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Estradiol is a critical regulator of food-reward behavior

Jennifer E. Richard, Lorena López-Ferreras, Rozita H. Anderberg, Kajsa Olandersson, Karolina P. Skibicka*

Department of Physiology/Metabolic Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden

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ABSTRACT

Food intake is reduced by estrogenic hormones, levels of which vary throughout life and fluctuate throughout the ovarian cycle in females. However, estrogens have also been shown to increase reward derived from drugs of abuse, where motivational properties of drugs and progression to addiction are potentiated by estrogens. Whether reward derived from food, and specifically motivational properties of food, are altered by estrogens remains unknown. Here we investigated the effect of the estrous cycle on food reward behavior and show estrous cycle dictated variability in food motivation, measured by progressive ratio operant conditioning, in female rats. Reward behavior was lowest on days associated with high estrogen signaling. We therefore also examined the actions of subcutaneously administered β-estradiol on food reward and found that β-estradiol reduced food reward behavior. The ventral tegmental area (VTA) is a crucial node of the neurocircuitry underlying motivated behavior and estrogen receptors are expressed in this nucleus. Thus, we examined whether the effects of estrogens on reward were exerted directly at the level of the VTA. Intra-VTA microinjection of β-estradiol led to a significant reduction in food-motivated behavior. Interestingly, this effect was not accompanied by a reduction in chow intake or body weight, nor did it alter locomotor activity. Importantly, removal of the ovaries produced a potent and lasting elevation in food reward and food-seeking behavior, suggesting that ovarian sex steroids are critical for maintenance of normal food reward behavior. These data reveal a novel role for estrogens in the control of food reward behavior.

1. Introduction

The obesity epidemic is continuing unabated (Flegal et al., 2016; Ng et al., 2014). Though the prevalence of obesity is similar between sexes, women have an increased risk of developing eating disorders and extreme obesity (Hoek, 2006 Yang and Colditz, 2015). Women with an increased risk of metabolic syndrome also have a higher risk of developing diabetes and cardiovascular diseases than men who display the same risk factors (Wenger, 2003). Moreover, women have a lower success rate for body weight loss while dieting (Keel et al., 2007). Yet most preclinical studies unraveling neural circuitry behind excessive eating and obesity are conducted exclusively in males (Miller, 2012). This gap in female-focused studies makes it evident that more research is needed to evaluate factors regulating body weight and food intake in females to facilitate development of more optimal pharmaceutical treatment strategies for weight loss (or weight management) for the female population.

Ovarian hormones play a key role in the regulation of food intake and they contribute to sex differences in the response to several food intake regulating hormones (Asarian and Geary, 2006, 2013; Perry et al., 2013a). Removal of sex steroids by ovariectomy results in weight gain (Wade et al., 1985). Estrogen is a critical factor in body weight control since development of obesity after ovariectomy can be prevented by estrogen treatment (Asarian and Geary, 2013). In addition, during naturally occurring behavioral estrus, food intake is lower than on other days of the estrous cycle indicating that food intake is also controlled cyclically and correlated with the levels of estrogens (Czaja and Goy, 1975; Eckel, 2004; Gong et al., 1989). The inhibitory effects of estrogens on food intake have been attributed to the actions of this hormone on estrogen receptors (ER) in the brain, for example in the hypothalamus or the nucleus tractus solitarius (NTS), areas classically associated with body weight and food intake control (Asarian and Geary, 2013).

In contrast to the well-established food intake-reducing role of estrogens, a large body of literature suggests that estrogens increase self-administration of psychomotor stimulants or alcohol

* Corresponding author at: Department of Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Medicinaregatan 11, PO Box 434, Gothenburg, SE-405 30 Sweden.
E-mail address: Karolina.Skibicka@neuro.gu.se (K.P. Skibicka).

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(Evans and Foltin, 2010; Festa and Quinones-Jenab, 2004; Hu and Becker, 2003; Hu et al., 2004). Female rats are more motivated to work for cocaine during the estrus phase compared to other phases of the estrous cycle (Carroll et al., 2002; Roberts et al., 1989) and estradiol treatment given to ovariectomized rats increases the motivation to self-administer cocaine (Becker and Hu, 2008). This estradiol-induced enhanced motivation for drugs of abuse is associated with an elevation in striatal dopamine release (Becker, 1990). These preclinical rodent studies are supported by human studies. For example women, who have higher levels of circulating estrogens, increase intake of drugs of abuse at a faster pace than men and show a faster progression to addiction (Carroll et al., 2004; Lynch et al., 2002). Women also show higher sensitivity to the rewarding effects of cocaine and amphetamine (Becker and Hu, 2008). The subjective effects of stimulating substances of abuse vary during the different phases of the menstrual cycle distinguished by fluctuating levels of estrogens (Justice and de Wit, 1999).

Food reward, and specifically food motivation, is regulated by neurocircuitry that is largely overlapping with neurocircuitry that regulates reward derived from drugs of abuse (Avena et al., 2008; Murray et al., 2014). Dopamine neurons, that reside in the ventral tegmental area (VTA) and project to the nucleus accumbens, form a core pathway for control of motivated behaviors (Koob, 1992; Wise, 2004a,b). Importantly, ER is expressed in the VTA (Shughrue et al., 1997). How estrogens affect food reward remains largely unexplored. One possibility is that estrogens enhance motivation for food as they do for drugs of abuse. This idea is supported by previous studies demonstrating similar effects on food and drug motivation with other peripheral hormones, for example glucagon-like peptide 1 or ghrelin (Dickson et al., 2011; Dickson et al., 2012; Skibicka et al., 2011). In line with potentially enhancing the effects of estrogens on food reward, estrogens have been shown to increase reinstatement behavior for a food reward in the Pavlovian conditioning task (Anderson and Petrovich, 2015). On the other hand, since estrogens reduce food intake, it also is possible that estrogens reduce food motivation. Here, we directly address the issue of estrogens' role in food motivation, and hypothesize that estrogens reduce food-motivated behavior as they do food intake.

2. Methods

2.1. Animals

Female Sprague-Dawley rats (5 weeks at arrival, weighing 135–165 g), Charles River, Germany) were housed in a 12 h light/dark cycle, in individual cages with *ad libitum* access to chow and water. Surgeries were performed at approximately 10 weeks of age and testing was conducted at 10–11 weeks of age. Each experiment (1–4) was conducted in a separate group of rats. All studies were carried out with ethical approval from the Animal Welfare Committee of the University of Gothenburg, in accordance with legal requirements of the European Community (Decree 86/609/EEC). All efforts were made to minimize suffering.

2.2. Drugs

17 β-estradiol was purchased from Tocris (Bristol, UK) and dissolved in artificial cerebrospinal fluid (aCSF; Tocris) with 20% DMSO (used as vehicle for intra-VTA injections) or sesame oil (Sigma-Aldrich, for subcutaneous administration). Drugs were stored as aliquots at −20 °C.

2.3. Ovariectomy and SHAM surgery

Bilateral incisions were made on the dorsolateral flank of the animal in parallel to the spinal cord under ketamine anesthesia. In animals undergoing cannula implantation surgery ovariectomy or SHAM procedures were carried out concomitantly. The ovaries were visualized and removed without harming surrounding tissues. In the SHAM procedure incisions were made and the ovaries visualized without removing any tissue. After all surgery procedures the muscle and skin were separately sutured with sterile non-absorbable silk sutures. Successful removal of the ovaries was confirmed by the absence of cycling after surgery. Body weight and food intake were measured daily after surgery.

2.4. Brain cannulation

Guide cannulas were implanted into the VTA using the following coordinates adapted from (Skibicka and Dickson, 2011): ±0.75 from the midline, 5.7/5.9 mm posterior to bregma, and 6.5 mm ventral from the surface of the skull, with injector aimed 8.5 mm ventral to skull. Cannula implantation surgery was performed under ketamine anesthesia. Dental acrylic and jeweler's screws were used to secure the cannula and the incision was closed using surgical staples as previously described (Skibicka et al., 2009). Placement was confirmed by micro-injection of India-ink post mortem at the same volume used throughout the study (0.5 μL). Only subjects with correctly placed cannulas were included in data analysis. Ink was found outside of the VTA in one rat and her data were excluded from the study.

2.5. Determination of estrous cycle phases

Estrous cycle phase was assessed using traditional stage assignments (Becker et al., 2005). Samples were collected each morning or immediately after operant conditioning on testing days, stained using Papanicolaou staining as described previously (Chateau et al., 1996), and visualized under a microscope.

2.6. Operant conditioning

The operant conditioning procedure is used to assess the motivation to obtain a reward, in this case food reward in the form of a sucrose pellet. Training and testing were conducted early in the early to mid light cycle in rat conditioning chambers (Med-Associates, Georgia, VT, USA) as described previously (Dickson et al., 2012; la Fleur et al., 2007). Training was conducted in four stages in *ad libitum* fed rats. Rats were first trained on the fixed ratio 1 (FR1) schedule in 30 min sessions (single press on the active lever resulted in the delivery of one sucrose pellet (45 mg)), followed by FR3 and FR5 (3 and 5 presses per pellet respectively), where a minimum of 39 responses per session on the active lever was required for advancement to the next schedule. Finally the rats were trained in progressive ratio (Wang et al. 2009) until stable responding was achieved (approximately 3–5 sessions). In the progressive ratio (PR) schedule the cost of a reward is progressively increased for each following reward, in order to determine the amount of work the rat is willing to put in to obtaining the reward. Responding was considered stable when the number of pellets earned per session did not differ more than 15% between three consecutive sessions. All operant response testing was performed after the responses stabilized. All operant conditioning training was done before surgery for experiments 2 and 4. Each PR session lasted for 60 min. The specific number of times the rats were tested in the operant conditioning PR procedure differed between experiments, as described below for each experiment.

2.7. Food seeking

Food-seeking behavior was defined as the amount of head entries into the food dispenser during the PR operant condition-