GLP-1 receptor signaling contributes to anorexigenic effect of centrally administered oxytocin in rats

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Rinaman, Linda, and Elizabeth E. Rothe. GLP-1 receptor signaling contributes to anorexigenic effect of centrally administered oxytocin in rats. Am J Physiol Regulatory Integrative Comp Physiol 283: R99–R106, 2002. First published March 29, 2002; 10.1152/ajpregu.00008.2002.—The present study examined possible interactions between central glucagon-like peptide-1 (GLP-1) and oxytocin (OT) neural systems by determining whether blockade of GLP-1 receptors attenuates OT-induced anorexia and vice versa. Male rats were acclimated to daily 4-h food access. In the first experiment, rats were infused centrally with GLP-1 receptor antagonist or vehicle, followed by an anorexigenic dose of synthetic OT. Access to food began 20 min later. Cumulative food intake was measured every 30 min for 4 h. In the second experiment, rats were infused with OT receptor blocker or vehicle, followed by synthetic GLP-1 [(7–36) amide]. Subsequent food intake was monitored as before. The anorexigenic effect of OT was eliminated in rats pretreated with the GLP-1 receptor antagonist. Conversely, GLP-1-induced anorexia was not affected by blockade of OT receptors. In a separate immunocytochemical study, OT-positive terminals were found closely apposed to GLP-1-positive perikarya, and central infusion of OT activated c-Fos expression in GLP-1 neurons. These findings implicate endogenous GLP-1 receptor signaling as an important downstream mediator of anorexia in rats after activation of central OT neural pathways.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Zivic Miller, Zelionople, PA or Harlan Laboratories, Indianapolis, IN) were initially housed individually in hanging wire cages in a controlled environment (24°C, lights on from 0700 to 1900). In this environment, rats had ad libitum access to water and pelleted rat chow (Purina). All experimental procedures were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Cannulation procedures. Rats weighing 225–250 g were anesthetized with halothane (1.5–2% in oxygen) and placed into a stereotaxic frame with the incisor bar positioned 3.3 mm below horizontal zero. Rats were fitted with chronic indwelling 26-gauge stainless steel guide cannulas (Plastics One) aimed at the lateral ventricle. Guide cannulas were positioned 1.5 mm lateral to bregma on the coronal suture with the tip protruding 4.5 mm below the surface of the skull.

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Cannulas were fixed to the skull with anchor screws and dental acrylic and fitted with removable obturators that extended 0.5 mm beyond the tip of the guide cannula.

Automated food intake measurements. At least 1 wk after surgery, rats with ventricular cannulas were moved in groups of 6–10 to a new environment (24°C, lights on from 0300 to 1500). Rats were housed individually in clear Plexiglas boxes (25 × 30-cm floor, 22-cm height) with stainless steel rod floors, each equipped with a computer-driven pellet delivery and monitoring system (Med Associates). Drinking water was available ad libitum from sipper tubes within each box, but food access was restricted to a daily 4-h period beginning at lights out (1500). At lights out, a single 45-mg chow pellet (Precision Dustless Pellets, Bio-Serv, Frenchtown, NJ) was delivered automatically to a shallow feeding trough in each cage, thereby breaking a photobeam crossing the base of the trough. A new pellet was delivered automatically each time the rat removed the preceding pellet. The cumulative number of pellet deliveries (photobeam breaks) was recorded automatically every 30 min for the 4-h feeding period. Cage trays beneath the open rod floors were routinely inspected to ensure that delivered pellets were consumed. Data were collected and stored using Med PC software (Med Associates). Rats were acclimated to the new environment and feeding schedule for at least 1 wk before experimental manipulations, by which time stable 4-h daily food intakes of ~7–8% body wt were achieved. Rats were handled every 2 days for a brief period between 0100 and 0200 to record body weights, which remained steady or increased throughout each study. Because the rats’ body weights ranged from 261 to 293 g on testing days in these studies, food intake is expressed as percent body weight in each experiment.

Evaluation of antagonist efficacy and selection of peptide doses. Lyophilized peptides were dissolved in sterile 0.15 M NaCl no more than 30 min before infusion and were delivered intracerebroventricularly in a volume of 5 μl. The efficacy of the antagonists used to block OT receptors ([d(CH2)5, Tyr(Me)2, Orn8]vasotocin; Peninsula Laboratories) and GLP-1 receptors (des His1 Glu9-exendin 4; American Peptides) has been reported (14, 15, 25, 29). The potency of each batch of the antagonists used to block OT receptors ([d(CH2)5, Tyr(Me)2, Orn8]vasotocin; Peninsula Laboratories) and GLP-1 (7–36) amide (1 μg; Peninsular Laboratories) and GLP-1 receptor antagonist (10 μg) followed 10 min later by OT (1 μg; n = 6) or vehicle (0.15 M NaCl) (n = 5) or OT receptor blocker alone (10 μg; n = 5). All rats were tested as a group in each condition in the order indicated, with 3 or 4 baseline recovery days between each test. One rat was removed from the study because of a loose cannula on the vehicle infusion day, and so data for the vehicle and OT blocker alone conditions were collected from 5 rats rather than 6.

Food intake was significantly inhibited only after intracerebroventricular infusion of OT (*P < 0.05 compared with each other treatment). B: in the second experiment, a separate group of 5 rats received no infusion or intracerebroventricular infusion of oxytocin (OT; 1 μg) (n = 6) or OT receptor blocker (10 μg) followed 10 min later by OT (1 μg; n = 6) or vehicle (0.15 M NaCl) (n = 6) or OT receptor blocker alone (10 μg; n = 5). All 5 rats were tested in each condition in the order indicated, with 3 or 4 recovery days between each test. Food intake was significantly inhibited only after intracerebroventricular infusion of GLP-1 (*P < 0.05 compared with each other treatment). BW, body weight.

Feeding experiment 2. A separate group of rats (n = 9) was infused centrally with either 0.15 M NaCl vehicle or OT receptor antagonist (10 μg) followed 15 min later by infusion of GLP-1 (7–36) amide (1 μg; Peninsular Laboratories) (Fig. 1B). Food access began 20 min after OT infusion, at lights out (1500). Cumulative pellet intake values were collected every 30 min for 4 h. Rats were tested twice, so that all 9 rats were included in both treatment conditions over 2 testing days, with 6 baseline days intervening. Baseline 4-h intakes were recorded on the day before each of the 2 testing days.

Food intake data analysis. Pellet delivery data for each rat were converted to grams (number of pellets × 45 mg), ex-

Fig. 1. Results of 2 pilot experiments to verify the efficacy of synthetic peptides and receptor antagonists used in these studies. Cumulative 4-h food intake (displayed as group means ± SE) was recorded in rats deprived of food for the preceding 20 h and given access to food at lights out. A: in the first experiment, rats received no infusion or intracerebroventricular infusion of oxytocin (OT; 1 μg) (n = 6) or OT receptor blocker (10 μg) followed 10 min later by OT (1 μg; n = 6) or vehicle (0.15 M NaCl) (n = 5) or OT receptor blocker alone (10 μg; n = 5). All rats were tested as a group in each condition in the order indicated, with 3 or 4 baseline recovery days between each test. One rat was removed from the study because of a loose cannula on the vehicle infusion day, and so data for the vehicle and OT blocker alone conditions were collected from 5 rats rather than 6.

Food intake was significantly inhibited only after intracerebroventricular infusion of OT (*P < 0.05 compared with each other treatment). B: in the second experiment, a separate group of 5 rats received no infusion or intracerebroventricular infusion of glucagon-like peptide-1 (GLP-1; 1 μg) or GLP-1 receptor blocker (10 μg) followed 10 min later by GLP-1 (1 μg) or GLP-1 receptor blocker alone (10 μg). All 5 rats were tested in each condition in the order indicated, with 3 or 4 recovery days between each test. Food intake was significantly inhibited only after intracerebroventricular infusion of GLP-1 (*P < 0.05 compared with each other treatment). BW, body weight.
pressed as percent body weight (% BW), and then combined by experimental treatment group. Values are expressed as group means ± SE. Treatment-related differences in food intake within each experiment were tested for statistical significance by using one-way ANOVA, with the first infusion substance [vehicle, receptor blocker, or no infusion (baseline day)] as the independent variable. When F values indicated significant overall main treatment effects, ANOVA was followed up with post hoc t-tests using Dunn’s (Bonferroni) correction procedure to control for multiple comparisons. Differences were considered significant when P ≤ 0.05.

Verification of intraventricular cannula placement. At the conclusion of each feeding study, rats were anesthetized (83 mg ketamine and 17 mg xylazine ip; Fort Dodge Labs), infused centrally with 5 μl of India ink (50% in distilled water), and perfused transcardially with 0.15 M NaCl (50 ml) followed by 4% paraformaldehyde (300 ml). Brains were removed from the skull and inspected visually for evidence of ventricular diffusion of ink. Ink was observed in the lateral, third, and fourth ventricles in each animal included in this report.

Immunocytochemical study. A separate group of rats with chronic lateral ventricular guide cannulas was infused centrally with 5 μl of vehicle (0.15 M NaCl; n = 5) or vehicle containing 1 μg OT (n = 5) between 1000 and 1100. Three additional rats were handled and had their guide cannula obturators loosened and retightened, but they received no central infusions. Four hours later, rats were anesthetized, infused centrally with 5 μl of 50% India ink, and perfused transcardially with 0.15 M NaCl (50 ml) followed by fixative (2% paraformaldehyde, 1% acrolein; 250 ml). Fixed brains were removed from the skull, bisected coronally at the level of the pineal gland, and inspected visually for evidence of ventricular diffusion of ink. Ink was observed in the lateral, third, and fourth ventricles in each animal included in this report.

Fixed brains were postfixed overnight in 4% paraformaldehyde (4°C), cryoprotected overnight in 20% aqueous sucrose (4°C), frozen, and sectioned coronally at 25-μm intervals using a sliding microtome. Tissue sections were collected serially into six adjacent sets. With the use of methods described previously (19), one set of brain stem sections was processed for dual immunoperoxidase localization of nuclear c-Fos (1:50,000; rabbit polyclonal antiserum kindly provided by Dr. P. Larsen; see Ref. 21) and perikaryal GLP-1 (1:500; goat polyclonal antiserum). In each case, forebrain and brain stem sections were processed for dual immunoperoxidase localization of nuclear c-Fos, or OT immunolabeling. For this purpose, GLP-1-positive neurons that were closely apposed by one or more OT-immunolabeled fibers or terminals were present in close apposition. The proportion of GLP-1 neurons that were closely apposed by one or more OT-positive profiles was determined in each of the five cases. Combined values are presented as means ± SE.

RESULTS

Feeding experiment 1. Average cumulative baseline intakes on the days preceding each of the 2 experimental treatment days did not differ significantly at any time point; thus these were combined to generate a single set of baseline values. As shown in Fig. 2, central administration of saline vehicle followed by OT (1 μg) significantly suppressed food intake compared with baseline at each 30-min time point during the 4-h monitoring period (P ≤ 0.05 for each comparison). Central administration of GLP-1 receptor antagonist (10 μg) blocked the anorexigenic effect of OT, such that intake values in rats receiving the GLP-1 receptor blocker followed by OT (1 μg) were not significantly different from baseline at any time point.

Feeding experiment 2. As in experiment 1, average baseline intakes on the days preceding each experimental treatment day did not differ significantly, and so these data were combined to generate a single set of baseline values. As shown in Fig. 3, central administration of saline vehicle followed by GLP-1 (7–36) amide (1 μg) significantly suppressed food intake relative to baseline at each time point examined during the 4-h monitoring period (P ≤ 0.05 for each comparison). Prior administration of OT receptor antagonist (10 μg) did not significantly alter the anorexigenic effect of GLP-1 (7–36) amide at any time point during the 4-h monitoring period, such that food intake re-
mained significantly depressed relative to baseline \( (P \leq 0.05\) for each comparison).

**Immunocytochemical study.** As shown in Fig. 4, relatively few GLP-1-positive cells \((4 \pm 1\%\) expressed c-Fos in control rats that were handled but received no intracerebroventricular infusion. Conversely, central administration of synthetic OT \((1 \mu g)\) activated c-Fos in the majority \((72 \pm 19\%)\) of GLP-1-immunoreactive neurons (Figs. 4 and 5A). The proportion of GLP-1-positive neurons expressing c-Fos after intracerebroventricular infusion of 0.15 M NaCl vehicle \((24 \pm 11\%;\) Fig. 5B) was significantly greater than the proportion activated in noninfused controls but was significantly less than the proportion activated in rats infused centrally with OT \((P \leq 0.05\) for each comparison; Fig. 4). The hindbrain distribution of c-Fos- and/or GLP-1-positive neurons in two representative rats (one receiving vehicle intracerebroventricularly and one receiving OT intracerebroventricularly) is illustrated schematically in Fig. 6. Although central OT infusion activated the majority of GLP-1 neurons, these comprised but a subset of the total population of activated medullary neurons (Fig. 6, right). Determining the chemical phenotypes of these additional activated neurons was not a goal of the present study, but our initial observations indicate that they include catecholaminergic neurons of the A2 and A1 cell groups \((23)\).

Microscopic inspection of brain stem sections immunolabeled for both OT and GLP-1 demonstrated that OT-positive fibers and terminals were coextensive with GLP-1-positive perikarya (Fig. 5C). Quantitative analysis revealed that OT-immunopositive fibers and/or terminals were closely apposed to the majority \((81 \pm 8\%)\) of GLP-1-immunolabeled perikarya identified in the DVC and adjacent reticular formation.
DISCUSSION

Results from this study indicate that the anorexigenic effect of centrally infused OT is significantly attenuated in rats after pharmacological antagonism of central GLP-1 receptors, whereas the anorexigenic effect of centrally infused GLP-1 is fully maintained after blockade of central OT receptors. Our immunocytochemical data demonstrate that the majority of hindbrain GLP-1 neurons are activated to express c-Fos after central infusion of an anorexigenic dose of OT. Furthermore, the close apposition of OT-immunoreactive fibers and terminals with GLP-1-positive perikarya suggests that GLP-1 neurons might respond directly to endogenous OT released at synapses in the caudal medulla (a possibility that must be confirmed by ultrastructural analysis). The new findings presented here support the hypothesis that central GLP-1 neurons and signaling pathways play a functionally important role in treatment-induced anorexia in rats, including the well-known anorexia that results from activation of central OT neural signaling pathways.

No abnormal motor effects were produced by the dose of OT used in the present report (1 μg). This dose significantly suppressed food intake relative to baseline, although the magnitude of this effect (~26% average suppression across all time points) was smaller than the 50–60% suppression reported after central infusion of a higher dose of OT (13). We elected to use the lower effective dose in the present work because we found that a higher dose of synthetic OT (10 μg) sometimes caused barrel-rolling and seizurelike activity lasting ~5–10 min.

The 4-h postinfusion survival time used in the c-Fos expression study was based on preliminary work in which lateral ventricular infusion of 0.15 M NaCl vehicle activated c-Fos expression in the majority of hindbrain GLP-1-immunopositive neurons in rats killed 60–90 min after the intracerebroventricular infusion (23). Subsequent experiments indicated that vehicle-induced c-Fos expression in GLP-1 neurons was significantly reduced when rats were perfused 4 h after the intracerebroventricular infusion. Because the anorexigenic effect of centrally administered OT persisted for at least 3–4 h (see Fig. 2), a 4-h postinfusion survival interval was considered valid for analysis of OT-induced c-Fos expression that was temporally related to OT-induced anorexia but separable from the nonspecific effects of the intracerebroventricular infusion itself. Even in rats killed 4 h after infusion, however, approximately six times as many GLP-1 neurons were activated in rats after intracerebroventricular infusion of saline vehicle compared with the proportion activated in control rats that were handled but received no infusion. Nevertheless, central infusion of OT activated significantly more GLP-1 neurons compared with either control condition (i.e., ~18 times more than in noninfused controls and 3 times more than in vehicle-infused controls; see Fig. 4).

Despite our findings that central infusion of saline vehicle activated c-Fos expression in GLP-1 neurons,
vehicle infusion did not inhibit food intake in our pilot study (Fig. 1A). It is possible that intracerebroventricular saline produced a relatively transient anorexigenic effect that was not apparent in this study. Alternatively, the neuronal activation produced by intracerebroventricular saline infusion may simply be insufficient to suppress feeding in the absence of some other necessary signal. In this regard, vehicle-induced c-Fos expression in GLP-1 neurons is not accompanied by significant activation of either hindbrain catecholaminergic neurons or hypothalamic OT-positive neurons (23). In contrast, GLP-1-positive neurons, hindbrain catecholaminergic neurons, and OT-positive neurons are commonly activated by several treatments that inhibit food intake, such as systemic administration of lithium chloride (LiCl), cholecystokinin, or lipo polysaccharide (16, 17, 19, 22, 32).

Two previous reports indicated that endogenous central GLP-1 receptor signaling is an important factor underlying LiCl-induced anorexia (18, 25). However, the precise brain location(s) of receptors that mediate the presumed anorexigenic and/or nauseogenic effects of endogenous GLP-1 remain unclear. The present study was inspired by previous work suggesting a possible interaction of GLP-1 signaling pathways with central OT neurons. Hypothalamic regions that contain OT neurons are specifically targeted by GLP-1-immunoreactive axon terminals (9), and OT neurons are activated after central infusion of synthetic GLP-1 (10). Furthermore, pretreatment of rats with a central OT receptor antagonist reduces the anorexic effects of LiCl (14). Because the anorectic effects of LiCl are also attenuated after blockade of central GLP-1 receptors, we hypothesized that GLP-1 may inhibit food intake due to activation/recruitment of central OT signaling pathways. However, blockade of central OT receptors did not attenuate the anorexigenic effect of centrally infused GLP-1 in the present study. Instead, the converse was true: the anorexigenic effect of centrally infused OT was completely eliminated after pharmacological antagonism of central GLP-1 receptors.

These findings suggest that anorexigenic responses in which central OT neural pathways have been implicated may also include a functionally “downstream” role for GLP-1. In addition to its role in LiCl-induced anorexia, central OT receptor signaling contributes to anorexia in rats after systemic administration of hypertonic saline (14) and after central infusion of corticotropin-releasing factor (CRF) (15). Importantly, these anorexigenic treatments also inhibit vagally mediated gastric motility and emptying (4, 5), as does central infusion of GLP-1 (7). The present study confirmed that OT-immunopositive nerve terminals are closely apposed to GLP-1-positive neurons in the DVC and that these neurons are activated by central infusion of an anorexigenic dose of OT. The OT-containing projection from the PVN to the DVC provides a tonic inhibitory influence over vagally mediated gastric motility that is further amplified by anorexigenic treatments (3, 4). Thus experimental treatments that activate central OT signaling pathways may inhibit gastric motility and food intake, at least in part, due to OT-mediated recruitment/activation of hindbrain GLP-1 neurons. If so, then functional blockade of central GLP-1 signaling pathways should also attenuate the gastric inhibitory effects of centrally infused OT and other anorexigenic treatments, such as systemically administered LiCl or centrally infused CRF, parallel with attenuation of treatment-induced anorexia. This has not yet been examined. However, the ability of restraint stress to stimulate colonic motility and fecal output in rats is completely reversed after blockade of central GLP-1 receptors (6), implicating GLP-1 more generally as a candidate neurotransmitter in stress-induced changes in gut motility.

Perspectives

“Treatment-induced anorexia” refers to a significant suppression of food intake, relative to baseline conditions, that is directly associated with a controlled experimental condition intended (ideally) to model some natural circumstance associated with inhibition of food
intake. For example, to the extent that LiCl treatment is a valid experimental model of toxemia and nausea and considering evidence that both OT and GLP-1 central signaling mechanisms are involved in the inhibition of feeding produced by LiCl, we interpret our findings as evidence that GLP-1 is an important downstream mediator of toxemia/nausea-related suppression of food intake. We do not know whether OT and/or GLP-1 signaling pathways are involved in the inhibition of feeding associated with more natural physiological conditions or whether these central pathways are involved in anorexigenic responses to social or cognitive stressors. We are not aware of any experimental anorexias for which central OT or GLP-1 signaling has been demonstrated not to play a role. However, in most cases, the potential involvement of these neural pathways has not been investigated.

Among the many brain areas that potentially mediate treatment-induced anorexia, it is likely that the DVC (including its GLP-1 neural constituents) plays a central role. As the major brain stem relay center for visceral sensory information and descending projections from the hypothalamus and limbic forebrain, the DVC is well positioned to integrate interoceptive cues with cognitive/emotional states and thereby serve a "comparator function" for organizing appropriate behavioral and autonomic responses. Direct and delayed projections from the DVC to somatic and autonomic motor nuclei provide potential routes by which the DVC might exert context-dependent control over both the ingestion and digestion of food. The present findings should serve to focus greater attention on the DVC and particularly on its resident GLP-1 neurons as an integral component of the central neural circuits that underlie ingestive behavior.

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REFERENCES


R105


