Differences in the Central Anorectic Effects of Glucagon-Like Peptide-1 and Exendin-4 in Rats

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OBJECTIVE—Glucagon-like peptide (GLP)-1 is a regulatory peptide synthesized in the gut and the brain that plays an important role in the regulation of food intake. Both GLP-1 and exendin (Ex)-4, a long-acting GLP-1 receptor (GLP-1r) agonist, reduce food intake when administered intracerebroventricularly, whereas Ex4 is much more potent at suppressing food intake when given peripherally. It has generally been hypothesized that this difference is due to the relative pharmacokinetic profiles of GLP-1 and Ex4, but it is possible that the two peptides control feeding via distinct mechanisms.

RESEARCH DESIGN AND METHODS—In this study, the anorectic effects of intracerebroventricular GLP-1 and Ex4, and the sensitivity of these effects to GLP-1r antagonism, were compared in rats. In addition, the GLP-1r dependence of the anorectic effect of intracerebroventricular Ex4 was assessed in GLP-1r$^{-/-}$ mice.

RESULTS—Intracerebroventricular Ex4 was 100-fold more potent than GLP-1 at reducing food intake, and this effect was insensitive to GLP-1r antagonism. However, GLP-1r antagonists completely blocked the anorectic effect of intraperitoneal Ex4. Despite the insensitivity of intracerebroventricular Ex4 to GLP-1r antagonism, intracerebroventricular Ex4 failed to reduce food intake in GLP-1r$^{-/-}$ mice.

CONCLUSIONS—These data suggest that although GLP-1rs are required for the actions of Ex4, there appear to be key differences in how GLP-1 and Ex4 interact with central nervous system GLP-1r and in how Ex4 interacts with GLP-1r in the brain versus the periphery. A better understanding of these unique differences may lead to expansion and/or improvement of GLP-1–based therapies for type 2 diabetes and obesity. ***Diabetes* 58: 2820–2827, 2009**

Glucagon-like peptide (GLP)-1 is a product of the preproglucagon gene (1) that is synthesized in the distal ileum (2) as well as the caudal nucleus of the solitary tract (NTS) and ventrolateral medulla (3). Although GLP-1 is perhaps best known for its essential role in the regulation of peripheral glucose homeostasis, multiple lines of evidence suggest that GLP-1 also acts in the central nervous system (CNS) to regulate food intake. In support of this hypothesis, long-acting GLP-1 receptors (GLP-1rs) are expressed in brain regions known to regulate energy balance, such as the mediobasal hypothalamus and the caudal brainstem (3,4), and consistent with a role for GLP-1 as a putative satiety signal, central administration of GLP-1 potently reduces short-term food intake (5,6). Conversely, central administration of the GLP-1r antagonist exendin (Ex) (9-39) (Ex9) increases food intake and body weight (7), suggesting that endogenous GLP-1 has a physiological role in the regulation of energy balance.

Recently, the GLP-1 system has emerged as a novel therapeutic target for type 2 diabetes, as peripheral GLP-1 infusion effectively lowers blood glucose levels and improves glucose tolerance in humans (8). However, because circulating active GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) (9–11), alternative strategies for targeting the GLP-1 system have been developed, including stable GLP-1 analogues and DPP-4 inhibitors. One such analog is Ex4, a peptide originally isolated from the saliva of the Gila monster (*Heloderma suspectum*), which is a highly potent, DPP-4–resistant GLP-1r agonist in vitro and in vivo (12,13). Recently, exenatide (a synthetic Ex4) and the DPP-4 inhibitor sitagliptin were Food and Drug Administration approved as therapies for type 2 diabetes. However, whereas both drugs effectively improved glycemic control in clinical trials (14,15), Ex4, but not sitagliptin, was also associated with significant weight loss (14,16).

The above finding is compelling in that it raises the possibility that Ex4, at doses used clinically, may have in vivo actions that are substantively different from those of intact GLP-1 achieved through DPP-4 inhibition. Although studies using GLP-1r knockout (GLP-1r$^{-/-}$) mice provide strong evidence that the GLP-1r is necessary for the in vivo actions of Ex4 (17–21), other studies using GLP-1r antagonists suggest that Ex4, particularly in the brain, may act, at least in part, independently of GLP-1r (22–24). Therefore, we tested the hypothesis that the central anorectic effect of Ex4 is different from that of GLP-1.

RESEARCH DESIGN AND METHODS

Adult male Long-Evans rats (Harlan, Indianapolis, IN), GLP-1r$^{-/-}$ mice, and their wild-type C57BL/6J littermates were housed individually in plastic rodent cages and maintained on a 12-h light/dark cycle with ad libitum access to water and pelleted rodent diet (Harlan Teklad). Rats and mice were outfitted with cannulas (Plastics One, Roanoake, VA) aimed at the third cerebral ventricle, and correct cannula placement was verified as previously described (25,26). All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Peptides. GLP-1 and Ex4 were obtained from Bio Nebraska (Lincoln, NE) and American Peptide (Sunnyvale, CA), respectively. The GLP-1r antagonists His1, Glu8 Ex4 (dHEx), and Ex (9-39) (Ex9) were obtained from Baylor College of Medicine Protein Synthesis Core (Houston, TX) and Tocris (Ellisville, MO), respectively. All peptides were dissolved in saline and administered either intracerebroventricularly in a volume of 1.0 μl or intraperitoneally in a volume of 1.0 ml/kg.

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Food intake studies. Rats and mice were fed ad libitum at all times except for the mornings of study days. During this time, food was removed from the animals' cages and weighed 4 h before lights off, and animals were assigned to weight-matched groups. Pretreatment (saline or GLP-1r antagonist) injections commenced 1 h before lights off, and treatment (saline or GLP-1r agonist) injections commenced 30 min before lights off. Injection order was counterbalanced across all experimental groups to evenly distribute subtle variations in timing of injections. Food was returned to the animals' cages at lights off, and food intake and body weight were measured at selected time points.

First, dose-response curves for anorexia induced by intracerebroventricular GLP-1 (0, 0.3, 1.0, 3.0, and 10.0 μg) and Ex4 (0, 0.01, 0.03, 0.1, and 0.3 μg) were established. Based on these results, time courses of anorexia induced by intracerebroventricular GLP-1 (3.0 nmol, ~10.0 μg) and Ex4 (0.03 nmol, ~0.1 μg) were compared, and conditioned taste aversion (CTA) to the same doses of intracerebroventricular GLP-1 and Ex4 was assessed as previously described (27).

To assess the ability of central GLP-1r antagonism to block anorexia induced by central GLP-1 and Ex4, rats were pretreated with intracerebroventricular saline, dHEx (10.0 μg, [28]), or Ex9 (100.0 μg) and then treated with intracerebroventricular saline, GLP-1 (10.0 μg), or Ex4 (0.1 μg). To assess the ability of peripheral GLP-1r antagonism to block anorexia induced by peripheral Ex4, rats were pretreated with intraperitoneal saline or dHEx (1.0 mg/kg) and then treated with intraperitoneal saline or Ex4 (10.0 μg/kg). Finally, to assess the ability of central Ex4 to reduce food intake in GLP-1r$^{-/-}$ mice, mice were treated with intracerebroventricular saline or Ex4 (1.0 μg).

c-Fos immunohistochemistry. To assess the ability of central GLP-1r antagonism to block neuronal activation induced by central GLP-1 and Ex4, rats were pretreated with intracerebroventricular saline or dHEx (10.0 μg) and then treated with intracerebroventricular saline, GLP-1 (10.0 μg), or Ex4 (0.1 μg). Two hours later, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.1 mol/l PBS followed by 4.0% paraformaldehyde/PBS. Brains were postfixed at 4°C for 24 h in 4.0% paraformaldehyde/PBS and stored at 4°C in 30.0% sucrose/PBS. Serial coronal forebrain sections and longitudinal hindbrain sections were collected at 35 μm using a freezing microtome and stored at −20°C in cryoprotectant.

After washing with PBS, sections were incubated in 1.0% hydrogen peroxide/PBS for 10 min, followed by 1.0% sodium borohydride/PBS for 30 min. Sections were blocked for 1 h in 0.1% BSA/0.4% Triton-X-100/PBS and incubated overnight at room temperature in blocking solution containing rabbit anti–c-Fos diluted at 1:5,000 (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA). The next morning, sections were washed and incubated at room temperature for 1 h in blocking solution containing biotinylated goat anti-rabbit IgG diluted at 1:200 (BA-1000; Vector Laboratories, Burlingame, CA) followed by 1 h in ABC solution diluted 1:800 in PBS (PK6100; Vector Laboratories) and 10 min in DAB-nickel solution. Finally, sections were washed with 0.1 mol/l phosphate buffer, mounted on gelatin-coated slides, and cover slipped.

For quantification of c-Fos immunoreactivity in the central nucleus of the amygdala (CeA), paraventricular nucleus of the hypothalamus (PVN), and nucleus of the solitary tract (NTS), digital images of sections were acquired using a digital camera attached to a Zeiss microscope (Zeiss, Thornwood, NY). For each brain, two sections per area were analyzed, and special care was taken to compare only sections within the same plane along the rostro-caudal (CeA and PVN) or dorso-ventral (NTS) axis. c-Fos immunoreactivity was quantified as optical density using the National Institutes of Health program Scion Image.

Tissue culture studies. INS-1 cells were seeded in 35-mm six-well plates at a density of 2×10^5 cell/well in 1.5 ml of media consisting of RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1.0 mmol/l sodium pyruvate, 2.0 mmol/l L-glutamine, 50.0 μmol/l β-mercaptoethanol, and 0.5 mg/ml gentamicin sulfate and grown in a 37°C incubator in an atmosphere of 5% CO_2 and 95% air and 100% humidity for 3 days until nearly confluent. On day 4, cells were washed with PBS and replaced with fresh media. On day 5, cells were preincubated for 2 h in 2.0 ml of buffer consisting of Krebs-Ringer bicarbonate buffer (KRB) supplemented with 0.1% BSA and 30 mg/dl glucose and then washed twice with 2.0 ml of the same buffer solution. Cells were then incubated for 1 h in 1.0 ml of KRB supplemented with 0.1% BSA, 200 mg/dl glucose, and 1.0 nmol/l GLP-1, 0.01 nmol/l Ex4, or 1.0 nmol/l Ex4 with or without 100 nmol/l dHEx. Finally, incubation buffer was harvested, centrifuged, decanted, and stored at −20°C for immunoreactive insulin (IRI) assay, and cells were washed once with 1.0 ml of preincubation buffer and then extracted with 1.0 ml of acid ethanol for 2 h at −20°C, after which acid ethanol was diluted 1:200 with Tris assay buffer for IRI assay in cell layer. IRI was measured using a radioimmunoassay as previously described (29).

Statistical analysis. All values are reported as means ± SE. Data were analyzed using one- or two-way ANOVA or two-way repeated-measures ANOVA. Post hoc multiple comparisons were made using Tukey's post hoc test. Significance was set at $P < 0.05$ for all analyses.

RESULTS

Comparison of intracerebroventricular GLP-1– and Ex4-induced anorexia. Consistent with previous reports, intracerebroventricular GLP-1 and Ex4 elicited potent, dose-dependent reductions in 4-h food intake (Fig. 1*A* and *B*; $P < 0.05$, one-way ANOVA with Tukey's post hoc test). However, Ex4 significantly reduced food intake at doses much lower than those of GLP-1. Specifically, 10.0 μg of GLP-1 and 0.1 μg of Ex4 produced comparable degrees of anorexia, reducing food intake to 56 and 45% of control values, respectively. These data indicate that, when administered into the third ventricle, Ex4 is roughly 100-fold more potent than GLP-1 at reducing food intake.

Figure 1*C* illustrates the time course of intracerebroventricular GLP-1– and Ex4-induced anorexia. Whereas 3.0 nmol (~10.0 μg) of GLP-1 and 0.03 nmol (~0.1 μg) of Ex4 both actively suppressed food intake up to 4 h, only Ex4 elicited persistent anorexia that remained detectable throughout the 24 h of observation ($P < 0.05$, two-way repeated-measures ANOVA with Tukey's post hoc test). Furthermore, these doses of GLP-1 and Ex4 both led to the formation of a CTA (Fig. 1*D*; $P < 0.05$, one-way ANOVA with Tukey's post hoc test). Interestingly, there was a strong trend toward a significantly lower preference ratio of Ex4-treated rats versus GLP-1–treated rats ($P = 0.052$), suggesting that the aversive effects of Ex4 were more pronounced than those of GLP-1.

Sensitivity of intracerebroventricular GLP-1 and Ex4 to GLP-1r antagonism. Although previous studies have reported an inability to block certain effects of Ex4 with GLP-1r antagonists, these studies did not necessarily account for the significantly greater potency of Ex4 over GLP-1. Therefore, we sought to compare the ability of GLP-1r antagonists to block anorexia and neuronal activation induced by doses of intracerebroventricular GLP-1 and Ex4 that produce effects of comparable magnitude. Pretreatment with either 10.0 μg of dHEx or 100.0 μg of Ex9 caused near-complete blockade of anorexia induced by 10.0 μg of GLP-1 (Fig. 2*A* and *C*; $P < 0.05$ by two-way ANOVA with Tukey's post hoc test). However, whereas 0.1 μg of Ex4 and 10.0 μg of GLP-1 elicited comparable degrees of anorexia, the doses of dHEx and Ex9 that nearly abolished GLP-1–induced anorexia failed to block the anorectic effect of Ex4 (Fig. 2*B* and *D*), although a nonsignificant trend was observed with dHEx ($P = 0.148$).

To determine whether neuronal activation in response to GLP-1 and Ex4 was also differentially sensitive to GLP-1r antagonism, the effect of intracerebroventricular dHEx to block c-Fos immunoreactivity induced by intracerebroventricular GLP-1 and Ex4 was compared. At the same doses as used above, GLP-1 and Ex4 both induced c-Fos immunoreactivity in identical brain regions, including the CeA, PVN, and the NTS (Fig. 3*A*–*C*; $P < 0.05$ by two-way ANOVA with Tukey's post hoc test). The magnitude of c-Fos immunoreactivity induced by GLP-1 and Ex4 was similar in the PVN and NTS, whereas GLP-1 induced slightly more c-Fos immunoreactivity than Ex4 in the CeA. In the CeA, dHEx significantly blocked c-Fos immunoreactivity induced by GLP-1 ($P < 0.05$); however, in the PVN and the NTS, this difference failed to reach statistical significance. Nonetheless, for all three regions, the amount of c-Fos immunoreactivity in brains treated with dHEx and Ex4 was significantly greater than that of brains treated

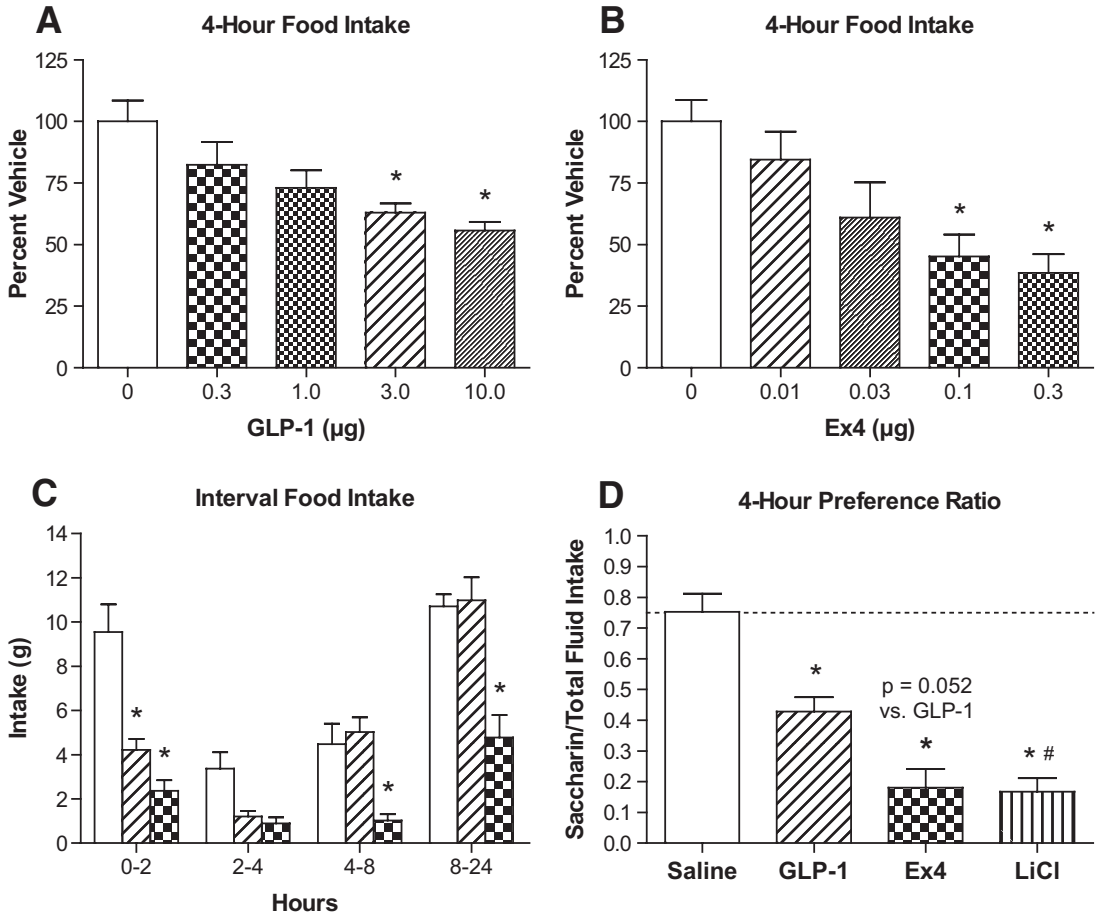

FIG. 1. Comparison of anorectic effects of intracerebroventricular GLP-1 and Ex4. *A* and *B*: Dose-response curves for intracerebroventricular GLP-1 (*A*) and Ex4 (*B*). Cumulative 4-h food intake is shown. *C*: Time course of anorectic effects of intracerebroventricular GLP-1 (3.0 nmol) and Ex4 (0.03 nmol) over 24 h. □, Saline; ▨, GLP-1 (3.0 nmol/l); ▩, Ex4 (0.03 nmol/l). *D*: Preference ratios for 0.1% saccharin versus total fluid intake during the 4-h two-bottle access to saccharin and water. Saccharin was previously paired with intracerebroventricular saline, intracerebroventricular GLP-1 (3.0 nmol), intracerebroventricular Ex4 (0.03 nmol), or intraperitoneal LiCl (0.15 mol/l administered at 2.0% body wt). Dotted line represents preference ratio for saline-treated rats. Data are represented as means ± SE. *$P < 0.05$ vs. saline. #$P < 0.05$ vs. GLP-1.

with saline, dHEx alone, or dHEx and GLP-1 ($P < 0.05$). These results, combined with the food intake data, suggest that CNS actions of Ex4 are relatively insensitive to competitive GLP-1r antagonism.

Potency of Ex4 and sensitivity to GLP-1r antagonism in vitro. Because dHEx, a validated but lesser used GLP-1r antagonist (28,30,31), failed to block anorexia and neuronal activation induced by intracerebroventricular Ex4, we sought to determine whether dHEx is an effective antagonist of Ex4 in vitro by assessing its ability to block insulin secretion induced by Ex4 in the rat pancreatic islet cell line INS-1. As expected, 1.0 nmol/l GLP-1 significantly augmented insulin secretion above that of glucose alone, and this effect was completely blocked by coincubation with 100 nmol/l dHEx (Fig. 4; $P < 0.05$ by two-way ANOVA with Tukey's post hoc test). However, in contrast to our in vivo data, 0.01 nmol/l Ex4 failed to augment insulin secretion, whereas 1.0 nmol/l Ex4 had an effect that was comparable to 1.0 nmol/l GLP-1. Moreover, this effect was completely blocked by coincubation with 100 nmol/l dHEx ($P < 0.05$).

Sensitivity of intraperitoneal Ex4 to GLP-1r antagonism. To determine whether the insensitivity of Ex4 to GLP-1r antagonism was specific to CNS administration, we assessed the ability of dHEx to block anorexia induced by intraperitoneal Ex4. As expected, 10 μg/kg of intraperitoneal Ex4 significantly reduced food intake at 4 h (Fig. 5; $P < 0.05$ by two-way ANOVA with Tukey's post hoc test). Surprisingly, pretreatment with 1.0 mg/kg i.p. dHEx, the same 100-fold excess of antagonist that failed to block anorexia induced by intracerebroventricular Ex4, significantly attenuated this effect ($P < 0.05$).

Effect of intracerebroventricular Ex4 in wild-type and GLP-1r$^{-/-}$ mice. The insensitivity of CNS Ex4 effects to GLP-1r antagonism raises the possibility that Ex4 may act in part via a GLP-1r–independent mechanism. To determine whether the GLP-1r is required for the central anorectic effect of Ex4, intracerebroventricular Ex4 was administered to wild-type and GLP-1r$^{-/-}$ mice. In wild-type mice, 1.0 μg of intracerebroventricular Ex4 elicited profound anorexia such that daily food intake and body weight were significantly reduced for up to 48 and 72 h, respectively (Fig. 6*A* and *B*; $P < 0.05$ by two-way repeated-measures ANOVA with Tukey's post hoc test). Conversely, this same high dose of intracerebroventricular Ex4 had no effect on food intake or body weight in GLP-1r$^{-/-}$ mice (Fig. 6*C* and *D*).

DISCUSSION

Because Ex4 (14), but not the DPP-4 inhibitor sitagliptin (16), produces weight loss in patients, it is critical that we

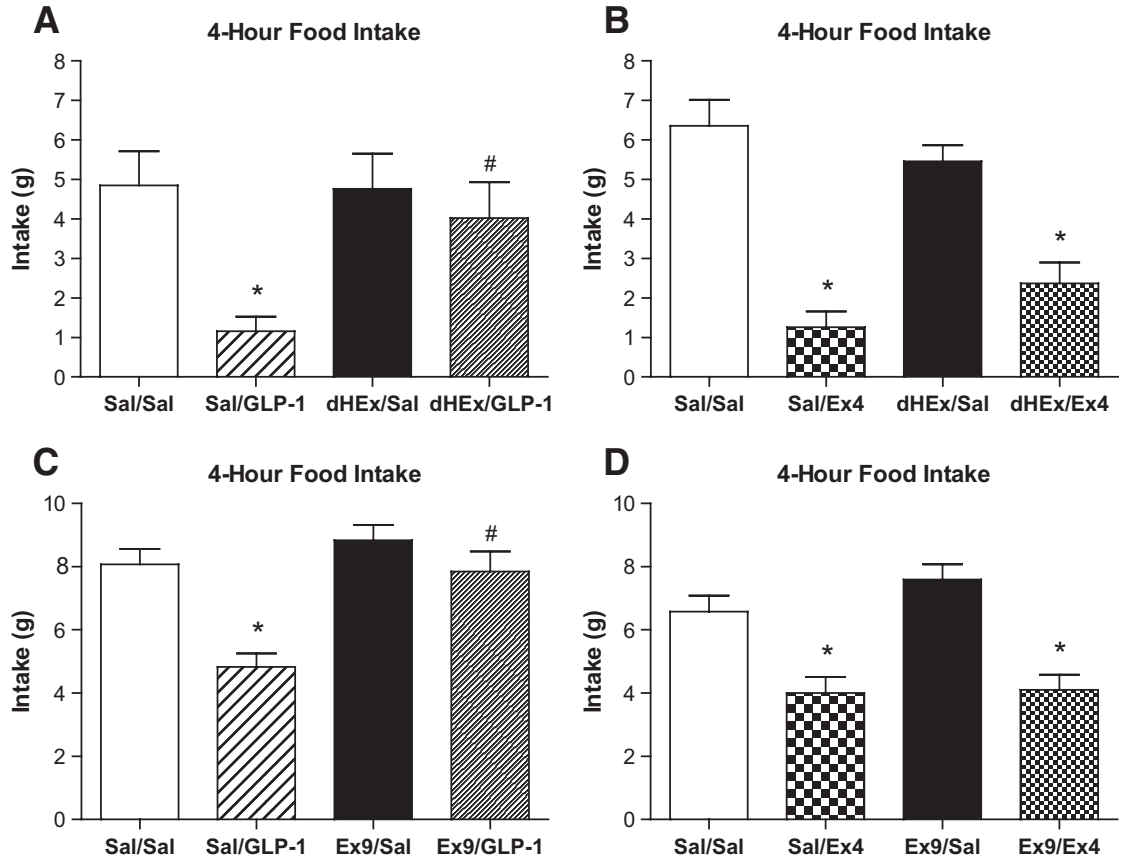

FIG. 2. Effect of GLP-1r antagonists on anorexia induced by intracerebroventricular GLP-1 and Ex4. *A* and *B*: Rats were pretreated with intracerebroventricular dHEx (10 μg) followed by intracerebroventricular GLP-1 (10 μg) (*A*) or Ex4 (0.1 μg) (*B*). *C* and *D*: Rats were pretreated with intracerebroventricular Ex9 (100 μg) followed by intracerebroventricular GLP-1 (10 μg) (*C*) or Ex4 (0.1 μg) (*D*). Cumulative 4-h food intake is shown. Data are represented as means ± SE. *$P < 0.05$ vs. Sal/Sal. #$P < 0.05$ vs. Sal/GLP-1.

better understand the unique anorectic properties of Ex4. To this end, we report key distinctions between the central anorectic effects of Ex4 and native GLP-1. Not only do our data confirm that central GLP-1 and Ex4 differ significantly in potency and duration of action, but they also reveal novel differences between the two peptides regarding sensitivity to GLP-1r antagonism.

Ex4, when administered into the CNS, reduces food intake in a manner distinct from that of GLP-1. Consistent with previous reports (32,33), central Ex4 reduced 4-h food intake at doses 30- to 100-fold lower than those required by GLP-1 to cause equivalent anorexia. Importantly, this difference in potency at 4 h cannot simply be explained by differences in duration of action, as both 3.0 nmol of GLP-1 and 0.03 nmol of Ex4 reduced food intake to a comparable extent from 0 to 2 h and 2 to 4 h. However, in contrast to GLP-1, Ex4 dynamically reduced food intake over 24 h of observation, indicating that even at significantly lower doses, central Ex4 exhibits a significantly longer duration of action.

Consistent with our food intake data, 0.1 μg of central Ex4 produced an almost identical degree of neuronal activation as 10.0 μg of GLP-1 in the PVN and the NTS but interestingly not in the CeA. It is possible that because

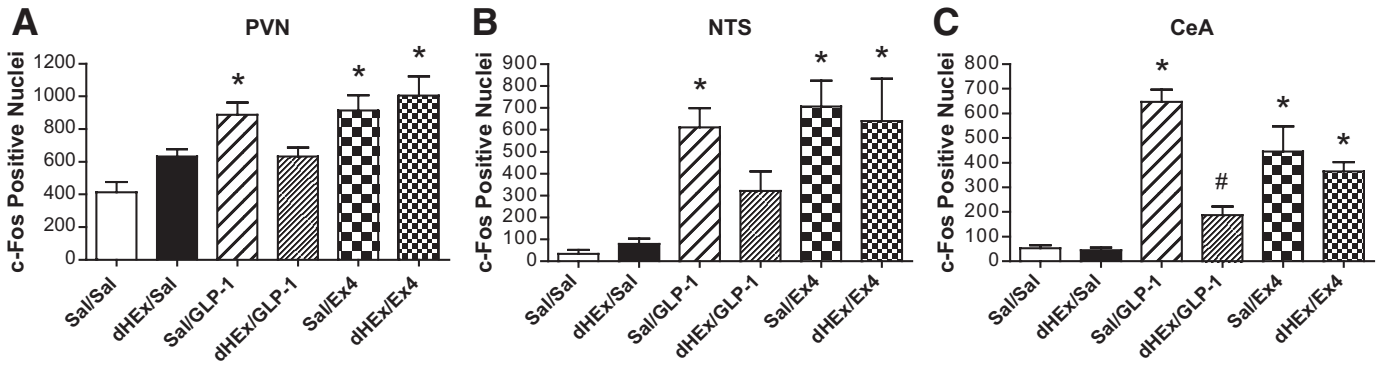

FIG. 3. Effect of dHEx on c-Fos immunoreactivity induced by intracerebroventricular GLP-1 and Ex4. Quantification of c-Fos–positive nuclei in the PVN (*A*), NTS (*B*), and CeA (*C*) of rats that were treated with intracerebroventricular saline or dHEx (10 μg) followed by intracerebroventricular saline, GLP-1 (10 μg), or Ex4 (0.1 μg) and killed 2 h later. Data are represented as means ± SE. *$P < 0.05$ vs. Sal/Sal. #$P < 0.05$ vs. Sal/GLP-1.

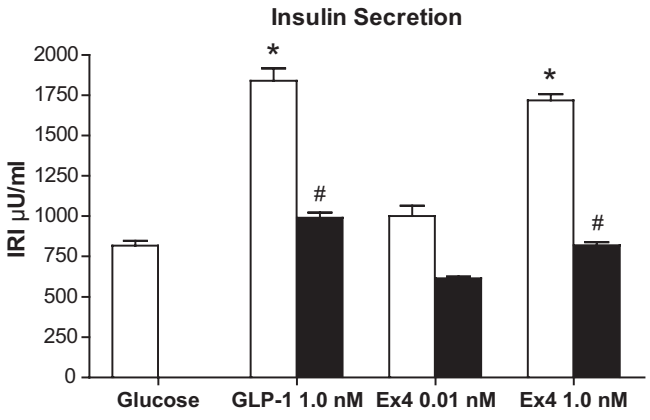

FIG. 4. Effect of dHEx (100 nmol/l) on insulin secretion induced by GLP-1 (1.0 nmol/l) and Ex4 (0.01 and 1.0 nmol/l) in the presence of glucose (200 mg%). Data are represented as means ± SE. *$P < 0.05$ vs. glucose. #$P < 0.05$ vs. glucose + GLP-1 (1.0 nmol/l) or glucose + Ex4 (1.0 nmol/l). □, Saline; ■, 100 nmol/l dHEx.

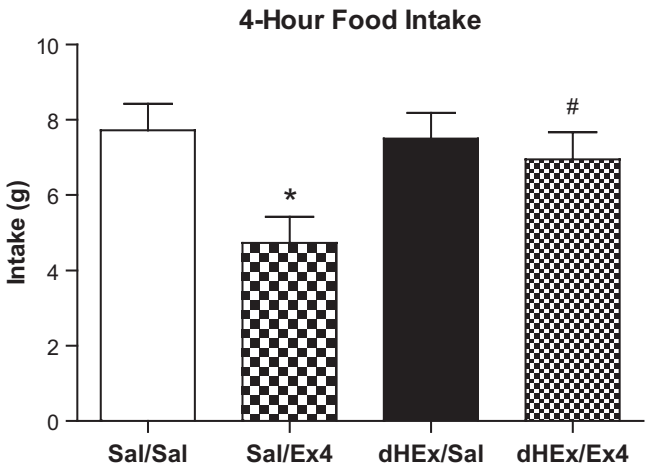

FIG. 5. Effect of intraperitoneal dHEx (1.0 mg/kg) on anorexia induced by intraperitoneal Ex4 (10.0 μg/kg). Cumulative 4-h food intake is shown. Data are represented as means ± SE. *$P < 0.05$ vs. Sal/Sal. #$P < 0.05$ vs. Sal/Ex4.

100-fold less Ex4 than GLP-1 was administered, less peptide diffused through the neuropil to the CeA, which, unlike the PVN and the NTS, does not abut the ventricular system. Despite producing less neuronal activation in the CeA, an area important for the formation of GLP-1–mediated CTA (34), central Ex4 produced a lower preference ratio for saccharin than GLP-1, suggesting that Ex4 induced a greater visceral illness response. Although a proportional relationship between GLP-1r–mediated neuronal activity and behavioral responses has yet to be established, these data are significant because they suggest that enhanced visceral illness or aversive learning may in part underlie the potent anorectic effect of central Ex4.

The above data and those of others support a role for Ex4 as a highly potent, long-acting CNS GLP-1r agonist, yet the mechanism for this unique pharmacological profile remains unknown. One possibility is that GLP-1 and Ex4 bind differently to CNS GLP-1r. However, in vitro and ex vivo comparisons of binding affinity have yielded equivocal results (35–38), and it remains unclear whether GLP-1 and Ex4 remain bound to CNS GLP-1r for different periods of time. A second possibility is that GLP-1 and Ex4 differentially desensitize CNS GLP-1r. However, data from Baggio et al. (39) revealed no difference in the ability of GLP-1 and Ex4 to desensitize the GLP-1r in vitro. Moreover, because GLP-1 and Ex4 were administered as boluses, this hypothesis fails to adequately explain the present results. Finally, it is possible that differential clearance and/or degradation of GLP-1 versus Ex4 account for their distinct pharmacological profiles within the CNS.

Perhaps the most striking difference between central GLP-1 and Ex4 revealed by our data are their sensitivity to GLP-1r antagonism. Whereas dHEx almost completely blocked anorexia and neuronal activation induced by GLP-1, it failed to significantly block that induced by an equipotent dose of Ex4. This phenomenon is not specific to dHEx, as Ex9 also failed to block anorexia induced by central Ex4. However, dHEx is an effective antagonist of Ex4 in vitro, as it completely blocked the enhancement of glucose-stimulated insulin secretion induced by Ex4 in INS-1 cells. In addition, dHEx is an effective antagonist of Ex4 in vivo, as intraperitoneal dHEx completely blocked anorexia induced by intraperitoneal Ex4. Taken together, these data indicate that compared with GLP-1, Ex4 is relatively insensitive to GLP-1r antagonism. Moreover, this phenomenon seems to be specific for central effects but not peripheral effects, many of which have been reported to be blocked by Ex9 (40–42).

Certainly, previous studies (22–24) have reported an inability to block Ex4 effects with GLP-1r antagonists. However, they may not have adequately accounted for the increased potency of Ex4 versus GLP-1. Here, we closely controlled for this difference and found that pretreatment with GLP-1r antagonists significantly blocked anorexia induced by central GLP-1 but not an equipotent and, importantly, 100-fold lower dose of Ex4. Moreover, this phenomenon is not secondary to differences in agonist duration of action, as it was observed at early time points when both GLP-1 and Ex4 dynamically reduced food intake. Nor is it secondary to the antagonist duration of action, as timing of pretreatment and treatment injections was consistent across all experiments, and similar trends were observed with both c-Fos and food intake (data not shown) at 2 h.

While intriguing, these data are difficult to reconcile with our other experiments. Specifically, we found no differences between GLP-1 and Ex4 in either potency or sensitivity to dHEx in vitro, although this discrepancy might easily be explained by obvious differences between animal models and immortalized cell lines. More difficult to explain, however, is the comparison to our peripheral Ex4 food intake study, in which the same 100-fold excess of dHEx, this time administered intraperitoneally, completely blocked intraperitoneal Ex4-induced anorexia. Consequently, it is possible that fundamental differences exist between central and peripheral GLP-1r, which may occur at the level of posttranslational processing, protein–protein interactions, or coupling to second-messenger systems.

Perhaps the most obvious explanation for the discrepancies between central GLP-1 and Ex4 is that the latter acts in part independently of the GLP-1r. However, consistent with previous reports (18), central Ex4 had no effect on either food intake or body weight in GLP-1r$^{-/-}$ mice, suggesting that the GLP-1r is required for these effects. Although the lack of Ex4 effects in GLP-1r$^{-/-}$ mice provides a strong basis to rule out GLP-1r independence, there is some evidence for both functional (43) and structural (44) differences between the GLP-1 systems of mice and

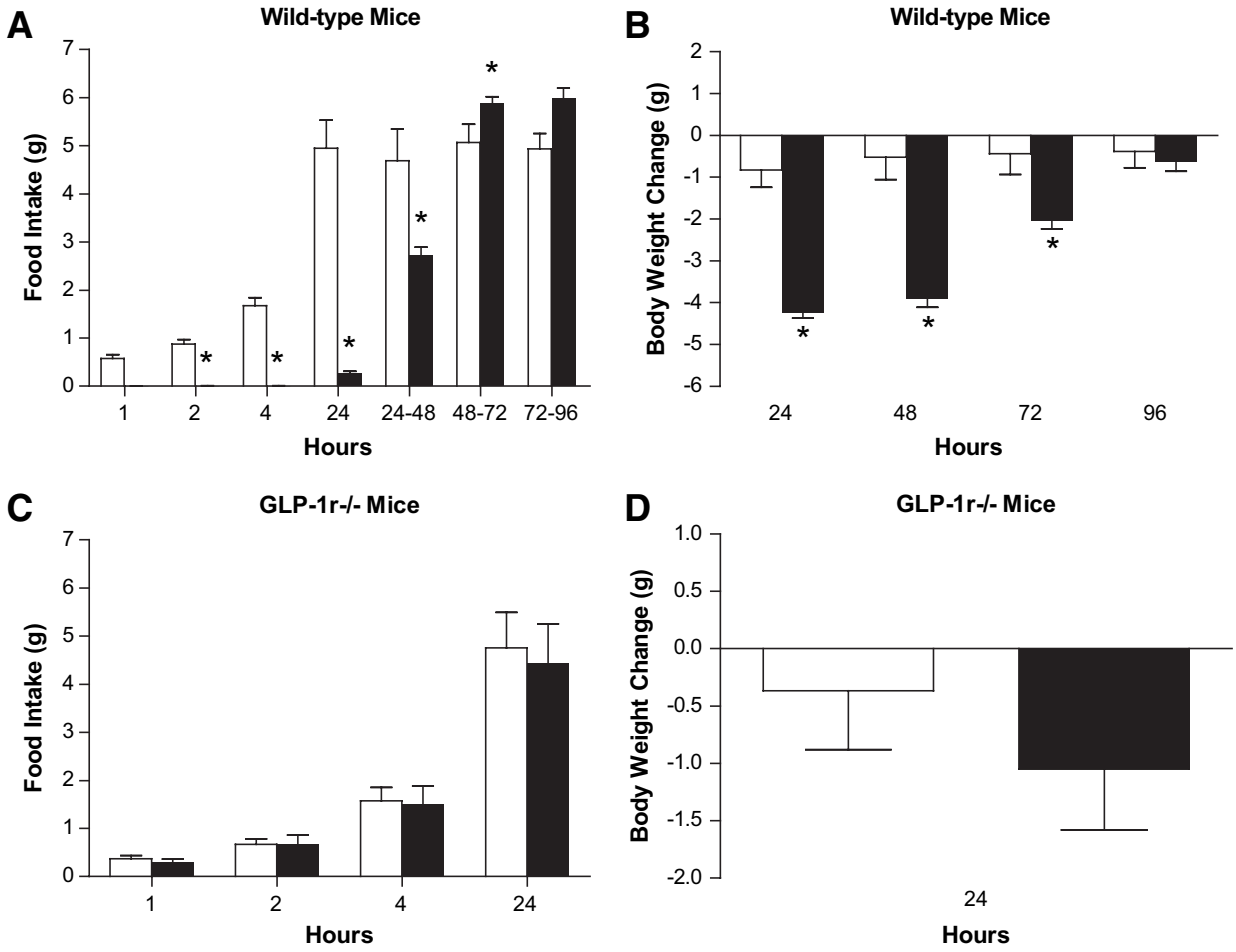

FIG. 6. Effect of intracerebroventricular Ex4 in wild-type and GLP-1r$^{-/-}$ mice. *A* and *B*: Wild-type mice received intracerebroventricular saline (□) or Ex4 (1.0 μg) (■). Food intake (*A*) and body weight change (*B*) were measured over 96 h. *C* and *D*: GLP-1r$^{-/-}$ mice received intracerebroventricular saline or Ex4 (1.0 μg). Food intake (*C*) and body weight change (*D*) were measured over 24 h. Data are represented as means ± SE. *$P < 0.05$ vs. saline.

rats. Recently, Sowden et al. (20) reported that Ex4 increases heart rate in rats but not mice. Finally, other studies reporting an inability to block Ex4 effects with GLP-1r antagonists have all been conducted in rats (22–24). Although none of these observations provides definitive evidence for GLP-1r–independent effects of Ex4, they do raise the possibility that Ex4 may interact with the GLP-1r in a species-dependent manner.

Although difficult to reconcile with the above data, our findings regarding central Ex4 and GLP-1r antagonists are consistent with several reports of in vivo effects of Ex4 that are insensitive to GLP-1r antagonists (22–24). Recently, it was reported that central Ex4 decreases ghrelin secretion in fasted rats (24). Not only was this effect insensitive to Ex9 blockade, it was also elicited by Ex9 alone, consistent with several in vitro reports of independent Ex9 effects (45–50). Whereas these data, like ours, fail to prove GLP-1r independence of Ex4, they are nonetheless significant in that they provide potential mechanistic insight into the unique anorectic properties of central Ex4, particularly its duration of action. For instance, ongoing GLP-1r signaling by Ex4 may prevent circulating ghrelin levels from rising in response to Ex4-mediated reductions in food intake, leading to an attenuation or delay in the subsequent drive to eat and thus a prolonged duration of anorexia. However, because our experiments used ad libitum–fed rats, whose circulating ghrelin levels should be low, and because Ex4 is more efficacious in fed versus fasted rats (51), it seems unlikely that Ex4's effects on ghrelin secretion underlie either its increased potency acutely or its insensitivity to GLP-1r antagonists in the present studies.

Because studies have generally found no effect of Ex4 in GLP-1r$^{-/-}$ mice (17–21), it seems reasonable to cite strictly pharmacological differences when explaining discrepancies between in vivo effects of GLP-1 and Ex4. However, in many ways, the existing data fail to adequately support this hypothesis. For instance, some in vitro studies have found Ex4 to have greater potency and affinity for the GLP-1r than native GLP-1 (35), but these differences, at least in potency, are significantly smaller than those reported here. Regarding antagonist sensitivity, one potential explanation for our findings is that Ex4 is more able to displace antagonists from the GLP-1r. However, studies have generally reported little to no difference in the ability of GLP-1 versus Ex4 to displace radio-labeled Ex9 (52–55). Taken together, our data, combined with the existing literature, provide conclusive evidence for distinct pharmacological profiles of GLP-1 and Ex4, yet further studies are needed to understand whether pharmacological differences alone are sufficient to explain the unique in vivo effects of Ex4.

In conclusion, our data indicate that the central, but not peripheral, anorectic effect of Ex4 is insensitive to GLP-1r

antagonism, yet GLP-1r is required for this effect. These data suggest that there are important differences between the in vivo pharmacological properties of GLP-1 and Ex4 within the CNS. Moreover, they underscore the need for a greater understanding of how these ligands interact with CNS GLP-1r, particularly in light of recent data revealing novel roles of CNS GLP-1r activity in the regulation of peripheral glucose homeostasis and cardiovascular function (56,57). Such an understanding is critical if we are to maximize the therapeutic benefit of Ex4 and other GLP-1–based therapies.

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