Hindbrain nucleus tractus solitarius glucagon-like peptide-1 receptor signaling reduces appetitive and motivational aspects of feeding

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Alhadeff AL, Grill HJ. Hindbrain nucleus tractus solitarius glucagon-like peptide-1 receptor signaling reduces appetitive and motivational aspects of feeding. Am J Physiol Regul Integr Comp Physiol 307: R465–R470, 2014. First published June 18, 2014; doi:10.1152/ajpregu.00179.2014.—Central glucagon-like peptide-1 receptor (GLP-1R) signaling reduces food intake by affecting a variety of neural processes, including those mediating satiation, motivation, and reward. While the literature suggests that separable neurons and circuits control these processes, this notion has not been adequately investigated. The intake inhibitory effects of GLP-1R signaling in the hindbrain medial nucleus tractus solitarius (mNTS) have been attributed to interactions with vagally transmitted gastrointestinal satiety signals that are also processed by these neurons. Here, behavioral and pharmacological techniques are used to test the novel hypothesis that the reduction of food intake following mNTS GLP-1R activation by microinjection of exendin-4, a long-acting GLP-1R agonist, reduced intake of a palatable high-fat diet, 2) operant responding for sucrose under a progressive ratio schedule of reinforcement, and 3) the expression of a conditioned place preference for a palatable food. Together, these data demonstrate that the intake inhibitory effects of mNTS GLP-1R signaling extend beyond satiation and include effects on food reward and motivation that are typically ascribed to midbrain and forebrain neurons.

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is released from gastrointestinal (GI) enteroeendocrine cells and neurons of the hindbrain nucleus tractus solitarius (NTS) in relation to food ingestion and contributes to the control of food intake, GI motility, and glycemia (19). The GLP-1 receptor (GLP-1R) is widely distributed throughout the brain (25), and GLP-1R activation in a variety of brain regions, including some nuclei of the hypothalamus (28), mesolimbic system (2, 8, 9), and hindbrain (1, 16), reduces food intake. Previous work from our laboratory shows that targeted GLP-1R activation in the medial subnucleus of the NTS (mNTS) (16) or systemic GLP-1R agonist treatment in rats whose caudal brain stems were surgically isolated from communication with forebrain neurons (17) reduces chow intake and body weight. These effects are mediated, at least in part, by interactions with the neural processing of GI satiation signals (i.e., gastric distension) (13).

Accumulating evidence supports a role for central GLP-1R signaling in food reward and appetitive and motivational aspects of feeding, but the anatomical basis of these effects has thus far been limited, focusing on GLP-1R activation in the ventral tegmental area (VTA) or the nucleus accumbens (NAC) (2, 8). The role of mNTS GLP-1R signaling in these appetitive aspects of feeding is unexplored. Thus, the present studies evaluate the novel hypothesis that the intake inhibitory effects of mNTS GLP-1R signaling include effects on appetitive and motivated aspects of feeding, in addition to the effects on satiety signal processing. Results described show that direct mNTS delivery of exendin-4, a long-acting GLP-1R agonist, significantly reduced 1) intake of a palatable high-fat diet, 2) operant responding for sucrose under a progressive ratio schedule of reinforcement, and 3) the expression of conditioned place preference for a palatable high-fat food, without causing malaise or alterations in activity. These data highlight a novel role for mNTS GLP-1R signaling on food reward and appetitive behavior.

MATERIALS AND METHODS

Subjects and Drugs

Adult 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 and had ad libitum access to standard pelleted chow (Purina Rodent Chow, 5001) and water except when otherwise noted. All procedures were approved by the University of Pennsylvania Animal Care and Use Committee. The long-acting GLP-1R agonist exendin-4 (American Peptide, Sunnyvale, CA) was dissolved in artificial cerebrospinal fluid.

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. Bilateral guide cannulas with 1.0-mm spacing (Plastics One; Roanoke, VA) were stereotaxically implanted in the medial NTS using the following coordinates: midline, 1.9 mm anterior to occipital, and 6.8 mm ventral from skull surface using a 15° 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 via a 100-nl injection of Chicago Sky Blue solution (2%). A representative image of the injection site is depicted in Fig. 1. Animals with injection sites that were not within the mNTS (based on Ref. 26) were excluded from analyses.

Experimental Procedures

Experiment 1: effects of mNTS GLP-1R signaling on high-fat diet intake. Rats (n = 11) maintained on ad libitum high-fat diet (HFD; 45% kcal/fat, Research Diets, New Brunswick, NJ) for 5 days received a 100-nl unilateral injection of exendin-4 (0.025 or 0.05 μg) or vehicle immediately before onset of the dark cycle in a within-subjects, counterbalanced design. Dose selection was designed to include doses suprathreshold (0.05 μg) and subthreshold (0.025 μg) for an effect on chow intake when administered to the 4th ventricle.
HFD intake was recorded at 1 h, 3 h, 6 h, and 24 h postinjection, accounting for spillage. Body weight was also measured 24 h postinjection. At least 48 h elapsed between drug injection conditions.

Experiment 2: effects of mNTS GLP-1 signaling on progressive ratio operant responding for sucrose pellets. Rats (n = 10) maintained ad libitum on standard chow were habituated to 45-mg sucrose pellets (Bio-Serv; Frenchtown, NJ) in their home cages and were trained, as previously described (1, 20) to press a lever to obtain pellets on a FR5 schedule of reinforcement (five lever presses required to receive one pellet). For all training sessions, the right lever was the active lever, and an inactive left lever served as a control for nonconditioned increases or decreases in operant responding.

Once rats established a stable FR5 baseline, they were given two tests using a progressive ratio (PR) schedule of reinforcement in a within-subjects, counterbalanced design and received one FR5 session on the intervening day between PR tests. A 100-nl unilateral mNTS injection of 0.025 g exendin-4 or vehicle was delivered 3 h prior to each PR test session. The 3-h time point was chosen on the basis of previous studies that demonstrated this as the earliest time point that 0.025 g exendin-4 significantly reduces food intake (16). Animals were returned to their home cages for the 3 h between injection and test session; food was withheld during this period. During the PR test, the effort required to obtain each pellet increased exponentially throughout the session, as previously described (1, 20), using the formula: 

$$F(i) = 5e^{0.21i} - 5,$$

where F(i) is the number of lever presses required to obtain the next pellet at i = pellet number. The PR session ended when a 20-min period elapsed without the rat earning a pellet.

Experiment 3: effects of mNTS GLP-1R signaling on conditioned preference for a palatable food. Rats (n = 17) maintained ad libitum on standard chow were trained for conditioned preference (CPP), as previously described (20). All CPP training and testing sessions were performed in a dimly lit room. Animals were trained in an apparatus consisting of two identical Plexiglas environments (74 cm long, 57.4 cm wide, and 24.7 cm high) separated by a divider wall with a door that was open in habituation and testing and closed during training. 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 the door open for one 15-min session. The 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 food. CPP training consisted of 16 consecutive days of 15-min training sessions: 8 days of training in a food-paired environment (when the divider door was closed and rats were restricted to the lesser-preferred environment), where 5 g of a HFD (60% kcal/fat; Research Diets) was divided into 10 aliquots and scattered throughout the environment, alternating with 8 days of training in the preferred environment (divider door closed) without food.

CPP testing commenced the day after the training was completed using a between-subjects design. Rats were matched for baseline preference (n = 9 exendin-4, n = 8 vehicle) and given a 100-nl unilateral injection of either exendin-4 (0.025 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 in the CPP apparatus for the 15-min test period. 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 calculated. As a control for potential exendin-4–induced effects on locomotor activity parameters, total time active and total distance traveled were also analyzed via ANY-maze software.

Experiment 4: pica, body weight, and Chow intake effects of mNTS GLP-1R activation. To determine whether reductions in feeding resulting from mNTS GLP-1R activation might be attributable to malaise/nausea, pica (the ingestion of nonnutritive substances) was assessed as previously reported (1, 2) in rats (n = 20) maintained ad libitum on standard chow and habituated to ad libitum access to kaolin pellets (aluminum silicate; Research Diets; New Brunswick, NJ) for 4 days. For testing, rats received a 100-nl unilateral mNTS injection of exendin-4 (0.025 µg) or vehicle in a within-subjects, counterbalanced experimental design immediately before onset of the dark cycle. Kaolin intake, chow intake, and body weight were measured 24 h postinjection, accounting for spillage. At least 48 h elapsed in between drug injection conditions.

Statistical Analyses

Data for each experiment were analyzed separately using Statistica (version 7; StatSoft, Tulsa, OK) and expressed as means ± SE. For all 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: mNTS GLP-1R Activation Reduced High-Fat Diet Intake

mNTS GLP-1R stimulation significantly reduced HFD intake. There was a significant main effect of mNTS exendin-4 on cumulative HFD intake at 6 h [F(2,20) = 10.6, P < 0.05] and 24 h [F(2,20) = 10.6, P < 0.001]. Post hoc comparisons showed that each dose of exendin-4 significantly reduced cumulative HFD intake at 6 h and 24 h (Fig. 2A). There was also a significant main effect of mNTS exendin-4 delivery (reduction) on 24-h change in body weight [F(2,20) = 13.6, P < 0.001] (Fig. 2B).

Experiment 2: mNTS GLP-1R Activation Reduced Progressive Ratio Responding for Sucrose Pellets

When ad libitum-fed rats were examined under a PR schedule of reinforcement, mNTS GLP-1R activation significantly reduced the number of active lever presses [F(1,9) = 6.0, P < 0.05] (Fig. 3A) and the number of sucrose reinforcers earned [F(1,9) = 5.57, P < 0.05] (Fig. 3B) compared with vehicle treatment. The number of presses on the inactive control lever...
was not influenced by mNTS exendin-4 delivery \(F(1,9) = 1.11\) (Fig. 3A).

**Experiment 3: mNTS GLP-1R Activation Reduces Conditioned Place Preference for a Palatable Food**

mNTS GLP-1R activation significantly reduced the CPP for palatable food (increased time spent in an environment previously associated with palatable food) compared with that observed in vehicle-treated rats \(F(1,15) = 5.68, P < 0.05\) (Fig. 4, A and B). A possible effect of mNTS exendin-4 on activity was examined, but the treatment had no effect on total time active \(F(1,15) = 0.022\) (Fig. 4C) or total distance traveled \(F(1,15) = 0.080\) (Fig. 4D) during CPP testing.

**Experiment 4: mNTS GLP-1R Stimulation Reduces Chow Intake and Body Weight but Does Not Induce Pica**

mNTS GLP-1R activation significantly reduced 24-h chow intake \(F(1,19) = 14.5, P < 0.01\) (Fig. 5B) and 24-h change in body weight \(F(1,19) = 10.9, P < 0.01\) (Fig. 5C) but did not affect 24-h kaolin intake compared with vehicle-treated rats \(F(1,19) = 1.56\) (Fig. 5A).

**DISCUSSION**

Peripheral and central GLP-1R signaling reduces food intake, in part, by increasing satiation (13) and by reducing appetitive processes, such as food reward and motivation to feed (1, 8); however, the brain regions involved in mediating these functional aspects of feeding control are not fully defined. To date, the literature suggests that these effects on food intake are mediated by different neural circuits (29); for example, mNTS GLP-1R signaling reduces food intake by interacting with satiation signal processing (13), and VTA and NAc GLP-1R signaling are thought to reduce feeding via effects on food reward and the motivation to feed (8). Data presented here support a different perspective and a novel role for mNTS GLP-1R signaling in the reduction of food-motivated appetitive behaviors. mNTS GLP-1R activation reduced 1) intake of a palatable high-fat diet, 2) operant responding for sucrose under a PR schedule of reinforcement, and 3) the expression of a CPP for a palatable food. These results are the first to implicate mNTS GLP-1R signaling with the control of appetitive, reward-related feeding behavior.

Consistent with previous data (16), mNTS exendin-4, at a dose subthreshold for effect when delivered to the 4th ventricle (16) or cerebral aqueduct (1), significantly reduced chow intake and body weight. We report here for the first time that mNTS GLP-1R activation also robustly reduced cumulative intake of a HFD, demonstrating that GLP-1R action in the mNTS on the control of food intake extends beyond the consumption of standard chow to include palatable foods. Our data suggest that the magnitude of food intake and body weight suppression is similar in animals maintained on HFD compared with those maintained on chow. A direct comparison of the potentially differential effects of mNTS GLP-1R stimulation on chow and HFD intake in animals given simultaneous access to both food types requires further investigation. It is interesting to note the time delay between exendin-4 injection and the manifestation of its effects on HFD intake. That centrally administered exendin-4 takes several hours to affect food intake is a phenomenon that has been documented in several brain nuclei for animals maintained on both chow (1, 15) and on a HFD (1, 2). A potential explanation for this observation is that mNTS GLP-1R stimulation-induced effects on food intake may involve changes in gene transcription and protein synthesis, processes that may take many hours to occur. This idea has been discussed previously in more detail (11, 12, 14) and should be directly tested in this context.

To test whether mNTS GLP-1R signaling affects motivated, appetitive feeding behavior, two paradigms were used: PR operant responding for sucrose pellets, and CPP for a palatable HFD. In the PR task, mNTS GLP-1R activation significantly reduced the total number of active lever presses, as well as the number of reinforcers earned during the PR test, suggesting that mNTS GLP-1R signaling reduces the motivation to work for a palatable food. The PR test captures the rats’ willingness to work increasingly more to obtain each subsequent reinforcer. At the same time, the animal consumes sucrose pellets intermittently and that consumption triggers postingestive effects, whose impact may also contribute to the observed reduction in operant responses. By contrast, the HFD-CPP paradigm is not influenced by the postingestive effects of food.
consumption, as there is no food available during testing. The finding that exendin-4 delivered to the mNTS attenuated the preference for an environment previously associated with palatable food consumption demonstrates that mNTS GLP-1R signaling suppresses food-motivated appetitive behaviors independently of satiation processing.

While the current data support a role for mNTS GLP-1R agonist delivery in the control of food-motivated behaviors, it is unknown whether or how endogenous GLP-1 or systemically delivered GLP-1R agonists affect these feeding behaviors through direct mNTS signaling. NTS GLP-1-producing neurons are stimulated following gastric distension (30) and CCK signaling (18). Thus, GLP-1 may be released locally in the NTS as a result of postprandial satiation signaling, in turn, activating mNTS GLP-1R to reduce appetitive feeding. It is also possible that systemic GLP-1R agonists that are resistant to the GLP-1-degradative enzyme dipeptidyl peptidase-IV, such as exendin-4 (Byetta, the GLP-1R agonist used in the current studies) and liraglutide (Victoza), act in an endocrine fashion and signal directly in the mNTS. Indeed, these agonists have been shown to enter the brain (10) and activate central GLP-1R to reduce food intake (21). Given that the aforementioned two GLP-1R agonists are Food and Drug Administration-approved for Type 2 diabetes mellitus, this untested idea may have clinical implications for the development of pharmacotherapies for obesity.

Our data demonstrate that mNTS exendin-4 reduces PR responding and CPP for a palatable food, consistent with a role for mNTS GLP-1R signaling in the control of food reward. Here, we consider alternate interpretations of our findings. Given that peripheral GLP-1R agonists are associated with nausea in humans (6, 7) and can produce conditioned taste avoidance in rodents (24), we examined the possibility that mNTS GLP-1R activation elicits malaise to suppress the motivation to seek food. Pica, the consumption of nonnutritive substances (e.g., kaolin clay) in response to nausea-inducing agents (3), was examined. Here, we showed that exendin-4 (0.025 μg) delivery to the mNTS did not induce pica but noted that delivery of a higher dose (2 μg) did cause pica (22). We conclude that the reduction in motivated feeding behaviors following mNTS leptin delivery in the current studies is not likely due to nausea/malaise.

To test whether mNTS GLP-1R activation may reduce appetitive feeding by altering activity and, thereby, performance, we directly measured activity parameters during the CPP test, in which the mNTS exendin-4 and vehicle-treated rats freely explored the CPP box. These analyses showed that mNTS exendin-4-treated rats were comparably active and active and
traveled a similar total distance compared with vehicle-treated animals, suggesting that mNTS GLP-1R-mediated effects on food-motivated behaviors are not explained by reductions in overall activity. It is also possible that mNTS GLP-1R stimulation reduces several motivated behaviors (e.g., drug taking, sexual behavior), including food motivation. This interesting concept is not directly tested in the current experiments but should be addressed in future studies.

The current data lead to conclusions about mNTS receptor signaling and appetitive behaviors that are similar to those of a recent study (20), in which mNTS leptin signaling reduced food-motivated behavior in PR and CPP paradigms. Collectively, these findings support a broader role for the mNTS in mediating food reward processing. While in the current study, direct mNTS injection of a GLP-1R agonist was sufficient to reduce food-motivated behavior, it is likely that mNTS projections engage other brain nuclei to exert effects on food reward. Motivational aspects of feeding have been linked with midbrain and forebrain circuits, including the mesolimbic system (VTA dopamine projections to the NAc) (4, 23); this pathway mediates operant responding for food, as well as CPP (5). Thus, these brain regions are likely direct or indirect targets of mNTS GLP-1R-expressing neurons. There are ascending monosynaptic projections from the mNTS to the VTA and the NAc (2, 9, 27), providing potential pathways by which mNTS neurons may engage neurons in the mesolimbic system. Whether mNTS GLP-1R-expressing neurons project to these regions is unknown.

**Perspectives and Significance**

Overall, these data are provocative and provide compelling support for the idea that mNTS GLP-1R signaling reduces food intake through a reduction in food-motivated appetitive behaviors and not exclusively via an interaction with satiation signal processing, long deemed the province of hindbrain neural processing. The current studies expand the range of brain nuclei involved in the GLP-1R-mediated control of food reward and begin to focus attention on the influence of mNTS on appetitive and motivated aspects of feeding. Future studies are required to elucidate the precise neural circuits and mechanisms (intracellular and neurochemical signals) mediating the effects of mNTS GLP-1R signaling on food-motivated appetitive behaviors.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**


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