A functional role for central glucagon-like peptide-1 receptors in lithium chloride-induced anorexia

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Rinaman, Linda. A functional role for central glucagon-like peptide-1 receptors in lithium chloride-induced anorexia. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1537–R1540, 1999.—The present study sought to determine whether central glucagon-like peptide-1 (GLP-1) receptor signalling contributes to the anorexigenic effects of systemically administered lithium chloride (LiCl). Male Sprague-Dawley rats with chronic intracerebroventricular (ICV) cannulas were acclimated to a feeding schedule that included daily 30-min access to palatable mash. In the first experiment, ICV infusion of a GLP-1-receptor antagonist [exendin-4-(3–39)] significantly attenuated (10 µg dose) or completely blocked (20 µg dose) the inhibition of food intake produced by subsequent ICV infusion of GLP-1-(7–36) amide (5 µg). In the second experiment, rats were infused with 0, 10, or 20 µg of the GLP-1-receptor antagonist, followed by injection of 0.15 M LiCl (50 mg/kg ip) or the same volume of 0.15 M NaCl. The ability of LiCl treatment to suppress food intake was significantly attenuated in rats that were pretreated with the GLP-1-receptor antagonist. These results support the view that central mechanisms underlying LiCl-induced anorexia include a prominent role for endogenous GLP-1 neural pathways.

exendin-4-(3–39); nausea; food intake; conditioned taste aversion

SYSTEMIC ADMINISTRATION of the nauseogenic agent lithium chloride (LiCl) suppresses food intake and produces conditioned taste aversion (CTA) in rats (4, 7, 8, 13). Because central administration of synthetic glucagon-like peptide-1 (GLP-1) also suppresses food intake and can produce CTA (5, 16–19), it is possible that treatment-induced activation of endogenous central GLP-1 neural pathways might contribute to the anorexigenic effects of LiCl. Indeed, a recent study showed that systemically administered LiCl activates expression of the immediate-early gene product, c-fos, in GLP-1-immunoreactive hindbrain neurons, including those that project to the hypothalamus (12). It has also been reported that pharmacological antagonism of central GLP-1 receptors significantly attenuates LiCl-induced c-fos expression in the rat brain stem (16). The goal of the present study was to determine whether pharmacological antagonism of central GLP-1 receptors attenuates the ability of LiCl treatment to suppress food intake. These results have been presented in abstract form (11).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Zivic Miller, Zelienople, PA) were housed individually in hanging wire cages in a controlled environment (24°C, lights on from 0700 to 1900). Rats had ad libitum access to water and pelleted rat chow (Purina), except as noted. 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 a 7:1 mixture of ketamine:xylazine (Fort Dodge Labs; 3 mg/ml; 0.1 ml/100 g body wt ip) 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. 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. Each rat received an intramuscular injection of 0.125 ml gentamicin sulfate (100 mg/ml; Fort Dodge Laboratories) during surgery as a prophylactic measure.

Correct cannula placement was verified 1 wk after surgery. For this purpose, water-replete rats were injected intracerebroventricularly with 10 µl of artificial cerebrospinal fluid (aCSF; see Ref. 9) containing 10 ng of ANG II (Sigma). All rats used in the experiments described below drank at least 10 ml of water within 30 min after ANG II administration, evidence for accurate cannula placement (e.g., see Ref. 14).

Evaluation of antagonist efficacy. The efficacy of the GLP-1-receptor antagonist used in this work [exendin-4-(3–39), Bachem] was evaluated by testing its ability to block the anorexia produced by central injection of synthetic GLP-1. For this purpose, rats with intracerebroventricular (ICV) cannulas (n = 30, 230–275 g) were acclimated for 5–7 days to a feeding schedule that included 3-h afternoon access to pelleted chow and 30-min morning access to a palatable mash (a 1:1 mixture of powdered chow and peanut butter). Water was available ad libitum. Stable intakes of mash were achieved by day 4 or 5, during which time the rats’ body weights remained stable or increased. On the morning of the test (day 6 or 7 of the acclimation period), rats were injected intracerebroventricularly with 10 µl of aCSF containing 0, 10, or 20 µg of the GLP-1-receptor antagonist followed 10 min later by ICV injection of 10 µl aCSF containing 0 or 5 µg synthetic GLP-1-(7–36) amide (Bachem; n = 5 for each
treatment combination). Lyophilized peptides were freshly dissolved in aCSF shortly before each experiment. Rats received mash 15 min after the second ICV injection. Cumulative 30-min food intakes were recorded.

Rats were kept on the same feeding schedule for the next 3 days without any injections. On the morning of the fourth day, the drug efficacy experiment was repeated in a limited crossover design, in which each rat received the same dose of receptor antagonist as in the first trial but the alternate dose of synthetic GLP-1. Thus 10 data points were achieved for each treatment combination. The outcome of this drug efficacy analysis indicated that the anorexigenic effect of 5 µg GLP-1 administered centrally was significantly attenuated by the 10-µg dose of receptor antagonist and was blocked by the 20-µg dose (see RESULTS). Thus the same doses of receptor antagonist were used in the LiCl experiment.

LiCl experiment. A different group of rats with ICV cannulas (n = 38; 240–280 g) was acclimated to the same feeding schedule described in Evaluation of antagonist efficacy. Rats were injected intracerebroventricularly with 10 µl aCSF containing 0, 10, or 20 µg of the GLP-1-receptor antagonist followed 10 min later by injection of 0.15 M LiCl (50 mg/kg ip) or the same amount of 0.15 M NaCl (n = 6 or 7 for each treatment combination). In previous work, similar doses of LiCl significantly inhibited food intake in rats for at least 1 h after intraperitoneal administration (7) and activated c-fos expression in hindbrain GLP-1-immunoreactive neurons (12). Rats received mash 15 min after the intraperitoneal injection. Cumulative 30-min food intakes were recorded. Rats were kept on the same feeding schedule for the next 3 days without any injections. On the morning of the fourth day, the experiment was repeated in a limited crossover design, in which each rat received the same ICV injection as in the first trial, but the alternate intraperitoneal injection (e.g., LiCl or NaCl). Thus at least 12 data points were achieved for each treatment combination.

Data analysis. Food intakes were converted to percent body weight and combined by treatment group. Values are expressed as means ± SE. Treatment-related differences in food intake were tested for statistical significance by using two-way ANOVA. When F values indicated significant overall main treatment effects and interactions, the ANOVA was followed up with planned post hoc comparisons using Dunn’s (Bonferroni) correction to control for repeated-measures analysis. Differences were considered significant when P < 0.05.

RESULTS

Antagonist efficacy. In rats that first received only aCSF intracerebroventricularly [i.e., the 0-µg dose of exendin-4-(3—39)-receptor antagonist], subsequent ICV administration of 5 µg GLP-1-(7—36) amide suppressed 30-min intake of mash by ~72% (Fig. 1). The magnitude of this effect is consistent with previous reports (15, 16, 18). In contrast, the same dose of GLP-1-(7—36) amide reduced intake by only 43% in rats that first received the 10-µg dose of GLP-1-receptor antagonist and did not reduce food intake in rats that first received the 20-µg dose of receptor antagonist. Thirty-minute food intake in this paradigm was not affected by either the 10- or 20-µg dose of exendin-4-(3—39) alone (Fig. 1), similar to previous reports using different GLP-1-receptor antagonists (15, 16).

The amount of water consumed by rats during the 30-min feeding test (prandial drinking) was not measured. However, all rats periodically stopped eating to drink water, regardless of treatment condition. Rats that ate relatively little mash during the feeding test also spent little time engaged in drinking behavior. Similar observations were made in the LiCl experiment.

LiCl experiment. As shown in Fig. 2, intraperitoneal administration of LiCl suppressed 30-min intake of mash by ~64% in rats that first received ICV aCSF (0
µg antagonist). The magnitude of the observed inhibitory effect of LiCl treatment on food intake was similar to previous reports (4, 7) and also was similar to the effect produced by central infusion of GLP-1(7–36) amide (see Fig. 1).

Pharmacological antagonism of central GLP-1 receptors significantly attenuated but did not block the inhibitory effect of LiCl treatment on 30-min food intake (Fig. 2). A dose-related effect of the GLP-1-receptor antagonist was observed, such that the 20-µg dose was significantly more effective than the 10-µg dose to attenuate LiCl-induced anorexia.

DISCUSSION

The principal finding of this study is that inhibition of food intake by LiCl treatment is significantly attenuated by pharmacological antagonism of central GLP-1 receptors. This new observation provides strong support for the idea that endogenous GLP-1 neural pathways play an important functional role in mediating LiCl-induced anorexia.

GLP-1 is a major splicing product of the proglucagon peptide precursor. In the rodent brain, GLP-1 is expressed only by short-axon cells in the glomerular layer of the olfactory bulb and by a discrete group of neurons located in and around the dorsal vagal complex in the caudal hindbrain (1, 6). GLP-1-receptor gene expression and binding sites have been localized to specific nuclei in the hypothalamus and other forebrain and brain stem regions that receive inputs from hindbrain GLP-1-positive neurons (6, 12).

A potential role for central GLP-1 as a neurochemical mediator of anorexia has been inferred from demonstrations that ICV administration of synthetic GLP-1 significantly decreases food intake and, in some cases, promotes the formation of CTA (5, 15–19). Supportive evidence that endogenous GLP-1 pathways are involved in anorexia was suggested by a recent c-Fos study in which GLP-1-positive neurons (including those that project to the hypothalamus) were specifically activated by LiCl and other treatments that inhibit food intake and produce visceral malaise (12). The results of the present study offer additional and more direct support for the view that central GLP-1-receptor signalling is an important factor underlying LiCl-induced anorexia. The present results also are consistent with a recent report that LiCl-induced c-Fos expression in the brain stem is attenuated by central infusion of a GLP-1-receptor antagonist (16). Other unpublished data from the same research group suggest that antagonism of central GLP-1 receptors can blunt LiCl-induced CTA (Ref. 16, p. 169). Such a result, if confirmed, would support a role for central GLP-1 neural pathways in the aversive (nauseogenic) properties of LiCl treatment.

LiCl apparently exerts both its anorexigenic and aversive effects by acting at chemoreceptors in the area postrema (AP) (2, 4, 13). In this regard, however, it is interesting to note that ablation of the AP in rats blocks the ability of LiCl to induce CTA (and thus, presumably, to induce nausea) but does not interfere with the ability of LiCl to reduce food intake (4). As pointed out in that study, LiCl treatment has two separate effects on food intake; one to diminish appetite for food, and the other to increase aversion for food (3). AP lesions appear to eliminate the increased aversion without affecting the decreased appetite (4). Conversely, antagonism of central GLP-1 receptors may affect both types of behavioral responses to LiCl treatment.

The precise brain location(s) of GLP-1 receptors relevant to the anorexigenic effects of LiCl treatment remains unclear. However, an interaction with central oxytocin (OT) neurons seems likely. Hypothalamic regions that contain OT neurons are specifically targeted by GLP-1-immunoreactive axon terminals (6, 12), and OT neurons are activated both by LiCl treatment (10) and by central infusion of synthetic GLP-1 (15). Furthermore, pretreatment of rats with a central OT-receptor antagonist markedly blunts, but does not eliminate, the anorectic effects of LiCl (9). Similarly, in the present study, pharmacological antagonism of GLP-1 receptors completely blocked the anorexigenic effect of centrally administered synthetic GLP-1, but did not completely block the anorexigenic effect of LiCl treatment. In this regard, it is possible that additional neurochemical systems account for the remaining LiCl-induced inhibition of food intake and/or that the receptor antagonist was unable to access the full complement of central GLP-1 receptors that potentially mediate LiCl-induced anorexia. Additional work is needed to address these questions.

In summary, the new data reported here indicate that central GLP-1 neural systems participate in LiCl-induced anorexia. It will be important to determine whether central GLP-1-receptor signalling plays a role in other experimental and physiological circumstances that are associated with inhibition of food intake.

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