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Estradiol modulates the anorexic response to central glucagon-like peptide 1

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ABSTRACT

Estrogens suppress feeding in part by enhancing the response to satiation signals. Glucagon-like peptide 1 (GLP-1) acts on receptor populations both peripherally and centrally to affect food intake. We hypothesized that modulation of the central GLP-1 system is one of the mechanisms underlying the effects of estrogens on feeding. We assessed the anorexic effect of 0, 1, and 10 μg doses of GLP-1 administered into the lateral ventricle of bilaterally ovariectomized (OVX) female rats on a cyclic regimen of either 2 μg β-estradiol-3-benzoate (EB) or oil vehicle 30 min prior to dark onset on the day following hormone treatment. Central GLP-1 treatment significantly suppressed food intake in EB-treated rats at both doses compared to vehicle, whereas only the 10 μg GLP-1 dose was effective in oil-treated rats. To follow up, we examined whether physiologic-dose cyclic estradiol treatment influences GLP-1-induced c-Fos in feeding-relevant brain areas of OVX females. GLP-1 significantly increased c-Fos expression in the area postrema (AP) and nucleus of the solitary tract (NTS), and the presence of estrogens may be required for this effect in the paraventricular nucleus of the hypothalamus (PVN). Together, these data suggest that modulation of the central GLP-1 system may be one of the mechanisms by which estrogens suppress food intake, and highlight the PVN as a region of interest for future investigation.

1. Introduction

Sex differences in food intake have been reported in the literature as far back as the early twentieth century (Wang, 1925). In intact cycling female rats, the peri-ovulatory rise in estradiol secretion is followed by a reduction in food intake that extends throughout the subsequent dark phase, when the rat is in estrus (Blaustein and Wade, 1976). Elimination of endogenous estrogens by ovariectomy results in an increase in food intake and body weight compared to intact rats (McElroy and Wade, 1987), whereas cyclic estradiol treatment after ovariectomy attenuates these increases (Asarian and Geary, 2002). Estradiol, a common estrogen produced by the ovaries, acts to selectively suppress meal size through interactions with factors that directly promote satiation during a meal (Eckel, 2004).

Research on interactions between estrogens and factors that influence meal size has been limited, but a few candidates have emerged. Of these interactions, the most is known about cholecystokinin (CCK) and 5-hydroxytryptamine (5-HT) (Asarian and Geary, 1999; Eckel et al., 2002; Eckel and Geary, 1999; Geary and Asarian, 1999; Rivera et al., 2012; Rivera and Eckel, 2005; Tecott et al., 1995). These findings suggest that modulation of central mechanisms controlling food intake may underlie estradiol-induced hypophagia in females. Here, we focused on the potential interaction between estrogens and glucagon-like peptide 1 (GLP-1), which acts both peripherally as a hormone and centrally as a neurotransmitter to affect food intake, body weight, and glucose homeostasis (Larsen and Holst, 2005).

Peripherally, GLP-1 is an incretin hormone secreted by L cells in the distal intestine, where it acts on nearby receptors to promote satiation and satiety, thus reducing food intake (Williams et al., 2009). Centrally, GLP-1 is produced by neurons in the caudal NTS, which project throughout the brain to several feeding-relevant regions and are activated by nutrient ingestion, gastric distension, CCK, and vagal afferent stimulation (Larsen et al., 1997; Rinaman, 2010). Pharmacologic studies support the role of central GLP-1 in the control of food intake. Stimulation of GLP-1 receptors (GLP-1R) in the brain suppresses food intake, whereas blockade of the receptor with GLP-1R antagonist Exendin-(9-39) (Ex9) or NTS GLP-1R mRNA knockdown increases food intake, revealing the endogenous action of central GLP-1 (Barrera et al., 2011; Turton et al., 1996). Given that GLP-1 in the brain is a central mediator of incoming satiation and satiety signals from nutrient presence in the gut, we asked whether estradiol might interact with the central GLP-1 system to influence feeding.

Recent findings suggest that estradiol may interact with the GLP-1 system to influence feeding. The satiating potency of endogenous peripheral GLP-1 was tested in OVX female rats maintained on a cyclic regimen of either estradiol or sesame oil, that had also undergone Roux-en-Y gastric bypass (RYGB) surgery or sham bypass surgery (Asarian et al.,

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2012). RYGB is a highly effective weight loss surgery, after which food intake is strongly suppressed. Clinical and basic research studies report that one of the consequences of RYGB is an increase in intestinal GLP-1 release (Morínigo et al., 2006; Pournaras et al., 2012). Current speculation in the field is that this elevation in GLP-1 is at least partially responsible for the RYGB-induced suppression of food intake. Interestingly, there was no effect of bypass surgery on GLP-1-mediated satiation, yet there was a significant effect of estradiol treatment. In both RYGB and sham bypass rats, the effect of peripheral GLP-1R blockade on Ensure intake in a 1-h feeding test was greater in estradiol-treated rats compared to oil-treated rats, suggesting an interaction between estradiol and the endogenous peripheral GLP-1 system in the control of food intake. In another recent study, Finan and colleagues found that in gonadally intact male and female mice, an estradiol/GLP-1 conjugate molecule was more effective at suppressing food intake and body weight than both GLP-1 in its native form and a dissociated conjugate mixture of estradiol and GLP-1 (Finan et al., 2012). Furthermore, new evidence has emerged suggesting that estrogens may be involved in the effect of central GLP-1R activation on food-motivated behavior (Richard et al., 2016). However, given that these studies were not designed to control for estrous cycle stage when testing females, and utilized the GLP-1R agonist Ex4, rather than GLP-1 itself, the conclusions that can be drawn regarding an interaction between estrogens and GLP-1 are limited. Taken together, these studies suggest that estrogens may potentiate the feeding effects of GLP-1, and here we assess this hypothesis in a more physiologic context.

To evaluate the potential for the GLP-1 system to mediate EB-induced hypophagia, we examined the effect of physiologic-dose cyclic estradiol treatment on sensitivity to the anorexic effect of central GLP-1 in an OVX female rat model. Additionally, we assessed whether cyclic estradiol treatment modulates GLP-1-induced c-Fos in several feeding-relevant brain areas. Our data indicate that modulation of the central GLP-1 system may be one of the mechanisms underlying the effect of estrogens on food intake and highlight the paraventricular nucleus (PVN) as a potential contributor to this effect.

2. Methods

2.1. Subjects

Naïve female Wistar rats weighing 175–200 g (Harlan, Indianapolis, IN) were single-housed in a temperature-controlled vivarium on a 12 h light: 12 h dark cycle in plastic chambers fitted with the Research Diets BioDaq continuous food intake monitoring system (Experiment 1) or standard plastic cages (Experiment 2). Rats had ad libitum access to distilled water and rat chow (Purina 5001) except where otherwise noted. Animals were handled daily and underwent habituation to experimental procedures prior to study onset. All experimental procedures were approved by the Florida State University Institutional Animal Care and Use Committee and conformed to the standard of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2. Surgery

Under 2–4% isoflurane delivered at a rate of 1 l/min, rats underwent bilateral ovariectomy using an intra-abdominal approach, and were implanted with unilateral 26G guide cannulas (Plastics One, Roanoke, VA) targeting the lateral ventricle (LV). Coordinates for the LV were: 1.5 mm lateral to midline, 0.9 mm posterior to bregma, and 2.27 mm ventral to skull surface. Injectors extending 2.5 mm below the end of the cannula guide were used. Carprofen (10 mg/kg sc) (Butler Schein Animal Health Supply, Dublin, OH) was administered with the onset of surgery and for the first two days of post-operative recovery. Cannula placements were verified prior to experiment onset via successful induction of water intake (>5 ml ingested during a 45 min test) induced by 40 μg Angiotensin II (Sigma-Aldrich, St. Louis, MO) administered intra-LV.

2.3. Drugs and injections

β-Estradiol-3-benzoate (EB) (Sigma-Aldrich, St. Louis, MO) was dissolved in sesame oil (Sigma-Aldrich, St. Louis, MO). One week following surgery, rats were separated into weight-matched groups and placed on a cyclic regimen of either 2 μg β-estradiol-3-benzoate (EB) delivered in 0.1 ml sesame oil or oil vehicle alone. This dose of EB was chosen because it has been shown to produce plasma estradiol levels comparable to that which is achieved physiologically during proestrus in intact cycling rats (Geary and Asarian, 1999). Furthermore, this dose has been shown to induce a transient decrease in food intake, similar to the decrease observed on estrus in cycling rats (Asarian and Geary, 2002). Angiotensin II (Sigma-Aldrich, St. Louis, MO), and GLP-1 (American Peptide, California, USA) were each dissolved in sterile 0.9% saline. Intra-LV injections were administered using a 10-μl syringe (Hamilton, Reno, NV) connected to a 33-G injector extending 2.5 mm past the guide cannula (Plastics One, Roanoke, VA) via Tygon tubing (VWR, Radnor, PA). Injections were delivered at a rate of 1.0 μl/min to the LV, and injections were 2.0 μl in volume.

2.4. Experiment 1: feeding effects of central GLP-1

To assess EB's ability to enhance the anorexic effect of central GLP-1, we conducted feeding tests the day after rats received their weekly estradiol or oil injection (Fig. 1A). This allowed us to examine EB's effect on GLP-1 sensitivity at a time during which EB-induced suppression of food intake is expressed. The weekly EB treatment timing was selected to accommodate the scheduling requirements of the experimenters, and has been previously shown to be an effective regimen (Asarian and Geary, 1999; Geary et al., 1994; Geary and Asarian, 1999; Santollo and Eckel, 2008). From days 1–4, this regimen mimics the changes in plasma estradiol levels across the ovarian cycle in gonadally intact females (Asarian and Geary, 2002). That is, estradiol levels are low, then peak on the day of EB treatment, and rapidly fall the subsequent day, which models estrus (Becker et al., 2005).

A within-subjects counterbalanced design was used to assess the feeding response to intra-LV GLP-1 in oil- and EB-treated rats (n = 10 oil & 12 EB). On test days, food was removed from the rats' cages 3 h before dark onset. 30 min before dark onset, rats received an intra-LV injection of 0, 1, or 10 μg of GLP-1 in 2 μl of saline. These doses are suprathreshold for an effect when administered into the LV of male rats, with the 10 μg significantly suppressing intake and the 1 μg dose being near the threshold for significant effect (Kinzig et al., 2002). Food was returned to the rats' cages immediately before dark onset, and food intake was measured continuously by the BioDaq system for the next 21 h.

Analysis of meal pattern was performed using BioDaq Data Viewer Software (Research Diets, New Brunswick, NJ). A meal was defined as any feeding episode measuring at least 0.25 g separated from subsequent intake by at least 15 min (Eckel et al., 2000, 1998; Eckel and Geary, 1999). Rats ate very little chow during the light phase, therefore, we only analyzed dark phase meal patterns. From this meal pattern analysis, we obtained data on the following meal pattern variables: latency to begin the first meal, meal size, number of meals, meal duration, and inter-meal intervals (IMI). Latency to begin the first meal was defined as the number of min between the time when food access resumed and the start of the first meal, and inter-meal interval was defined as the time (*sec*) between the end of one meal and the onset of the subsequent meal. To examine intake over time, we analyzed hourly cumulative intake throughout the 12 h dark phase, as well as non-cumulative intake during each 4-h tertile of the dark phase.

Throughout the experiment, body weights and food intake were measured daily. Drug treatments were separated by seven days.

2.5. Experiment 2: GLP-1-induced c-Fos

OVX female rats (n = 4–5 per group) with cannulas targeting the LV were maintained on a cyclic regimen of either 2 μg EB or oil,