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A Functional Inhibitory Role of Habenular Glucagon-Like Peptide-1 (GLP-1) in Forebrain Reward Signaling

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Abstract

There is emerging evidence implicating glucagon-like peptide-1 (GLP-1) in reward, including palatable food reinforcement and alcohol-based reward circuitry. While recent findings suggest that mesolimbic structures, such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc), are critical anatomical sites mediating the role of GLP-1's inhibitory actions, the present study focused on the potential novel impact of GLP-1 within the habenula, a region of the forebrain expressing GLP-1 receptors. Given that the habenula has also been implicated in the neural control of reward and reinforcement, we hypothesized that this brain region, like the VTA and NAc, might mediate the anhedonic effects of GLP-1. Rats were stereotaxically implanted with guide cannula targeting the habenula and trained on a progressive ratio 3 (PR3) schedule of reinforcement. Separate rats were trained on an alcohol two-bottle choice paradigm with intermittent access. The GLP-1 agonist exendin-4 (Ex-4) was administered directly into the habenula to determine the effects on operant responding for palatable food as well as alcohol intake. Our results indicated that Ex-4 reliably suppressed PR3 responding and that this effect was dose-dependent. A similar suppressive effect on alcohol consumption was observed. These findings provide initial and compelling evidence that the habenula may mediate the inhibitory action of GLP-1 on reward, including operant and drug reward. Our findings further suggest that GLP-1 receptor mechanisms outside of the midbrain and ventral striatum are critically involved in brain reward neurotransmission.

Keywords

Alcohol, Anhedonia, Appetitive Motivation, Brain Reward, Ethanol, Exendin-4, GLP-1 Receptors, Operant Responding, Palatable Food Intake, Reward Salience

1. Introduction

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide produced in intestinal L-cells and in the nucleus tractus solitarius (NTS) of the brain [1]. GLP-1 receptor (GLP-1R) expression has been identified in regions throughout the central nervous system (CNS), including hypothalamic nuclei as well as mesolimbic and other forebrain structures [1] [2]. While studies investigating the effects of exogenous GLP-1 may be difficult to interpret given the short half life of the peptide [3], other work has shown that ventricular or hypothalamic injection of the GLP-1 agonist, exendin-4 (Ex-4), reduces food intake in freely feeding rats [4] [5] [6] [7]. Ventricular administration of Ex-4 results in elevated expression of interleukin-1 (IL-1) and interleukin-6 (IL-6) in the hypothalamus and hindbrain as well as reduced food intake. Furthermore, blockade of IL-1 and IL-6 signaling attenuates the Ex-4-induced feeding and weight loss effects [7]. Ex-4 treatment within the arcuate nucleus (ArcN) and the paraventricular nucleus (PVN) also alters energy metabolism and substrate oxidation [4] [8]. In fact, the ArcN, the PVN, and the lateral hypothalamus (LH) are all believed to mediate the anorexigenic and/or metabolic activity of GLP-1 [4] [5] [6]. Moreover, the orexigenic and metabolic effects of hypothalamic ghrelin and neuropeptide-Y (NPY) are antagonized by discrete, localized injection of Ex-4 [4]. The interaction of GLP-1 with other endogenous neuropeptide signals is consistent with evidence showing that Ex-4 activates c-fos expression of ghrelin and NPY immunoreactive neurons in the ArcN and PVN [7].

More recent work has demonstrated that GLP-1R activation may be implicated in hedonic eating behavior and reward. Administration of Ex-4 into mesolimbic areas, including the ventral tegmental area (VTA) and nucleus accumbens (NAc), attenuates operant responding and conditioned place preference (CPP) for palatable food in rats [5] [8] [9] [10] [11] [12]. GLP-1 has further been implicated in the rewarding effects of drugs of abuse, namely cocaine and alcohol [13] [14] [15]. Prior work has found that peripheral and intra-VTA Ex-4 injections decrease cocaine seeking and self-administration [14] [15] [16]. Ex-4 suppresses alcohol-mediated CPP [17] and VTA or accumbal treatment reduces alcohol intake via two-bottle choice as well as self-administration [5] [13] [17] [18]. While such preclinical research indicates that GLP-1R stimulation attenuates alcohol consumption in rodent models, ongoing work examining the potential therapeutic effects in humans is now under investigation [19].

GLP-1R expression has additionally been identified within the habenula, an epithalamic structure that links forebrain limbic and mesolimbic regions to subcortical nuclei [20] [21] [22] [23]. The habenula projects to mesolimbic neurons within the VTA and the rostromedial tegmental nucleus (RMTg) and receives inputs from the PVN and NAc [21] [24] [25]. Recent studies suggest that the habenula plays a role in reward [21] [23] [25]-[30]. Stimulation of lateral habenula neurons decreases appetitive motivation and dopaminergic signaling in the RMTg, whereas inhibition of the same neurons has the opposite effect [25]; moreover,

habenula neuronal activity increases in response to aversive stimuli [23]. Similarly, inhibition of lateral habenula neurons projecting to the dorsal raphe nucleus (DRN) decreases licking behavior, a proxy for perseverance, in a reward omission paradigm [26]. Inhibition of cAMP-dependent protein kinase (PKA) signaling in medial habenula neurons in a *Prkar2a* knockout mouse results in decreased hedonic eating [29]. Finally, the glutamatergic pathway between the LH and the lateral habenula appears to mediate food reward, with neuronal inactivation resulting in increased food intake [31]. Still more work implicates the habenula in drug reward [21]. Cocaine stimulates habenular activity shortly after administration, resulting in temporary aversion, but lesions in the rostral entopeduncular nucleus (rEPN), which projects to the lateral habenula, reduce habenular c-fos expression and avoidance of cocaine in a conditioned place aversion (CPA) paradigm [27]. Stimulation of the direct pathway between the lateral habenula and the VTA can result in increased VTA dopaminergic signaling, which contrasts with how stimulation of the lateral habenula to RMTg pathway indirectly decreases the activity of dopamine neurons in the VTA [24] [25].

Despite mounting evidence for the habenula's involvement in reward, no study has yet directly assessed a possible role of habenular GLP-1 receptor mechanisms in food and alcohol reinforcement. Therefore, in order to better understand the impact of habenular GLP-1 in reward signaling, we examined the effects of localized injections of Ex-4 on both alcohol intake and operant responding for palatable food using a two-bottle choice paradigm and operant schedule of reinforcement, respectively. The additional focus on operant behavior allowed us to assess a potential impact on reward salience as well as appetitive motivation and effort-based behavior.

2. Methods

2.1. Animals

Female (N = 15) Long Evans rats (Envigo, Indianapolis, IN) were individually housed in polypropylene cages and maintained on a 12 h light/dark cycle (lights off at 13:00 h). Food (LabDiet, St. Louis, MO) and water were provided ad libitum. All behavioral paradigms were conducted during the nocturnal active cycle. Colony temperature was maintained at 22˚C ± 2˚C in a humidity-controlled environment. All experimental procedures were approved by the Reed College Institutional Animal Care and Use Committee.

2.2. Stereotaxic Surgery

Rats were anesthetized via isoflurane gas and placed into a Kopf stereotaxic frame once anesthesia was achieved. The incisor bar was set 3.5 mm below the interaural line and all rats were cannulated unilaterally with a stainless steel guide cannula (26 gauge; P1 Technologies, Roanoke, VA). Cannulae were placed 1 mm dorsal to the habenula (posterior − 4.0 mm, lateral ± 0.6 mm, and ventral − 3.9 mm relative to bregma) and secured with anchor screws, acrylic cement, and

stainless steel stylets. Animals were administered carprofen (5 mg/kg SC, Covetrus, Portland, ME) at the midpoint of surgery in order to minimize discomfort and were given a period of 14 days of postoperative recovery.

2.3. Experimental Paradigms

Operant training and alcohol administration were performed as described previously [5]. Rats were injected with either Ex-4 or saline vehicle in a 0.2 µl volume. Doses of Ex-4 were 0.05 and 0.10 µg. Injections were administered at the onset of the dark cycle and delivered into the habenula over a period of 4 minutes. In the operant protocol, rats (n = 8) were initially trained on a progressive ratio 3 (PR3) schedule using banana flavored sucrose pellets (Bio-Serve, Lancaster, NH). Training sessions were conducted daily for three weeks in order to establish stable response rates. The precise protocol outlining manual shaping and training is described previously [13] [32]. Each test session, yielding a measure of the total number of reinforcers acquired, lasted 30 minutes. A repeated measures design was employed where rats were subjected to treatment or vehicle conditions, presented in randomized order. Each test session was separated by four non-injection days.

A separate group of female rats (n = 7) was habituated to alcohol using a two-bottle intermittent choice paradigm [5] [13]. In this study, rats were allowed access to increasing concentrations of alcohol until intake of 6% alcohol (v/v) had stabilized. Alcohol exposure was provided every other day while only water was available on intervening days. Intakes were measured over a period of 24 hours. On the day of testing, rats were administered saline vehicle, 0.05 µg of Ex-4 or 0.10 µg of Ex-4 and returned to their home cage which contained a bottle of 6% alcohol and a separate water bottle. Consumption was determined 24 h later. Again, a repeated measures design was used such that all rats were tested under each condition.

Finally, to ensure that results from operant sessions and in the alcohol two-bottle choice paradigm were due to an impact on motivation and not as a result of changes in locomotor function, we initially assessed behavior in the elevated plus maze and open field paradigm. We found no change or suppression of locomotor behavior resulting from Ex-4 treatment (data not shown) and we were therefore confident that any change in operant responding or in alcohol intake was due to an impact on reward signaling.

3. Results

Cannula placement was confirmed via histological examination as described previously [5] [12]. A schematic representation of habenular microinjection sites is shown in **Figure 1**. The effect of Ex-4 administration on operant responding for sucrose pellets is illustrated in **Figure 2**. ANOVA and post hoc Tukey testing indicated that both doses of Ex-4 suppressed operant responding as demonstrated by the reduction in the total number of reinforcers acquired. That is, each dose of Ex-4 reliably suppressed operant responding with the higher dose eliciting the

most robust inhibitory response ($P < 0.05$). With respect to Ex-4's effect on alcohol intake, one-way repeated measures ANOVA demonstrated that Ex-4 significantly reduced 24-h intake in a dose-dependent manner ($P < 0.05$). Specifically, 0.05 μg of Ex-4 decreased intake compared to vehicle and the highest dose of 0.10 μg elicited a greater suppression in intake when compared to either vehicle or the 0.05 μg dose. See Figure 3.

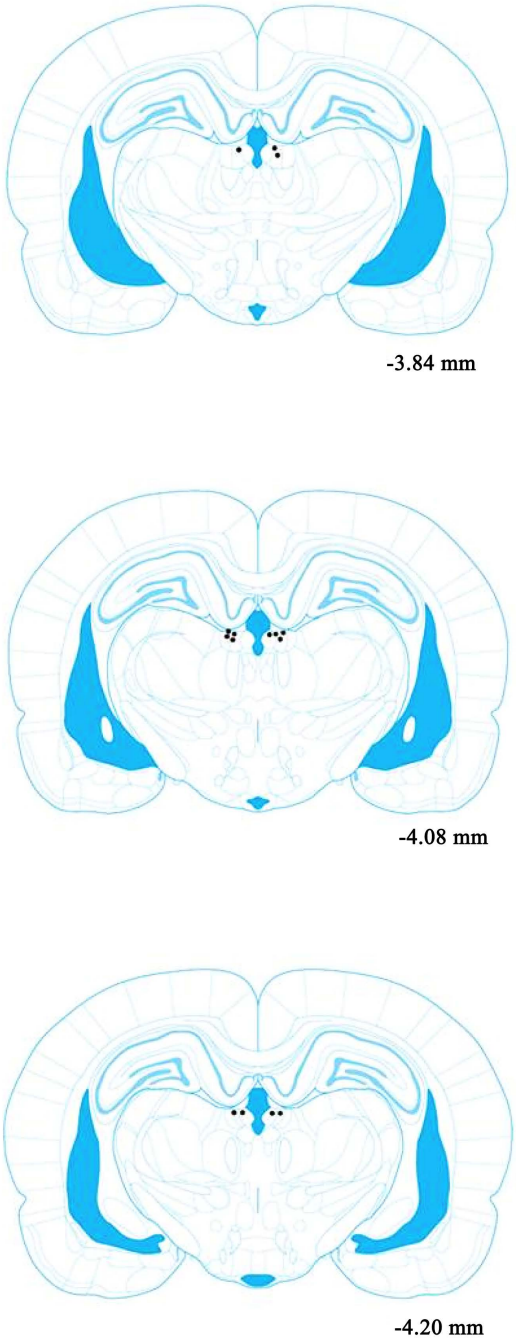

Figure 1. Coronal representation of injection sites where Ex-4 was unilaterally administered into the habenula. Microinjections were counterbalanced into the right and left hemispheres [33].

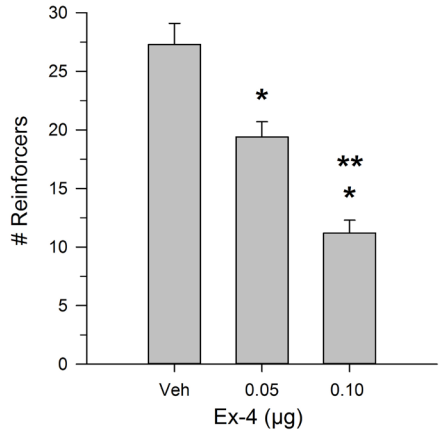

Figure 2. Ex-4 microinjection into the habenula dose-dependently decreased operant responding for palatable sucrose pellets as indicated by a reduction in the number of reinforcers received (n = 8). Testing occurred in the early nocturnal cycle. Values represent mean number of reinforcers ± SEM. *P < 0.05 compared to vehicle control; **P < 0.05 compared to 0.05 μg.

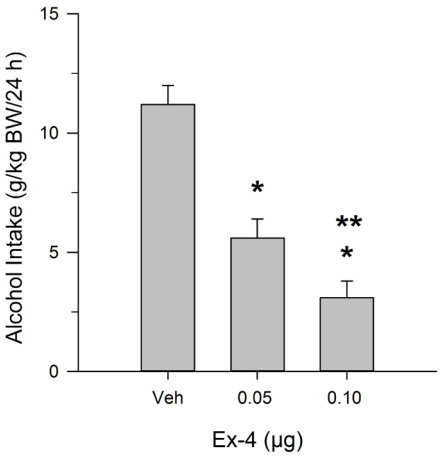

Figure 3. Ex-4 administration into the habenula dose-dependently decreased alcohol consumption (n = 7). Ex-4 was injected at dark onset and intakes were assessed 24 h postinjection. Values represent mean intake ± SEM. *P < 0.05 compared to vehicle; **P < 0.05 compared to 0.05 μg.

4. Discussion

In the present report we found that habenula microinjection of the GLP-1 agonist Ex-4 elicited a reliable suppression in alcohol intake and in operant responding for sucrose pellets. This suggests that the habenula may play an integral role in reward circuitry, particularly with respect to GLP-1 signaling. Since a reduction in operant behavior was observed, it seems reasonable to argue that habenular GLP-1 is involved in effort-based or motivated reward. While emerging evidence implicates mesolimbic GLP-1 neurons in appetitive and drug reward [5] [12] [34] [35] [36] [37], our findings suggest that forebrain structures such as the habenula are also important in mediating GLP-1's inhibitory action on appetitive motivation and incentive value.

Prior research has shown that peripheral administration of Ex-4 reduces mesolimbic dopamine release and amphetamine-induced locomotor stimulation [34] [38], as well as cocaine locomotor stimulation and cocaine self-administration [35]. As described above, VTA and accumbal GLP-1 have been reported to reduce palatable food consumption and alcohol intake, suggesting that the peptide plays an important role in reward processing. Moreover, the mesolimbic system receives anatomical projections from the habenula, further supporting a role of GLP-1 in this brain region in the control of motivational salience [24]. In addition, it is now documented that projections from the VTA and NAc innervate the habenula and the habenula itself is known to express GLP-1 receptors [2] [21]. Consistent with an integral role of GLP-1 in reward, we have recently reported that VTA Ex-4 inhibits the stimulatory effects of ghrelin on operant responding and alcohol consumption. The inhibitory action of Ex-4 is as robust as the serotonergic-2C receptor agonist Ro60-0175 [12].

In the present report we specifically investigated GLP-1 receptor agonism in female rats. Indeed we have recently observed that Ex-4 effectively suppresses alcohol intake in female rats when administered into the NAc core but not the shell region [13]. This is consistent with additional research indicating that co-activation of GLP-1 and estrogen receptors reduces female rat body weight and food reward. In fact, systemic, intracerebroventricular, or direct targeting of the supramammillary nucleus results in combined GLP-1-estrogen actions on metabolism and reward [39]. This work is in turn in agreement with putative sex differences in GLP-1 neuronal action in the LH in relation to food reinforcement, appetite, and body weight [6]. Future research should be directed at investigating potential male and female differences in habenular GLP-1 control of reward signaling.

As indicated above, GLP-1 has recently been implicated in energy metabolism and food intake. For example, direct hypothalamic injection into the PVN nucleus has been shown to reduce food intake and alter energy substrate oxidation [40]. Interestingly, lateral hypothalamic injection of GLP-1 is reported to decrease operant responding for sucrose pellets and alcohol intake in a two-bottle choice paradigm [5]. In contrast, no effect of GLP-1 was found on alcohol intake when Ex-4 was administered into the ArcN or PVN [5]. It is known that a majority of GLP-1 receptor-expressing neurons in the LH project directly to the VTA [24]. Therefore, like the LH, we hypothesize that habenular projections to mesolimbic neurons act to specifically impact reward processing. Consistent with an action on drug reward, other evidence suggests that habenular GLP-1 acts to control nicotine intake. Specifically, activation of GLP-1 receptors in the medial region of the habenula inhibits nicotine reward and decreases nicotine intake whereas knockdown or pharmacological antagonism increases nicotine intake [41]. In addition, prior work has shown that alcohol increases neuronal excitation in the lateral habenula [42]. While the results of our study clearly implicate habenular GLP-1 receptor mechanisms in reward, these latter findings suggest that the medial and lateral regions of the habenula may differentially or selectively

act on brain reward neurotransmission. More research is required in order to elucidate possible selective roles of GLP-1 signaling in specific subregions of the habenula in relation to natural and drug reward processing.

5. Conclusion

In summary, microinjection of the GLP-1 receptor agonist Ex-4 into the habenula suppressed operant responding for palatable food reward as well as alcohol intake as assessed in a two-bottle intermittent access paradigm. These findings are consistent with emerging evidence from mesolimbic mapping studies demonstrating that GLP-1 signaling exerts inhibitory control over natural reward, reward salience, and drug reward. Our findings further suggest that the habenula is critically involved in and is an integral part of the neural processing of brain reward signaling.

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Conflicts of Interest

The authors declare no conflict of interest.

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