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Central & peripheral glucagon-like peptide-1 receptor signaling differentially regulate addictive behaviors

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HIGHLIGHTS

- Mice received (Ex-4, a GLP-1 analog) following disruption of CNS GLP-1R signaling.
- Amphetamine reward, alcohol intake and hedonic feeding were examined thereafter.
- Ex-4 failed to reduce amphetamine reinforcement behavior and alcohol consumption.
- Hedonic feeding behavior was partially attenuated following Ex-4 pretreatment.
- Data elucidate mechanisms whereby GLP-1 signaling regulates reinforced behaviors.

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ABSTRACT

Recent data implicate glucagon-like peptide-1 (GLP-1), a potent anorexigenic peptide released in response to nutrient intake, as a regulator for the reinforcing properties of food, alcohol and psychostimulants. While, both central and peripheral mechanisms mediate effects of GLP-1R signaling on food intake, the extent to which central or peripheral GLP-1R signaling regulates reinforcing properties of drugs of abuse is unknown. Here, we examined amphetamine reinforcement, alcohol intake and hedonic feeding following peripheral administration of EX-4 (a GLP-1 analog) in FLOX and GLP-1R KD^{Nestin} (GLP-1R selectively ablated from the central nervous system) mice (n = 13/group). First, the effect of EX-4 pretreatment on the expression of amphetamine-induced conditioned place preference (Amp-CPP) was examined in the FLOX and GLP-1R KD^{Nestin} mice. Next, alcohol intake (10% v/v) was evaluated in FLOX and GLP-1R KD^{Nestin} mice following saline or EX-4 injections. Finally, we assessed the effects of EX-4 pretreatment on hedonic feeding behavior. Results indicate that Amp-CPP was completely blocked in the FLOX mice, but not in the GLP-1R KD^{Nestin} mice following EX-4 pretreatment. Ex-4 pretreatment selectively blocked alcohol consumption in the FLOX mice, but was ineffective in altering alcohol intake in the GLP-1R KD^{Nestin} mice. Notably, hedonic feeding was partially blocked in the GLP-1R KD^{Nestin} mice, whereas it was abolished in the FLOX mice. The present study provides critical insights regarding the nature by which GLP-1 signaling controls reinforced behaviors and underscores the importance of both peripheral and central GLP-1R signaling for the regulation of addictive disorders.

1. Introduction

Glucagon-like peptide-1 (GLP-1), a feeding peptide with anorectic properties, is secreted by the gastrointestinal tract [1,2] and released from neurons in the nucleus of the solitary tract (NTS) [3,4,5]. Both GLP-1 and Exendin-4 (EX-4, a synthetic GLP-1 analog) administration attenuate the reinforcing properties of food, alcohol and psychostimulants [6–8], suggesting a role for GLP-1 that extends beyond regulation of energy homeostasis. The appetite suppressive effects of GLP-1 require both vagal afferent and central nervous system (CNS) signaling mechanisms [7]. Recent studies indicate that peripheral administration of GLP-1 attenuates psychostimulant-reinforced behaviors [9] and that GLP-1R stimulation within brain reward circuitry reduces alcohol consumption and food reinforcement [3,8,10]. However, it is unknown if activation of peripheral or CNS GLP-1R signaling regulates the reinforcing properties of psychostimulant drugs. It is also unclear what role peripheral GLP-1R signaling plays in the regulation of alcohol and palatable food intake. We hypothesized that peripheral GLP-1R signaling (i.e. vagal afferent signaling) regulates alcohol consumption and hedonic feeding behavior whereas central GLP-1R

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signaling controls psychostimulant reinforcement. To test this hypothesis, we evaluated amphetamine reward, alcohol consumption and hedonic food intake following peripheral administration of EX-4 in GLP-1R KD^{Nestin} mice in which GLP-1R was selectively ablated from the CNS.

2. Methods and materials

2.1. Animals

GLP-1R KD^{Nestin} mice, where GLP-1R was selectively ablated from the CNS, along with their respective wild-type littermates were generated as reported previously [11]. Genetic ablation involved inserting *loxP* sites surrounding *glp1r* gene (FLOX) and crossbreeding with nestin-*Cre* mice, generating GLP-1R KD^{Nestin} mice. Study animals were derived from crosses between heterozygous animals back-crossed >10 generations onto a C57BL6/J genetic background. Current studies were performed with male mice, which were housed in a 12-h light/dark cycle with regular chow and water available ad lib, except when indicated. All animal procedures were carried out in accordance with NIH guidelines and were in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee at the University of Cincinnati.

2.2. Diets

All mice were maintained on ad libidum chow (Teklad, 3.41 kcal/g, 0.51 kcal/g from fat) and water unless noted. The hedonic feeding experiments utilized high-fat diet (HFD) (Research Diets, New Brunswick, NJ, 4.41 kcal/g, 1.71 kcal/g from fat). Dietary composition of standard rodent chow and HFD used in the present study has been described previously [12].

2.3. Drugs

The effects of GLP-1 manipulation were measured using the synthetic GLP-1 agonist exendin-4 (30 μg/kg) (Bachem, Torrance, CA), a dose selected based on its ability to reduce cocaine reward in mice [9].

2.4. Amphetamine-induced conditioned place preference

We utilized conditioned place preference (CPP) to examine effects of EX-4 on amphetamine reward in FLOX and GLP-1R KD^{Nestin} mice. The CPP studies were conducted as described previously [13,14]. All mice (n = 13/group) were habituated to the CPP apparatus for 15 min. On the next day (CPP-Day 1), mice (n = 6–7/group) received either saline or D-amphetamine (1.0 mg/kg, i.p.), were placed into one side of the chamber and were detained in the chamber for 30 min. On the following day (CPP-Day2), the treatment (saline or D-amphetamine) was reversed and mice were detained into the opposite side of the chamber. The treatment (amphetamine or saline) and side of chamber (black or white) were counterbalanced across 12 consecutive days of testing. On the test day (CPP-Day 13), mice were placed in the center chamber following saline or EX-4 (30 μg/kg; i.p.) injections and allowed free access to all chambers for 15 min. Time spent in each chamber and locomotor activity was determined using a computerized tracking system (TopScan, Clever Sys, Inc., Reston, VA). Data are presented as percent of total time spent in saline- or amphetamine-paired side following saline or EX-4 pretreatment for both FLOX and GLP-1R KD^{Nestin} mice.

2.5. Alcohol intake

FLOX and GLP-1R KD^{Nestin} mice (n = 13/group) were allowed to consume 10% alcohol solution or water in their home cage in a two-bottle choice paradigm and 24 h alcohol intake was recorded. Next, mice were divided in groups (n = 6–7/group), matched based on the baseline alcohol consumption, and alcohol (10%) intake was recorded for 90 min following saline or EX-4 (30 μg/kg; i.p.) injections. Alcohol intake is expressed as intake per kilogram of body weight (g/kg).

2.6. Hedonic feeding

We utilized a feeding paradigm in which rodents voluntarily consume a palatable test diet following a non-palatable preload to determine the effects of central or peripheral GLP-1R signaling on hedonic feeding behavior [15,16]. GLP-1R KD^{Nestin} mice along with their wild type littermates (n = 6–7/group) were pre-exposed to HFD to prevent neophobia. Subsequently, all mice were food deprived for twenty-one hours. The next day, chow food hoppers were weighed, placed in each cage and subsequently reweighed each hour for 2 h. To investigate the effects of GLP-1R signaling on hedonic feeding, mice received a single peripheral EX-4 injection after the first hour of chow exposure only. The nature of this manipulation allowed us to examine the effect GLP-1R activation on re-feeding that occurred during the second hour of chow exposure. Following the second hour of chow access, a separate set of food hoppers containing HFD was weighed and placed in each cage beside the previously placed chow hoppers. The opportunity to consume HFD after re-feeding on chow constitutes the hedonic portion of this test. At the conclusion of the test (4 h after food was returned), both sets of food hoppers (chow and HFD) were re-weighed to determine effects of EX-4 on HFD intake after re-feeding.

2.7. Statistical analysis

CPP data were analyzed by mixed-model two-way ANOVA to compare percent of total time spent in saline or amphetamine-paired side following saline or EX-4 treatment, with post-hoc paired-sample *t*-tests to compare within group effects. Alcohol intake data were analyzed using a univariate analysis of variance to compare the effect of saline or EX-4 on alcohol drinking. A mixed-model two-way ANOVA compared the effect of saline or EX-4 pretreatment on chow intake, with post-hoc paired sample t-tests to compare within group effects. A univariate analysis of variance was used to compare HFD intake in the FLOX and GLP-1R KD^{Nestin} mice following saline or EX-4 pretreatment. To determine the extent to which EX-4 pretreatment affected HFD intake in FLOX and GLP-1R KD^{Nestin} mice, we compared 3rd hour HFD intakes to zero (indicating no food intake) using one-sample t-test. All statistical comparisons were conducted at 0.05α level.

3. Results

3.1. GLP-1R regulation of amphetamine CPP

A mixed model ANOVA revealed a main effect of exposure during conditioning suggesting that amphetamine induced a strong CPP in both FLOX and GLP-1R KD^{Nestin} ($F_{1,11} = 7.493$, $p = 0.019$) mice. Following training, EX-4 pretreatment completely blocked the expression of Amp-CPP in the FLOX mice without affecting locomotion in either group. However, this treatment was ineffective at reducing Amp-CPP in the GLP-1R KD^{Nestin} mice (Fig. 1).

3.2. GLP-1R regulation of alcohol consumption

Baseline alcohol consumption did not differ among any of the groups (data not shown). Alcohol intake on the test day was not significantly different compared to baseline intake in saline injected FLOX ($t(5) = 2.041$, $p > 0.05$) or GLP-1R KD^{Nestin} ($t(5) = 0.4341$, $p > 0.05$) mice. However, Ex-4 pretreatment selectively blocked ($F_{1,11} = 8.7$, $p = 0.013$) alcohol consumption in the FLOX mice, but was ineffective in the GLP-1R KD^{Nestin} mice (Fig. 2). Furthermore, there was no difference in body weight 24 h following EX-4 injections in either group (Table 1).