Leptin enhances feeding suppression and neural activation produced by systemically administered bombesin

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Leptin enhances feeding suppression and neural activation produced by systemically administered bombesin. Am J Physiol Regul Integr Comp Physiol 289: R473–R477, 2005. First published April 28, 2005; doi:10.1152/ajpregu.00835.2004.—Leptin amplifies feeding inhibition and neural activation produced by either cholecystokinin or intragastric preloads, suggesting that leptin may increase the efficacy of gastrointestinal meal-related signals. To determine whether leptin would similarly potentiate the feeding inhibitory actions of another putative satiety peptide, we evaluated the effects of third ventricular leptin administration on food intake and c-Fos activation in response to systemically administered bombesin (BN). Leptin (3.5 μg) was administered 1 h before either 0.9% saline or BN (0.32 and 1.0 nmol/kg) followed by 30-min access to Ensure liquid diet. Although neither leptin nor 0.32 nmol/kg BN alone suppressed Ensure intake, the combination reduced intake by 28%. The higher BN dose (1.0 nmol/kg) produced a significant suppression by itself but was further enhanced in the presence of leptin. Consistent with the behavioral results, c-Fos activation in the nucleus of the solitary tract was increased by combined dosages of leptin and 0.32 nmol/kg BN beyond the individual response to either peptide. In the presence of leptin, BN produced a 3.4- to 5.2-fold increase in the number of c-Fos-positive cells in the nucleus of the solitary tract compared with when BN was given alone. These data provide further support for the hypothesis that the effect of leptin on food intake may be mediated, in part, by modulating meal-related satiety signals.

Gut peptides that are secreted in response to nutrient ingestion have been implicated in the control of food intake by eliciting signals that limit the size of individual meals and result in meal termination (11, 36). Other signals, such as leptin, are secreted in direct proportion to adiposity and convey long-term information related to body energy stores (22). Although these controls were long thought to be distinct, there is increasing evidence to support the idea that the overall regulation of food intake and body weight results from integration of meal-related satiety signals and long-term energy stores. Several laboratories have demonstrated that exogenous administration of leptin selectively decreases meal size without affecting meal frequency or intermeal interval (7, 9, 13). This pattern is similar to that produced by meal-related satiety signals, suggesting that leptin may exert its effects on food intake through a mechanism that alters the efficacy of within-meal satiety signals (8, 12, 27).

A number of studies have demonstrated a synergistic interaction between leptin and the meal-related satiety peptide cholecystokinin (CCK) in food intake and body weight (1, 8, 23, 24, 27). We (8) previously reported that feeding suppression by peripheral CCK administration was significantly increased by central leptin administration and that the combined treatment resulted in activation of brain stem neurons that were not responsive to either peptide individually. A complementary study by McMinn et al. (27) demonstrated that leptin deficiency generated by fasting decreased the ability of CCK to reduce food intake but that its satiety effect was restored by leptin replacement. Together, these results have lent further support to the overall hypothesis that leptin may participate in controlling food intake by either enhancing, or dampening, the responsiveness of neurons to meal-generated satiety signals.

Bombesin (BN) is an amphibian-derived peptide that is closely related to the mammalian peptide gastrin-releasing peptide (GRP) (25, 33). Like CCK, BN has been proposed as a meal-contingent satiety signal (10). BN-like immunoreactivity is found in abundance in the gastrointestinal tract and central nervous system of mammals (5, 29, 33, 34) and is increased in the stomach and several brain regions in response to food ingestion (14, 28, 32). Systemic administration of BN-related peptides inhibits food intake in a dose-related fashion, under a variety of experimental conditions and in a number of mammalian species, including humans (11, 21, 30). Exogenous administration of BN, like CCK, decreases food intake by reducing meal size and, at low doses, does not prolong the intermeal interval (35). Furthermore, mice with a targeted deletion of the receptor for the mammalian BN-like peptide, GRP, exhibit an increase in meal size (16). Because BN’s effects on feeding share many characteristics with those of CCK, we hypothesized that leptin may also influence food intake through an interaction with BN-like peptides. Therefore, in the present study, we addressed whether centrally administered leptin would alter the ability of peripherally administered BN to decrease food intake in a short-term feeding paradigm. In addition, we evaluated the effect of these treatments on neuronal activation in the nucleus of the solitary tract (NTS) and area postrema (AP), a brain region that is critically important to BN’s feeding effects and exhibits increased c-Fos expression following intraperitoneal BN administration (4, 17, 18, 20).

METHODS

Subjects. All experiments used male Sprague-Dawley rats (Charles River, Kingston, NY) weighing 250–300 g at the start of experimentation. They were individually housed in hanging wire mesh cages in a temperature-controlled room (22–23°C) on a 12:12-h light-dark
cycle. Unless otherwise specified, pelleted rat chow and tap water were available ad libitum. All behavioral testing was performed in the rat’s home cage in the light portion of the light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University.

Surgery. Rats were anesthetized with a mixture of ketamine (150 mg/kg)-xylazine (30 mg/kg) given intramuscularly and mounted into a stereotaxic apparatus with the skull level. Stainless steel 23-gauge guide cannulas aimed at the third cerebral ventricle were implanted at a 10° angle at a position 1 mm lateral to midline 1 mm posterior to bregma, and 5.5 mm ventral to dura. The cannulas were anchored to the skull using three stainless steel screws and acrylic dental cement. Cannulas were blocked with a 30-gauge stainless steel obturator when not in use.

Behavioral verification of cannula placement. Approximately 1 wk after surgery, cannula placement was verified by measuring water consumption in response to third ventricular infusion of ANG II (10 ng in 3 μl; Sigma, St. Louis, MO). Only animals that drank ≥5 ml in the 30 min after ANG II administration were included to have viable cannula placements and used in the subsequent experiment (n = 32).

Feeding paradigm. Rats were then adapted to a feeding schedule in which they had rat chow removed each day at 9:00 AM and returned at 4:00 PM. At 3:00 PM, they were presented with a nutritionally complete liquid diet (Ensure, Ross Products, Columbus, OH), and intake was measured for 30 min. Feeding experiments commenced when Ensure intake had stabilized.

To examine the effects of leptin on BN-induced suppression of food intake, rats received a third ventricular injection of either human recombinant leptin (3.5 μg in 3.5 μl; PeproTech, Rocky Hill, NJ) or its vehicle (0.9% saline) via a Gilmont microliter syringe attached to PE-10 tubing and a 30-gauge stainless steel injector that extended 1.5 mm below the cannula tip. One hour later, rats were injected intraperitoneally with either 0.9% saline or BN (0.32 nmol/kg and 1.0 nmol/kg; Bachem, King of Prussia, PA) in a volume of 1 ml/kg and were immediately given access to Ensure liquid diet for 30 min. Thus the experimental conditions for each animal were as follows: icv saline + ip saline, icv saline + ip BN (0.32 and 3.2 nmol/kg), icv leptin + ip saline, icv leptin + ip BN (0.32 and 3.2 nmol/kg).

Data analyses. Only animals that had a positive response to the ANG II test and correct cannula placement, as verified postmortem by histological examination, were included in the data analyses. Data were subjected to repeated-measures ANOVA (3 × 2) analyses. Differences between means from each experimental condition were determined by planned t-test comparisons.

c-Fos immunohistochemistry. At the conclusion of the behavioral experiments, a subgroup of rats (n = 17) was then evaluated for the activation of c-Fos protein in the caudal hindbrain (AP and NTS) in response to the leptin and BN treatments. In accordance with treatment group, rats were injected with either 0.9% saline or leptin (3.5 μg) into the third ventricle 1 h before intraperitoneal administration of either 0.9% saline or BN (0.32 nmol/kg). The four groups were as follows: icv saline-ip saline (n = 3), icv leptin-ip saline (n = 4), icv saline-ip BN (n = 4), and icv leptin-ip BN (n = 6). Because feeding and gastric distention induce c-Fos activation in the brain regions of interest, all injections were done in the absence of food, and food was not returned after the second injection. Seventy-five minutes after the intraperitoneal injection, rats were anesthetized with Enthasol (pentobarbital sodium and phenytoin; Delmarva Laboratories, Midlothian, VA) and perfused transcardially with 200 ml of 0.1 M PBS (pH 7.4) followed by 200 ml of 4% paraformaldehyde in PBS. The fixed brains were removed and placed in 4% paraformaldehyde containing 25% sucrose for 24–48 h. Serial 4-μm frozen sections were cut coronally through the brain regions of interest. All sections were stained for c-Fos activation with Histofine-SABC immunostaining kit (Nichirei, Tokyo, Japan). Briefly, sections were preabsorbed with 10% normal goat serum in 0.1 M PBS containing 0.1% Triton X-100. Sections were incubated for 48 h at 4°C in rabbit anti-c-Fos antisera (1:30,000; Oncogene Science, San Diego, CA) and sequentially incubated in biotinylated goat anti-rabbit IgG and avidin-biotin-peroxidase complex (Elite Vectastain kit, Vector Labs, Burlingame, CA). We visualized the reaction product using nickel sulfate-enhanced diaminobenzidine as the chromagen. Sections were mounted onto gelatin-coated slides, dehydrated in alcohols, and coverslipped.

Analyses of c-Fos in the AP-NTS. Images of tissue sections were digitized, and areas of interest were outlined based on cellular morphology. c-Fos-positive nuclei within the regions of interest were quantified with automated image analysis software (IpLab, ScancoMetrix, Fairfax, VA). The four caudal hindbrain regions evaluated were the mid-AP level and three levels of the NTS (one matched section from each level), corresponding to the coordinates in the brain atlas of Paxinos and Watson (31) of approximately −14.08, −13.68, and −13.3 mm caudal to bregma. The effect of leptin and BN on c-Fos activation at each brain site was then compared across treatment groups by 2 × 2 ANOVA (peptide treatment by leptin treatment) followed by post hoc Tukey’s tests to evaluate differences between individual means.

RESULTS

Effect of leptin on BN-induced suppression of food intake. The interaction of leptin with BN to reduce 30-min Ensure intake is demonstrated in Fig. 1. The ANOVA indicated a significant dose effect of BN [F(2,62) = 19.967, P < 0.0001], as well as a significant interaction between BN and leptin [F(2,62) = 8.496, P < 0.0005].

When leptin or the low BN dose (0.32 nmol/kg) was given alone, there was no significant suppression of Ensure intake (P > 0.05). However, when leptin was administered into the third ventricle 1 h before intraperitoneal 0.32 nmol/kg BN administration, Ensure intake was significantly reduced by 28% (P < 0.01).

The higher BN dose (1.0 nmol/kg) produced a significant suppression of Ensure intake (15.7%) compared with intake alone, there was no significant suppression of Ensure intake (P > 0.05).

Fig. 1. Effect of combined dosages of leptin and bombesin (BN) on 30-min Ensure intake. Data are expressed as means ± SE under the following conditions: icv saline-ip saline (SAL/SAL), icv saline-ip BN (SAL/BN-32), 3.5 μg icv leptin-ip saline (LEP/SAL), 3.5 μg icv leptin-0.32 nmol/kg ip BN (LEP/BN-32), icv saline-1.0 nmol/kg ip BN (SAL/BN1.0), and 3.5 μg icv leptin-1.0 nmol/kg ip BN (LEP/BN1.0). *Significantly different from SAL/SAL (*P < 0.01); #significantly different from SAL/BN-32 (P < 0.01); + significantly different from SAL/BN1.0 (P < 0.05).
after saline injection ($P < 0.01$). The combination of leptin and BN elicited a significantly greater reduction of Ensure intake (29.7%, $P < 0.05$) than when either peptide was given alone. This suppression did not represent a further reduction in intake from the combination of leptin and the low dose of BN ($P > 0.05$).

**Effect of leptin on BN-induced c-Fos activation in the AP-NTS.** The effect of combined treatments of intracerebroventricular leptin and intraperitoneal BN on c-Fos activation in the caudal hindbrain is shown in Fig. 2. The results were consistent with those of the food intake experiment. Post hoc analyses revealed that neither BN nor leptin significantly increased c-Fos expression compared with the comparable saline-treated condition ($P > 0.05$). However, when 3.5 μg of leptin were administered into the lateral ventricle 1 h before intraperitoneal injection of 0.32 nmol/kg BN, significant peptide-leptin interactions were seen at all levels of the NTS [NTS1, $F(1,16) = 5.32$, $P < 0.05$; NTS2, $F(1,16) = 4.77$, $P < 0.05$; NTS3, $F(1,16) = 6.46$, $P < 0.05$]. The number of c-Fos-positive cells in the caudal (NTS1), mid (NTS2), and rostral (NTS3) areas was significantly increased by 4.8-, 5.2-, and 3.4-fold, respectively, compared with when BN was given in the absence of leptin ($P < 0.05$).

The distribution of c-Fos-positive cells in the NTS was similar to that previously described in that most of the c-Fos-like immunoreactivity was confined to the medial subnucleus with minimal labeling in the commissural subnucleus (4, 20). There were no differences detected in the number of c-Fos-positive cells in the AP after any treatment ($P > 0.05$).

**DISCUSSION**

The results of this experiment demonstrate that, when leptin is administered centrally 1 h before peripheral administration of BN, the reduction of food intake is significantly greater than when either peptide is given alone. These results support the hypothesis that leptin, a long-term signal involved in energy homeostasis, may affect food intake and body weight by modifying meal-generated satiety signals such as BN.

A number of studies have supported the notion that either metabolic status or obesity can alter the ability of a variety of peptides to affect food intake, including BN. For example, studies examining the effects of food deprivation on BN satiety demonstrated that BN was less effective in suppressing food intake in food-deprived than in normally fed rats (2, 15). Moreover, rats chronically fed a high-fat diet are less responsive to the feeding inhibitory effects of BN than those fed a low-fat diet (6). Finally, genetically obese (ob/ob) mice have deficits in feeding suppression produced by exogenous BN administration (26). All of these studies represent a decrease in leptin signaling due to either diminished leptin levels (as in fasting) or reduced sensitivity to leptin (as with high-fat diets or obesity), suggesting that impaired sensitivity to BN may be the result of reduced BN and leptin interactions.

We chose to use BN in this study instead of the mammalian BN-like peptide GRP because it has a longer duration of action, similar to that of CCK. As mentioned previously, BN is an amphibian peptide that is structurally similar to the mammalian peptide GRP. Unlike GRP, BN binds with equal and high affinity to two mammalian BN receptor subtypes: BB2, which has a high affinity for GRP, and BB1, which has a high affinity for neurenomed B (38). Several lines of evidence suggest that BN-induced suppression of feeding is mediated by both receptor subtypes (19, 37). Therefore, by using BN in the present study, we cannot discern whether the feeding effects that we observed with our combined treatment was due to leptin enhancing suppression induced by BN’s interaction with one or both receptor subtypes.

A previous study examining the effects of combined doses of leptin and BN in mice reported a failure to detect an interaction to reduce food intake (1). There are several differences between that study and the present experiment that may explain this result. In the prior study, the earliest time point in which feeding was measured was 1 h after administration. Because the majority of feeding suppression by BN occurs in the first 30 min after administration and declines shortly thereafter, the 1-h time point may not have been optimal to observe an interaction. Moreover, the timing of the leptin injection and the route of administration may be critical factors in revealing a synergistic interaction. In their study, both the leptin and BN were injected intraperitoneally and were administered simultaneously, whereas in the present experiment the leptin was given centrally 1 h before intraperitoneal administration of BN. Although the mechanism(s) responsible for the interaction of leptin and BN on feeding suppression is unknown, it is possible that brain sites sensitive to leptin may require activation before gastrointestinal peptide stimulation to initiate a chain of neuronal events that would enhance sensitivity to neurons responsible for BN’s feeding effects. Thus our experimental design of providing leptin 1 h before BN administration would facilitate such an interaction. The importance of timing is consistent with other studies revealing positive interactions between leptin and CCK (3, 8).

Results from the combined treatment of leptin and BN on c-Fos activation in the caudal hindbrain paralleled those of the behavioral experiments. When leptin or BN was administered alone, there was no significant induction of c-Fos. However, when leptin and BN were given in combination,
there was a substantial increase in c-Fos immunoreactivity in the medial NTS. Unlike our previous results with CCK (8), there was no significant increase in c-Fos immunoreactivity in the AP after the combined treatment. The distribution of c-Fos in the NTS after the leptin-BN combination is consistent with previous reports examining c-Fos activation in the caudal hindbrain after higher doses of intraperitoneal BN that potently reduced food intake (4, 20). The similarity in distribution of combined leptin-BN treatment with that produced by a higher dose of BN alone suggests that leptin may increase the responsiveness of the same neuronal population.

Although these data support a role for the NTS in the interaction between leptin and BN, they do not address which leptin receptor population is responsible for mediating this effect. We have previously suggested that leptin and CCK’s synergistic effect on food intake may be mediated through a leptin-sensitive descending hypothalamic pathway that projects to caudal hindbrain nuclei to enhance the response to meal-related gastrointestinal stimuli (8). Because of the reliance of intraperitoneal BN’s feeding effects on NTS neurons (17, 18), this mechanism would also be concordant with the results of the present experiment. However, we cannot rule out the possibility of a local site of action of leptin because leptin administered into the third ventricle would also gain access to leptin receptors in the NTS.

In summary, we found that prior administration of intracerebroventricular leptin significantly enhanced the feeding inhibitory effects of systemically administered BN and produced parallel results on neuronal activation within the NTS. These results are consistent with those that we have previously reported examining leptin and CCK interactions and further support the hypothesis that one mechanism through which leptin may reduce food intake is by amplifying sensitivity to gastrointestinal satiety signaling.

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GRANTS
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