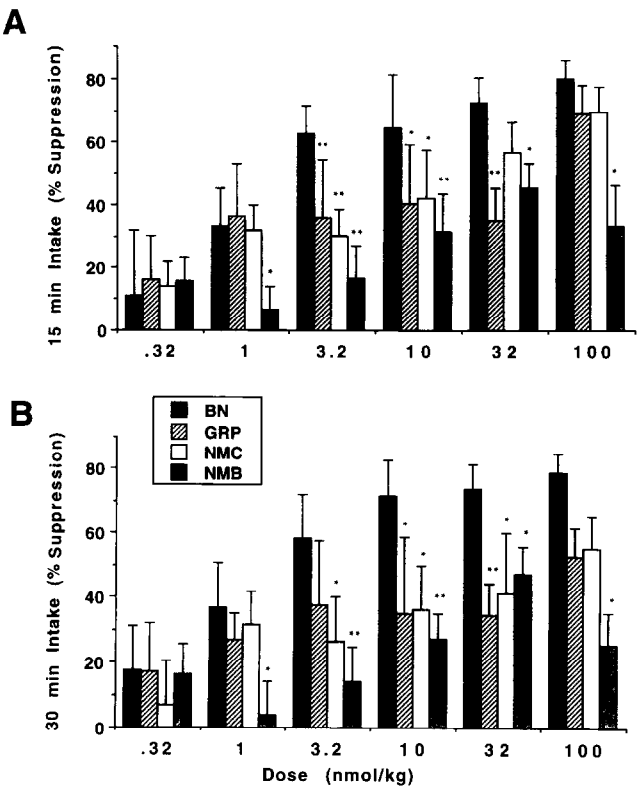

FIG. 1. Percent suppression of glucose intake produced by various doses of IP BN, GRP, NMC, and NMB. Data shown are for 15 min (A) and 30 min (B) after glucose presentation and are expressed as means ± SEM. Percent suppression = (intake after control injection − intake after peptide) × 100/intake after control injection. Asterisks indicate those points that are significantly different from BN (*$p < 0.05$, **$p < 0.01$).

at various doses. At 1 nmol/kg there were no differences in the magnitude of suppression between BN, GRP, and NMC. However, at the 3.2 and 10 nmol/kg doses BN administration resulted in significantly greater suppression than either GRP or NMC (Fig. 1). At 32 nmol/kg, GRP resulted in less suppression than BN, while no differences were found between BN and NMC at this dose level. At the highest dose tested (100 nmol/kg) there were no differences in the magnitude of suppression among BN, GRP, and NMC at the 15-min time point (Fig. 1A). However, by 30 min the magnitude of suppression produced by GRP and NMC began to decrease such that BN suppressed intake to a greater extent than either of the mammalian counterparts at this dose (Fig. 1B).

Suppression of glucose intake by NMB was consistently less than BN at all doses and at both time points. When compared to NMC and GRP, significant differences in suppression produced by NMB were observed only at the 1 nmol/kg and 100 nmol/kg dosages ($p < 0.05$).

The results of this experiment demonstrate that BN, GRP, NMC, and NMB all suppressed glucose intake in a dose-dependent manner. However, the potency with which the peptides reduced food intake and their overall efficacy were not equivalent. The rank order of potency for suppression of intake was BN > GRP = NMC> NMB in that at the intermediate doses, BN was more potent than either GRP and NMC. The efficacy for suppression of glucose intake by BN, GRP,

and NMC was greater than for NMB. A difference in the duration of action was also observed between BN and the mammalian peptides GRP, NMC, and NMB. While suppression of intake by BN was maintained at the 30-min time point, the efficacy of GRP, NMC, and NMB began to decline at this later time point. This time-related decline in efficacy may indicate that overall differences in potency and efficacy could be due to differences in bioavailability, possibly related to more rapid enzymatic degradation of the mammalian BN-like peptides.

EXPERIMENT 2

In this experiment, we tested the possibility that BN's greater potency and efficacy in the previous experiment may have been the result of increased bioavailability due to resistance to enzymatic degradation. Therefore, we examined the ability of the acetylated forms of NMC and NMB to suppress intake after intraperitoneal administration. The acetylation of peptides provides protection from degradation thus increasing their bioavailability.

Rats from the NMC group (n = 6) in Experiment 1 were tested with acetylated NMC (Ac-NMC), while those in the NMB group (n = 6) were tested with acetylated NMB (Ac-NMB). The experiment was conducted in the same manner as described in Experiment 1. Rats in the NMC group received intraperitoneal injections of either 0.9% saline or Ac-NMC at dosages of 0.32, 1, 3.2, 10, and 32 nmol/kg. Animals in the NMB group received intraperitoneal injections of 0.9% saline or 0.32, 1, 3.2, 10, 32, and 100 nmol/kg Ac-NMB. Glucose was presented 5 min later and 15 and 30 min intakes recorded. Statistical analyses were conducted using repeated measures ANOVA to compare the effects of the nonacetylated peptides to their acetylated counterpart. Between and within ANOVAs were used to compare the effect of the acetylated peptide with that of BN. Planned t comparisons (two tailed) were used to compare individual means.

Results

Peripheral administration of Ac-NMC produced a dose-dependent decrease in glucose intake ($p < 0.02$, Fig. 2). A comparsion of the magnitude of suppression of intake produced by nonacetylated NMC with that produced by Ac-NMC revealed a significantly greater suppression of intake by Ac-NMC at the 30-min time point with the 3.2 nmol/kg dose ($p < 0.05$, Fig. 2B).

A comparison between the effects of Ac-NMC and BN revealed no significant differences in suppression of glucose intake at any of the doses at either time point. Thus, while NMC was less potent than BN at 15 min with the 3.2 and 10 nmol/kg doses, Ac-NMC was not. At the 30-min time point, when suppression by NMC began to decline, no significant differences were observed between Ac-NMC and BN at 3.2, 10, and 32 nmol/kg ($p > 0.80$).

Figure 3 shows the suppression of glucose intake by Ac-NMB relative to that produced by NMB and BN. As with NMB, Ac-NMB produced a dose related suppression of intake at both the 15- and 30-min time points. In contrast to the results with NMC, acetylation of NMB did not alter its potency or efficacy when compared to NMB ($p > 0.15$).

These results demonstrate that at the 30-min time point the acetylated form of NMC provided a greater suppression of intake with the 3.2 nmol/kg dose than nonacetylated NMC, suggesting that acetylation of NMC increased its bioavailability such that its ability to suppress intake was comparable to that of BN. In contrast to Ac-NMC, the acetylation of NMB failed to enhance its activity either in terms of potency or duration of action. This lack of effect of acetylation may be attributable to the decreased NMB receptor affinity of this compound (Table 1).

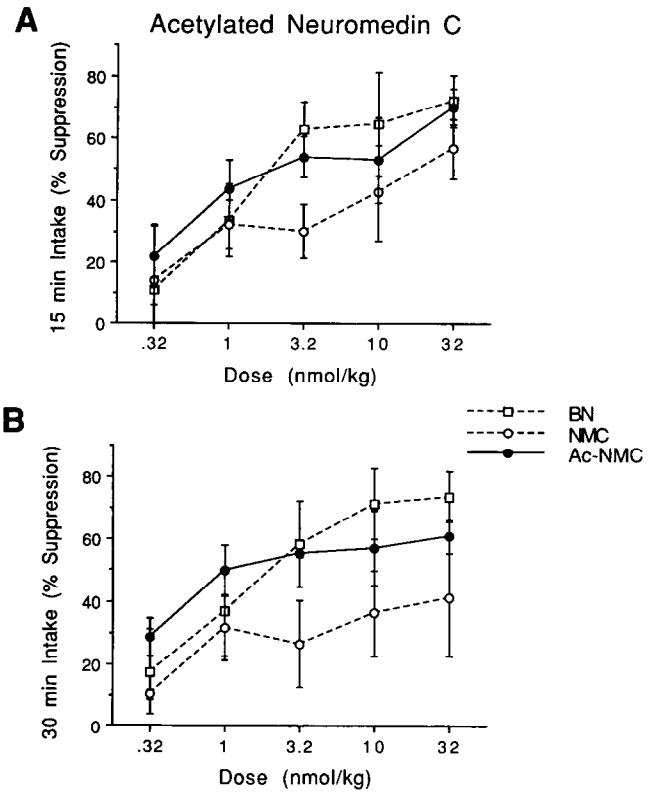

FIG. 2. Percent suppression of 15 min (A) and 30 min (B) glucose intake after IP administration of acetylated NMC (Ac-NMC). The dashed lines represent the values depicted in Fig. 1 (Experiment 1) from the suppression produced by BN and nonacetylated NMC. Data are expressed as means ± SEM. The suppression of intake produced by Ac-NMC did not differ significantly from BN at either time point ($p > 0.80$).

EXPERIMENT 3

Two approaches were used to test the possibility that the suppression of food intake produced by BN results from an additive effect produced by BN's interaction with both GRP and NMB preferring receptors. The dose–response curves with GRP and NMC in Experiment 1, indicating a plateau at the middle doses, are consistent with the overall feeding suppression resulting from two distinct components. At low doses, the mammalian analogs inhibited intake to the same degree as BN. BN's dose–response curve continued to increase at the 3.2 and 10 nmol/kg while the dose effect curves for NMC and GRP did not. At the 32 and 100 nmol/kg doses the NMC and GRP-induced suppressions again increased. BN, with equal affinity for both GRP- and NMB-preferring receptors, may inhibit intake through a combined activation of both receptor subtypes. NMC and GRP have higher affinity for GRP receptors and activation of these receptors may account for the initial suppression at low doses. GRP and NMC have roughly

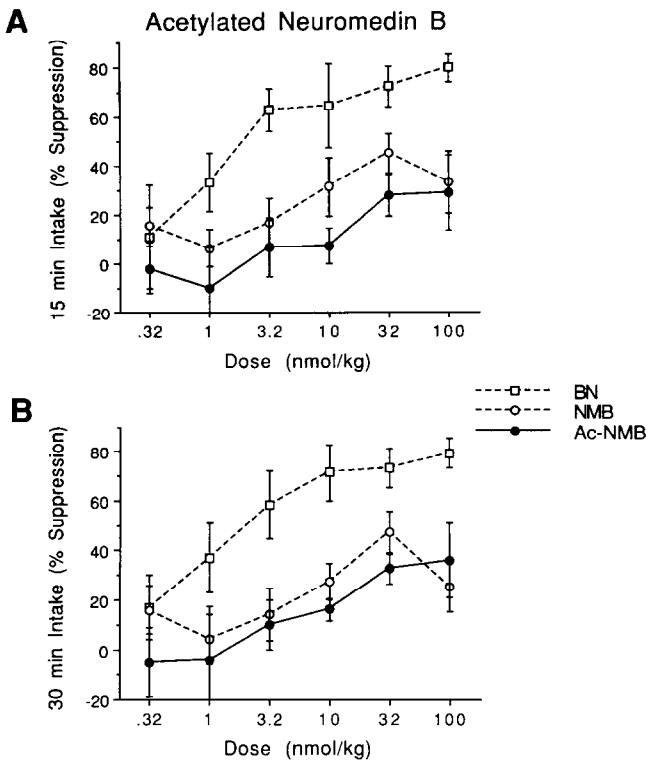

FIG. 3. Percent suppression of 15 min (A) and 30 min (B) glucose intake after IP administration of acetylated NMB (Ac-NMB). The dashed lines are the values from Fig. 1 (Experiment 1) showing the suppression produced by BN and nonacetylated NMB. Suppression of glucose intake by Ac-NMB was significantly different from BN ($p < 0.05$) but not significantly different from nonacetylated NMB ($p > 015$). Data are shown as means ± SEM.

a fivefold lower affinity for NMB than for GRP preferring sites and may only interact with these sites to produce additional suppression at the higher doses. To assess this possibility we compared the suppression produced by BN with the suppression produced by combined doses of NMC and NMB and GRP and NMB.

Rats from the GRP group ($n = 6$) in Experiment 1 served as the subjects for the examination of the effect of combined doses of NMC and NMB. Rats received either 10 nmol or 32 nmol/kg each of NMC and NMB. Separate injections of the two peptides were given with NMB always administered immediately prior to NMC. Five minutes later glucose was presented and intake was monitored at 15- and 30-min time points as previously described.

In an additional experiment, the combined effects of NMB and GRP were assessed. A naive group of rats ($n = 6$) were exposed to daily glucose access as described above. In these rats, the effects of a dose range of NMB on glucose intake were assessed to determine the dose that produced a maximal suppression of intake. Rats were administered 0.9% saline or 10, 32, 100, and 178 nmol/kg NMB intraperitoneally 5 min prior to glucose presentation. While in Experiment 1, a maximum suppression was obtained at a dose of 32 nmol/kg, in these rats, a maximum suppression was obtained at 100 nmol/kg. The reason for this discrepancy is unknown but could be attributed to individual rat or peptide batch differences. The 100 nmole/kg dose was then combined with a submaximal dose of GRP (3.2 nmol/kg) in a 2 × 2 design. That is, rats received a) saline + saline, b) saline + 3.2 nmol/kg GRP, c) 100 nmol/kg NMB + saline, and d) 100 nmol/kg NMB + 3.2 nmol/kg GRP. The injections were given sequentially 5 min prior to glucose access.

Results

As demonstrated in Fig. 4, coadministration of 10 nmol/kg NMB and NMC resulted in a suppression of 15-min glucose intake that was not significantly different from BN ($p > 0.20$). Because the suppression of intake by the combined dosages of 10 nmol/kg NMB and NMC began to decline while suppression by BN remained constant significant differences were observed between the combined dosages and BN at the 30 min time point (Fig. 4B, $p < 0.01$).

Although coadministration of 32 nmol/kg NMC and NMB elicited a significant increase in suppression above that of NMB alone ($p < 0.05$), there was no significant difference

TABLE 1

ABILITY OF THE PEPTIDES USED IN THIS STUDY TO INHIBIT BINDING OF ^{125}I-(Tyr4)BN TO PANCREATIC TISSUE SECTIONS AND ACINI (GRP-PREFERRING) AND TO ESOPHAGUS AND GLIOBLASTOMA C-6 CELLS (NMB-PREFERRING) (23,26).

	^{125}I-(Tyr4)Bombesin (Ki, nM)			
	GRP-Preferring		NMB-Preferring	
	Pancreas			
Peptide	Tissue Sections	Acini	Esophagus	C-6 Cells
Bombesin	5 ± 2	4 ± 1	2.2 ± 0.3	21 ± 6
GRP	7 ± 1	17 ± 5	30 ± 4	403 ± 60
Neuromedin C	18 ± 14	20 ± 12	36 ± 3	209 ± 17
Neuromedin B	156 ± 58	248 ± 5	0.3 ± 0.03	3.3 ± 1
Ac-NMC	–	8.6 ± 1.5	–	44 ± 9
Ac-NMB	–	135 ± 7	–	41 ± 4

Values represent the means ± SD from 3 experiments. K_i's were calculated for each peptide based on their ability to inhibit binding of ^{125}I-(Tyr^4)BN to rat pancreatic tissue and acini or rat esophagus or glioblastoma C-6 cells.

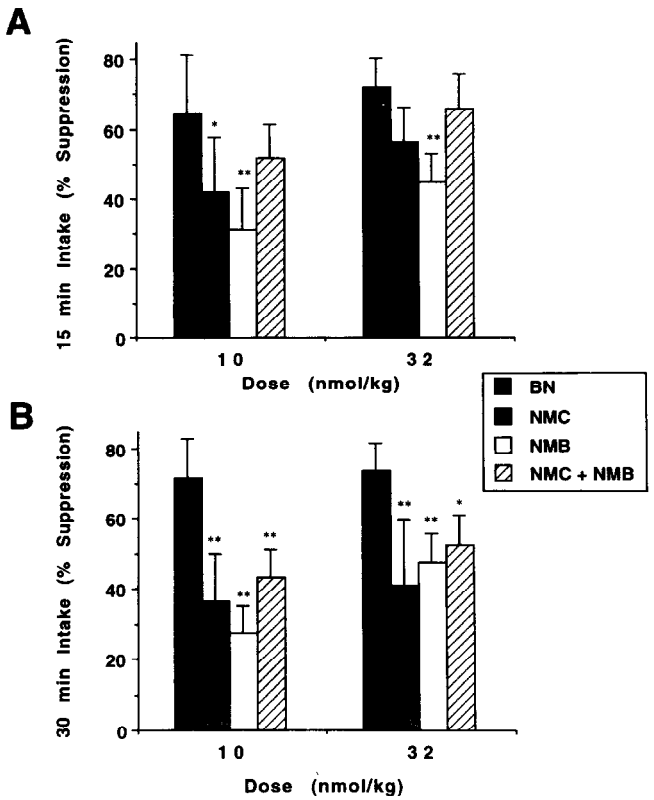

FIG. 4. Percent suppression of glucose intake following combined dosages of 10 and 32 nmol/kg each NMC and NMB. Data shown are at 15 min (A) and 30 min (B) after glucose presentation. For comparison, the values from Fig. 1 (Experiment 1) are included to show the effects of 10 and 32 nmol/kg BN, NMC, and NMB when given alone. Coadministration of NMC and NMB produced suppression that was not significantly different from BN ($p > 0.10$). Data are shown as means ± SEM. Asterisks indicate significant differences compared to BN (*$p < .05$), **$p < 0.01$).

from suppression produced by NMC alone ($p > 0.10$), nor did it result in an additional increase above that elicited by 32 nmol/kg of BN alone ($p > 0.10$).

As shown in Fig. 5, when a maximal dose of NMB (100 nmol/kg) was given in combination with a submaximal dose of GRP (3.2 nmol/kg) the suppression of intake was enhanced such that the combination of peptides elicited suppression that was significantly greater than either of the peptides administered alone ($p < 0.01$).

The results from these experiments demonstrate that coadministration of NMC and NMB can increase suppression of intake to a magnitude equivalent to, but not beyond, that produced by BN alone. The finding that the combined effects of NMC and NMB elicited an increase in suppression beyond that produced by either peptide alone suggests that both receptor subtypes can be stimulated simultaneously to suppress feeding and thus supports the idea that BN's action to suppress food intake results from its interaction with both receptor subtypes.

Similarly, when a submaximal dose of GRP was combined with a maximal dose of NMB, the suppression of intake was greater than when either peptide was given alone. Because this dose of NMB produced a maximal suppression of intake,

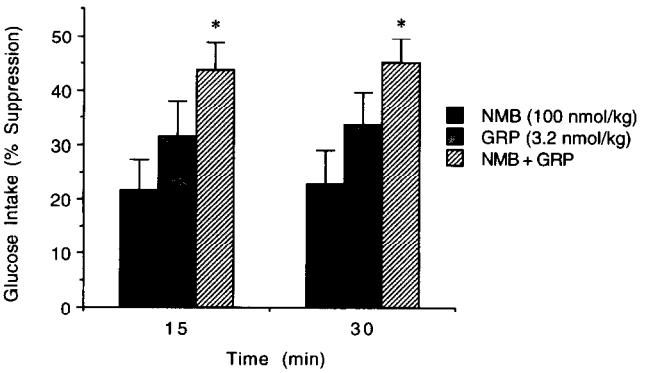

FIG. 5. Percent suppression of glucose intake at 15 and 30 min following administration of 100 nmol/kg NMB alone, 3.2 nmol/kg NMC alone, and the combination of these dosages of NMB and NMC. Data are represented as means ± SEM. The percent suppression of glucose intake produced by the combined dosages of GRP and NMB was significantly different from either peptide alone (*$p < 0.05$).

any increase in suppression produced by the addition of GRP must be due to GRP's interaction with GRP-preferring receptors. This result provides additional evidence that suppression of intake by BN-like peptides may be mediated by two distinct subtypes of BN receptors.

GENERAL DISCUSSION

The purpose of these experiments was to examine the suppression of food intake produced by BN-like peptides and to characterize the receptor subtypes(s) responsible for mediating this effect. The results from the comparative study in which we examined suppression of intake produced by BN and the mammalian peptides GRP, NMC, and NMB demonstrated that all peptides dose dependently reduced intake. However, the potency and efficacy for suppressing intake differed between peptides at various doses.

Previous studies in which BN's ability to suppress food intake was compared individually to that of GRP or NMC have concluded that BN is more potent than either mammalian peptide in suppressing food intake. A preliminary report by DiPaola et al. (4) demonstrated that NMC produced a dose-dependent reduction in food intake that was 40% less potent than BN. In a study comparing GRP and BN, Stein and Woods (21) showed that GRP was approximately 30% less potent than BN and that the nature of the dose–response curves for the two peptides suggested that BN and GRP were acting to suppress food intake through a common mechanism. In both studies the range of doses used (NMC = 1.8 to 14.3 nmol/kg, GRP = 0.7 to 5.6 nmol/kg) fall within our low range of doses where we saw the greatest differences in suppression between the mammalian peptides and BN. In that dose range, our results support the interpretation that BN is more potent than either GRP or NMC. However, by examining a wider range of doses we have demonstrated that differences in suppression between BN, GRP, and NMC are entirely dose dependent.

The characteristics of the dose–response curves for GRP and NMC suggest two components to their feeding effects that may be attributed to their interaction with GRP-preferring sites at low doses and with both GRP and NMB-preferring sites at the highest dose. The similarity in the shapes of the dose–effect curves for GRP and NMC was expected because NMC is the fragment of GRP that possesses full biological

activity. In both structure–activity analyses and binding studies, GRP and NMC have been shown to exhibit similar characteristics (23).

The result that NMB was consistently less potent than BN in suppressing intake is in agreement with previous studies examining the effects of both NMB and the structurally related amphibian peptide ranatensin on food intake (7,8,10). Both the shape of the dose–effect curve for NMB and the failure to induce suppression of a magnitude equivalent to BN and the other mammalian peptides suggest that, at these dosages, NMB is acting only at NMB-preferring receptors. This finding is not surprising based on the relative affinities of the peptides for each receptor subtype. In receptor binding studies it was demonstrated that the K_i for GRP to inhibit binding of ^{125}I-(Tyr^4)BN to rat esophagus (NMB-preferring) was 30 ± 4 nM, while for rat pancreas (GRP-preferring) it was 7 ± 1 nM [(23), see Table 1]. Thus, GRP has approximately fivefold greater affinity for GRP-preferring receptors over NMB-preferring receptors. The relative affinities of NMC for the two receptor subtypes were very similar to those of GRP (23). By comparison, the K_i for inhibition of ^{125}I-(Tyr^4)BN by NMB was 0.3 ± 0.03 nM in esophageal tissue and 156 ± 58 nM in pancreatic tissue [(23), see Table 1]. Thus, NMB has approximately 500-fold greater affinity for NMB-preferring receptors over GRP-preferring receptors. The differences in relative affinities for each receptor subtype make it more likely that GRP and NMC will interact with NMB-preferring receptors than that NMB will act at GRP-preferring receptors.

The finding that acetylation of NMC increased the magnitude of suppression to that of BN suggests that increased bioavailability may contribute, in part, to BN's increased potency compared to NMC and GRP. Previous studies examining the potency for suppression of glucose intake by AcGRP(20–27) compared with BN have found that, in contrast to GRP, AcGRP(20–27) possesses equivalent potency to that of BN with IC_{50} values of 3.24 ± 0.14 and 3.48 ± 0.95 nmol, respectively (10). These results suggest that differences in the magnitude of suppression of food intake produced by NMC and GRP when compared to BN may depend upon two factors, that of dose and bioavailability. However, because acetylation of NMC increased its affinity for both GRP and NMB receptors (Table 1), it is not clear whether suppression of food intake was enhanced because of decreased peptide degradation, thus increasing the amount of peptide available, or whether increased bioavailability provided the opportunity for the peptide to interact with both receptor subtypes. Because the acetylation of NMB reduced its receptor affinity (Table 1), we do not know if improved bioavailability would enhance its ability to suppress feeding .

Further evidence for the involvement of both receptor subtypes in BN's feeding effects was provided by the experiments examining additivity of the mammalian peptides. In a study by Stratford et al. (22) it was reported that coadministration of equimolar dosages of GRP and NMB dose dependently reduced food intake to the same degree as BN. In contrast to our results, they did not observe a significant supppression of intake with NMB at any dose tested. However, the result that combined dosages of GRP and NMB produced an increase in suppression equivalent to that of BN is in agreement with the present study. Although this finding is suggestive that BN's effects on food intake are mediated by an interaction with both GRP- and NMB-preferring receptors, this type of experiment in isolation cannot completely address that interpretation. It is possible that additivity could result from a combined action of both peptides at one receptor subtype and, thus, would represent a response equivalent to administering a larger dose of the same peptide. To rule out this possibility, we did the additional experiment of examining whether suppression of intake by a maximal dose of NMB could be bolstered by adding a submaximal dose of GRP. The finding that suppression of intake could be increased beyond that produced by NMB demonstrates that the actions of both receptor subtypes can be combined to increase the level of suppression and lends support to the involvement of both receptor subtypes in BN's feeding inhibitory effects.

We have previously reported that prior administration of an antagonist specific for NMB-preferring receptors blocked the suppression of food intake produced by NMB but had no effect on suppression produced by NMC (14). The results from the present study are consistent with this finding and support the interpretation that NMB can act independently to suppress food intake by its interaction with NMB-preferring receptors and not by acting with lower affinity at GRP-preferring receptors.

In a report by Kirkham et al. (11), it was demonstrated that a specific GRP-preferring receptor antagonist was effective in reversing the feeding suppression produced by peripherally administered BN. However, this study was not designed to evaluate the role of BN receptor subtypes in feeding suppression produced by BN-like peptides. The recent availability of antagonists with a high degree of specificity for each BN receptor subtype will enable us to compare the potencies of these antagonists to block suppression of food intake by BN and the related mammalian peptides. This type of analysis, in combination with results from agonist experiments, will be neccessary to determine the contribution of each receptor subtype to the feeding inhibitory effects of BN-like peptides.

Taken together, the results of this study support the hypothesis that the suppression of feeding by BN is due to its interaction with both BN receptor subtypes. Further studies will be needed to define the nature of this interaction and the relationship of BN receptor subtypes to the physiological and behavioral controls of food intake.

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