Leptin receptor signaling in the lateral parabrachial nucleus contributes to the control of food intake

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Submitted 8 August 2014; accepted in final form 4 October 2014

Alhadeff AL, Hayes MR, Grill HJ. Leptin receptor signaling in the lateral parabrachial nucleus contributes to the control of food intake. Am J Physiol Regul Integr Comp Physiol 307: R1338–R1344, 2014. First published October 8, 2014; doi:10.1152/ajpregu.00329.2014.—Pontine parabrachial nucleus (PBN) neurons integrate visceral, oral, and other sensory information, playing an integral role in the neural control of feeding. Current experiments probed whether lateral PBN (lPBN) leptin receptor (LepRb) signaling contributes to this function. Intra-lPBN leptin microinjection significantly reduced cumulative chow intake, average meal size, and body weight in rats, independent of effects on locomotor activity or gastric emptying. In contrast to the effects observed following LepRb activation in other nuclei, lPBN LepRb stimulation did not affect progressive ratio responding for sucrose reward or conditioned place preference for a palatable food. Collectively, results suggest that lPBN LepRb activation reduces food intake by modulating the neural processing of meal size/satiation signaling, and highlight the lPBN as a novel site of action for leptin-mediated food intake control.

METHODS

Subjects and Drugs

Male Sprague-Dawley rats (250–300 g upon arrival; Charles River Laboratories, Wilmington, MA) were individually housed in hanging metal cages on a 12:12-h light-dark cycle with ad libitum access to pelleted chow [Purina Rodent Chow, 5001; 28.5% (kcal) protein, 13.5% fat, 58.0% carbohydrate, 3.3 kcal/g] and water except when otherwise noted. All procedures conformed to and received approval from the institutional standards of the University of Pennsylvania Animal Care and Use Committee.

Leptin was purchased from Harbor University of California, Los Angeles Research and Education Institute, Torrance, CA, and was dissolved in sodium bicarbonate.

Surgery

Rats received intramuscular ketamine (90 mg/kg; Butler Animal Health Supply, Dublin, OH), xylazine (2.7 mg/kg; Anased, Shenandoah, IA), and acepromazine (0.64 mg/kg; Bitler Animal Health Supply) anesthesia and subcutaneous analgesia (2.0 mg/kg Metacam; Boehringer Ingelheim Vetmedica, St. Joseph, MO) for all surgeries.

Unilateral 26-gauge guide cannulas (Plastics One, Roanoke, VA) were stereotaxically implanted in the lPBN or the cerebral aqueduct, according to the following coordinates. lPBN guide cannulas were positioned ± 2.0 mm lateral from midline, 0.6 mm anterior to lambda, and 5.7 mm ventral from skull surface using a 20° angle (negative slope in anterior to posterior direction) with the injector aimed 2.0 mm below the end of the guide cannula. Cannula placements were histologically confirmed post mortem: Chicago sky blue ink (100 nl) was injected in the lPBN after animals were euthanized; brains were removed and postfixed in formalin, cut on a cryostat, mounted on glass slides, and analyzed for placement. A representative image of the injection site, as well as a schematic diagram representing injection placements for a cohort of rats, is depicted in Fig. 1. Rats with injection sites that were not within the lPBN were excluded from analyses. Aqueduct guide cannulas were positioned anterior to the lPBN, ± 2.0 mm medial from midline, 8.2 mm caudal anterior from bregma, and 3.85 mm ventral from skull using a 20° angle (negative slope in the lateral to medial direction). Cannula placements were functionally confirmed via measurement of the sympathoadrenal mediated glycemic response to 5-thio-D-glucose (210 µg/2 µL in artificial cerebrospinal fluid, aCSF) injected into the aqueduct, as previously described (28). A postinjection increase in blood glucose level of at least 100% from baseline was necessary for subject inclusion.
Experimental Procedures

Experiment 1: leptin effects in the cerebral aqueduct: parenchymal dose selection. To select leptin doses for IPBN experiments that were subthreshold for effect when delivered into the cerebroventricular system, rats \( (n = 9) \) that were habituated to experimental procedures received a 100-nl unilateral injection of leptin \((0.6, 0.3, \) or \(0.1 \mu g) \) or vehicle via an automated syringe pump (PHD Ultra; Harvard Apparatus; Holliston, MA) in the aqueduct in a within-subjects, counterbalanced experimental design immediately before the onset of the dark cycle. Chow intake was measured at 1 h, 3 h, 6 h, and 24 h, accounting for spillage. Body weight and water intake were measured 24 h postinjection. At least 48 h elapsed between drug injection conditions.

Experiment 2: IPBN leptin effects on chow intake and meal patterns. Rats \( (n = 18) \) were housed in a custom, automated feedometer consisting of hanging wire cages with a small access hole to a food cup resting on an electronic scale. The associated software (LabView) records the weight of food cups every 10 s. Following habituation to the cages and powdered standard chow for 5 days, rats received a 100-nl unilateral injection of leptin \((0.1, 0.3, \) or \(0.6 \mu g) \) or vehicle into the IPBN in a within-subjects, counterbalanced experimental design immediately before the onset of the dark cycle. As noted, the doses selected were first determined to be subthreshold for effect when delivered to the ventricular system (experiment 1). Automated food measurements were made for 24 h postinjection; body weight was recorded manually 24 h postinjection. In a subset of the total group of rats \( (n = 8) \), 24-h water intake was recorded manually. Meal patterns were subsequently analyzed for all rats, with a meal defined as any intake \( \geq 0.25 \, \text{g} \); \( \geq 10 \, \text{min} \) must elapse for feeding bouts to be considered two separate meals. At least 48 h elapsed between drug injection conditions.

Experiment 3: IPBN leptin effects on high-fat diet intake and meal patterns. Rats \( (n = 10) \) were subjected to the same procedures as in experiment 2, except that they were maintained on powdered high-fat diet [HFD; Research Diets, New Brunswick, NJ; 20% (kcal) protein, 45% fat, 35% carbohydrate, 3.73 (kcal/g)] throughout the duration of the experiment.

Experiment 4: IPBN leptin effects on progressive ratio responding. Rats \( (n = 11) \) maintained ad libitum on standard chow were habituated to 45 mg of sucrose pellets (Bio-Serv, Frenchtown, NJ) in their home cage and were trained, as we have previously described (19) to press a lever for pellets at a fixed ratio (FR)-3 schedule of reinforcement (three lever presses required to receive one pellet). For all training sessions, the right lever was active, and an inactive left lever served as a control for nonconditioned changes in operant responding.

Rats were given three tests in a within-subjects design using a progressive ratio (PR) schedule of reinforcement. A 100-nl unilateral IPBN injection of leptin \((0.3 \text{ or } 0.6 \, \mu g) \) or vehicle was delivered 3 h prior to each PR test session in a within-subjects, counterbalanced experimental design. Animals were returned to their home cage for the 3 h between injection and test session, and food was withheld. During the PR test, the effort required to obtain each pellet increased exponentially throughout the session, as we have previously described (1, 19), using the formula \( F_i = 5e^{0.25i} \), where \( F_i \) is the number of lever presses required to obtain the next pellet at \( i \), the pellet number. The PR session ended when a 20-min period elapsed without the rat earning a pellet.

Experiment 5: IPBN leptin effects on food-conditioned place preference expression. Rats \( (n = 21) \) maintained ad libitum on standard chow were trained for food-conditioned place preference (CPP), as we have previously described (19). All CPP training and testing sessions were performed in a dimly lit room. Animals were trained in an apparatus consisting of two identical Plexiglas compartments \((74 \, \text{cm long}, 57.4 \, \text{cm wide}, \) and \(24.7 \, \text{cm high}) \) separated by a divider wall with a door that was closed during training but open during habituation and testing. The two environments within the CPP box were made distinguishable by different wall color and design and floor texture. Rats were habituated to the CPP chamber (with access to both environments) for one 15-min video-recorded session. The differential time spent in each of the two environments was analyzed via ANY-Maze software (Stoelting, Wood Dale, IL), and a baseline environment preference was determined. For each rat, the environment that was least preferred during habituation was subsequently paired with the palatable food for all training, whereas the preferred side was never paired with palatable food. CPP training consisted of 16 consecutive days of training (15-min sessions); 8 days of training in a food-paired environment, where 5 g of a high-fat diet \((60\% \, \text{kcal/fat}; \) Research Diets, New Brunswick, NJ) was divided into 10 aliquots and scattered throughout the environment, alternating with 8 days of training in the other environment without food.

CPP testing commenced the day after the training was completed using a between-subjects design. Rats were matched for baseline

![Fig. 1](http://ajpregu.physiology.org/)

**Fig. 1.** Representative image of lateral pontine parabrachial nucleus (IPBN) injection site (black arrow) \((A)\), and a schematic diagram of approximate injection sites in a cohort of rats in this study \((B)\), numbers represent position \((\text{mm})\) relative to bregma. ● represents hits and X represents misses.

![Fig. 2](http://ajpregu.physiology.org/)

**Fig. 2.** Leptin administration to the cerebral aqueduct did not affect cumulative chow intake.
postinjection of leptin or vehicle.

emptied from the stomach prior to euthanasia, which occurred 5 h
night at 80°C, and the dry weight of the stomach contents was
stomach contents were collected. Stomach contents were baked over-
also analyzed via ANY-maze software.

motor activity parameters, total time active and total distance traveled
preference (from habituation baseline) for the food-paired side was
was analyzed via ANY-maze software, and the percentage shift in
with no food. The time spent in each environment
animals were returned to their home cages for the three intervening
prevention (version 7; StatSoft, Tulsa, OK) and expressed as means

preference (n = 11 vehicle; n = 10 leptin) and given a unilateral
injection of leptin (0.6 μg) or vehicle 3 h prior to CPP test. The
animals were returned to their home cages for the three intervening
hours, and food was withheld. With the divider door open and no food
present, the rats were videotaped for the 15-min CPP test to determine
the total time spent in the environments previously associated with
palatable food or without food. The time spent in each environment
was analyzed via ANY-maze software, and the percentage shift in
preference (from habituation baseline) for the food-paired side was

Cerebral aqueduct delivery of leptin did not significantly
affect cumulative chow intake at 1 h [F(3,24) = 1.69], 3 h
[ F(3,24) = 0.84], 6 h [ F(3,24) = 0.89], or 24 h [ F(3,24) =
1.46] compared with vehicle treatment (Fig. 2). Additionally,
post hoc comparisons revealed no significant drug effects for
any doses or time points.

Experiment 2: IPBN Leptin Significantly Reduces Chow
Intake Via a Reduction in Meal Size

There was a significant main effect of IPBN leptin (0.1, 0.3,
0.6 μg) on cumulative food intake at 5 h [F(3,51) = 3.80; P <
0.05], 12 h [F(3,51) = 3.91; P < 0.05], and 24 h [F(3,51) =
11.70; P < 0.001] postinjection (Fig. 3A). Post hoc compari-
sions revealed significant effects of 0.3 μg leptin at 24 h and 0.6
μg leptin at 5, 12, and 24 h postinjection compared with
vehicle treatment. There was also a significant main effect of
IPBN leptin on a 24-h change in body weight [F(3,51) = 9.11,
behavioral experiments, repeated-measures or one-way ANOVA and
post hoc Neumann-Keuls comparisons were made. Alpha levels were
set to α = 0.05 for all analyses.

RESULTS

Experiment 1: Evaluation of IPBN Effects in the Cerebral
Aqueduct

Statistical Analyses

Data for each experiment were analyzed separately using Statistica
(version 7; StatSoft, Tulsa, OK) and expressed as means ± SE. For all

Fig. 4. IPBN LepRb activation reduced average meal size (A) but had no effect on average meal number (B) in animals maintained on chow (means ± SE; *P <
0.05, **P < 0.01).
Experiment 3: IPBN Leptin Significantly Reduces High-Fat Diet Intake

There was a significant main effect of IPBN leptin (0.1, 0.3, 0.6 μg) on short-term cumulative HFD intake at 1.5 h \( F(2,18) = 33.82; P < 0.05 \) and 2 h \( F(2,18) = 6.67; P < 0.05 \) (Fig. 5A), as well as 24-h HFD intake \( F(2,18) = 4.03; P < 0.05 \) postinjection (Fig. 5B). Post hoc comparisons revealed significant effects of 0.3 μg leptin at 1.5 and 2 h, and 0.6 μg leptin at 1.5, 2, and 24 h postinjection. There was no effect of IPBN leptin on 24-h change in body weight \( F(2,18) = 2.26 \) (Fig. 5C) or on 24-h water intake \( F(2,18) = 0.21 \) (Fig. 5D). At the time points at which leptin significantly reduced cumulative HFD intake, there were no significant effects on average meal size or average meal number (Fig. 6, A–D), although there were trends for reductions in average meal size at various time points.

Experiment 4: IPBN Leptin Does Not Reduce Progressive Ratio Responding for Sucrose Pellets

There was no significant effect of IPBN leptin on active lever presses \( F(2,20) = 0.18 \) (Fig. 7A), inactive lever presses \( F(2,20) = 0.24 \) (Fig. 7A), or total pellets earned \( F(2,20) = 0.17 \) (Fig. 7B).
Experiment 5: IPBN Leptin Does Not Reduce Food-Conditioned Place Preference Expression

There was no significant effect of IPBN leptin on food-conditioned place preference expression \( [F(1,19) = 0.048] \) (Fig. 7C). Likewise, IPBN leptin did not affect total distance traveled during the CPP test \( [F(1,19) = 0.026] \) (Fig. 7D).

Experiment 6: IPBN Leptin Does Not Affect Gastric Emptying

There was no significant effect of IPBN leptin on 90-min gastric emptying of solid food \( [F(1,14) = 1.37] \) (Fig. 8).

DISCUSSION

The hindbrain PBN is critical for the integration of multimodal oral and visceral information, and signaling within the IPBN affects feeding behavior (1, 7, 33). IPBN neurons express LepRb, and the current studies investigated the role of leptin signaling in the IPBN on food intake control. Experiments reveal that acute leptin administration to the IPBN significantly reduced food intake and body weight. Consistent with studies on peripheral (9, 18) and central (21) LepRb signaling, meal pattern analyses showed that IPBN LepRb signaling reduced chow intake specifically via a reduction in meal size. Although LepRb signaling in other CNS nuclei has been shown to reduce food reward (11, 19), IPBN LepRb stimulation did not affect PR responding for sucrose or CPP for a palatable food. Collectively, these results suggest that IPBN LepRb activation reduces food intake likely by modulating the neural processing of meal size/satiation signaling rather than affecting the motivation to work for food.

Historically, nuclei of the hypothalamus have been the focus of attention for leptin’s effects on food intake (29). However, it is now clear that leptin contributes to the control of energy balance through action in a variety of brain nuclei distributed throughout the neuraxis [e.g., NTS (14, 15), VTA (12, 16), hippocampus (20)]. In fact, previous work from our laboratory has demonstrated that NTS LepRb signaling is required for the normal control of energy balance, as a LepRb knockdown virus targeted to the NTS causes hyperphagia and obesity (15). Our current data highlight another hindbrain region, the IPBN, in LepRb-mediated control of food intake. These data add to the growing body of literature focusing on the IPBN as a nucleus involved in the control of feeding.

IPBN LepRb stimulation reduced food intake in rats maintained on diets of differing palatability, but the temporal profiles differed. While IPBN leptin treatment reduced standard chow intake from 5–24 h postinjection, the effects of IPBN leptin on HFD intake were most robust within the first 2 h postinjection and waned at later time points. Given that the IPBN can process taste information (22) and is involved in the hedonic valuation of food, it is not entirely surprising that IPBN LepRb signaling has differential effects on the intake of foods of varying palatability. Longer-term intake suppression of HFD induced by IPBN leptin signaling may be overridden or masked by the high palatability of the HFD, a concept originally suggested by Ward and Simansky (32), who documented a similar phenomenon (only acute feeding effects on palatable food intake) with IPBN opioid receptor signaling. A direct comparison of IPBN LepRb signaling effects on chow and...
Peripheral leptin administration reduces food intake by reducing meal size without affecting meal number (9, 18). Additionally, our laboratory has shown that LepRb signaling in the NTS modifies meal size via a direct interaction with gastrointestinal (GI) satiation signals (15). Here, results showed that IPBN leptin injection reduced chow intake specifically via a reduction in meal size, suggesting that IPBN leptin reduces intake by enhancing the processing of satiation signals. Although no significant differences were observed, there were trends for reductions in average meal size when animals were maintained on a high-fat diet. The IPBN integrates GI and other visceral and sensory signals relayed by NTS projections; thus, it is possible that an interaction between leptin and GI signals occurs at the level of the IPBN; this idea warrants future investigation.

Given that 1) central LepRb signaling modifies appetitive and motivated feeding behavior (6, 11, 19) and 2) IPBN neural processing is involved in food reward and hedonics (1, 7, 31), we used two different paradigms to test the hypothesis that IPBN LepRb signaling reduces food intake, at least in part, by reducing food-motivated behaviors. Surprisingly, IPBN leptin injection had no effect on PR operant responding for sucrose or CPP for a HFD. This finding is especially interesting in light of the recent findings that LepRb signaling in another hindbrain region (NTS) both reduces PR responding and attenuates a CPP for HFD (19). Given that the current study focused on the IPBN, it is possible that LepRb signaling in more medial regions of the PBN may reduce reward-related feeding behavior, since the medial PBN is known to receive gustatory afferents from the oral cavity. Together with findings on LepRb-mediated effects on food intake and reward in other brain nuclei (VTA, NTS), these data encourage a broader and more systematic analysis of the behavioral mechanisms by which LepRb signaling controls for food intake in distributed brain nuclei.

That IPBN LepRb signaling reduces chow intake by affecting meal size is consistent with an interpretation that leptin signaling in the IPBN increases satiation. To examine whether the intake-suppressive effect of IPBN leptin injection was influenced by effects on locomotor activity, we analyzed the total distance traveled by animals during the CPP test. IPBN LepRb stimulation did not affect this activity parameter. Given that peripherally or centrally administered leptin reduces the rate of gastric emptying (4, 24, 30), potential effects on IPBN LepRb signaling on gastric emptying were also examined. However, intra-IPBN leptin administration did not inhibit gastric emptying; thus, it appears that IPBN LepRb signaling reduces meal size-independent of effects on gastric emptying rate.

Though LepRb is expressed on neurons in the IPBN (14, 17), the neurochemical phenotypes and target projection site(s) of these neurons are not fully characterized. Systemic leptin activates (as measured by c-Fos immunoreactivity) cholecystokinin (CCK)-containing neurons in the IPBN (10), providing a potential neuronal phenotype for IPBN LepRb-expressing neurons. However, this must be directly examined using alternative electrophysiological and immunohistochemical techniques, as c-Fos immunoreactivity within the IPBN could represent activation of CCK neurons by leptin through indirect and second-order responses. In addition to CCK, a variety of other neurochemical signals, including calcitonin gene-related peptide, glutamate, neurotensin, substance P, enkephalin, and somatostatin, among others, are expressed in the IPBN (3, 5). Additionally, though the IPBN projects to brain nuclei such as those of the hypothalamus (2, 27), amygdala (2, 5, 27), and nucleus accumbens (23), it is unknown to which brain regions IPBN LepRb-expressing neurons project. Thus, a comprehensive characterization of the phenotypes and anatomical targets of IPBN LepRb neurons is warranted.

**Perspectives and Significance**

Collectively, these data demonstrate for the first time a role for IPBN LepRb signaling in the control of food intake. IPBN LepRb signaling reduced cumulative chow intake by reducing meal size, with no effect on reward-related feeding behavior, suggesting that IPBN leptin signaling is likely modulating the neural processing of within-meal satiation signaling. Future studies should examine the neural interactions (neurochemical and intracellular signals) and circuits mediating IPBN LepRb stimulation-induced reduction in feeding. Overall, these novel findings on IPBN LepRb add to the growing body of literature highlighting IPBN signaling in the control of food intake and energy balance.

**ACKNOWLEDGMENTS**

This work was funded by National Institutes of Health Grants DK-21397 (to H. J. Grill), DK-096139 (to M. R. Hayes), and F31NS084633 (to A. L. Alhadeff). The authors would like to thank Richard Ritacco for his contributions to data collection.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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