Hormones and Behavior 105 (2018) 128–137

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Estrogen effects on oxytocinergic pathways that regulate food intake

Dusti K. Sloan[a,b,*], Diana S. Spencer[b], Kathleen S. Curtis[a]

[a] Department of Pharmacology and Physiology, Oklahoma State University-Center for Health Sciences, Tulsa, OK 74107, USA
[b] Department of Science and Mathematics, Tulsa Community College, Tulsa, OK 74133, USA

ARTICLE INFO

Keywords:
Paraventricular nucleus
Nucleus of the solitary tract
Body weight
Microstructural analysis
Oxytocin antagonist
Intracerebroventricular injection

ABSTRACT

Multiple stimulatory and inhibitory neural circuits control eating, and these circuits are influenced by an array of hormonal, neuropeptide, and neurotransmitter signals. For example, estrogen and oxytocin (OT) both are known to decrease food intake, but the mechanisms by which these signal molecules influence eating are not fully understood. These studies investigated the interaction between estrogen and OT in the control of food intake. RT-qPCR studies revealed that 17β-estradiol benzoate (EB)-treated rats showed a two-fold increase in OT mRNA in the paraventricular nucleus of the hypothalamus (PVN) compared to Oil-treated controls. Increased OT mRNA expression may increase OT protein levels, and immunohistochemistry studies showed that EB-treated rats had more intense OT labeling in the nucleus of the solitary tract (NTS), a region known to integrate signals for food intake. Food intake measurements showed that EB treatment reduced food intake, as expected. EB-treated rats lost weight over the course of the experiment, as expected, and EB-treated rats that received the highest dose of OT lost more weight than EB-treated rats that did not receive OT. Finally, OT antagonist administered to EB-treated rats reversed the effect of EB on food intake, suggesting that estrogen effects to decrease food intake may involve the oxytocinergic pathway.

1. Introduction

The neural circuitry involved in the control of eating is extremely complex and includes numerous interconnected brain regions that communicate via hormonal, neuropeptide, and neurotransmitter signals (reviewed by Schwartz et al., 2000). A wealth of data shows that estrogen decreases food intake and body weight (Asarian and Geary, 2013; Wade and Gray, 1979). Female rats eat the least amount of food during the estrous phase of their cycle, following peak estrogen levels in proestrus (Blaustein and Wade, 1976; Drewett, 1974). Furthermore, when ovarian hormones are eliminated by ovariectomy, rats increase food intake and gain weight, an effect that is reversed by estrogen replacement (Blaustein and Wade, 1976; Geary and Asarian, 1999). Estrogen effects on food intake are thought to be due to actions at estrogen receptors (ERs) within the central nervous system (Geary et al., 2001; Heine et al., 2000; Roesch, 2006; Santollo and Eckel, 2009; Santollo et al., 2010; Thammacharoen et al., 2009). Both the α and β subtypes of nuclear ERs are located in regions of the brain known to regulate food intake (Laflamme et al., 1998; Osterlund et al., 1998; Shima et al., 2003; Shughrue et al., 1997; Stern and Zhang, 2003). Many studies show that ERα is the primary receptor subtype involved in eating and body weight regulation (Heine et al., 2000; Roesch, 2006; Santollo and Eckel, 2009; Santollo et al., 2010; Thammacharoen et al., 2009); however, ERβ are located in OT neurons (Alves et al., 1998; Hrabovszky et al., 1998; Shughrue et al., 2002; Simonian and Herbison, 1997), and are the predominant ER subtype in the PVN (Laflamme et al., 1998; Shughrue et al., 1997; Simonian and Herbison, 1997), a hypothalamic area involved in eating. Furthermore, EB treatment induces c-Fos expression (an indicator of neuronal activation) in the PVN (Eckel and Geary, 2001). These studies suggest the possibility that ERβ also play a role in estrogen-induced inhibition of feeding.

The oxytocinergic pathway from the PVN to the hindbrain NTS has been implicated in the control of feeding (Flanagan et al., 1992; McCann and Rogers, 1990; Moran and McHugh, 1982; Peters et al., 2008; Travagli and Anselmi, 2016; Verbalis et al., 1986), suggesting an interaction between estrogen and OT in the estrogen-induced inhibition of food intake, perhaps via ERβ regulation of the oxytocinergic pathway from the PVN to the NTS. OT released from PVN projections binds to OT receptors, including those that terminate in the hindbrain NTS (Peters et al., 2008; Travagli and Anselmi, 2016), which integrates information from the PVN and from the GI tract to regulate feeding and gastrointestinal function (Flanagan et al., 1992; Moran and McHugh, 1982; Travagli and Anselmi, 2016; Verbalis et al., 1986). Studies involving administration of OT, an OT agonist, or an OT antagonist (Arletti et al.,

* Corresponding author at: Tulsa Community College, 10300 East 81st Street, Tulsa, OK 74133, USA.
E-mail address: dusti.sloan@tulsacc.edu (D.K. Sloan).

https://doi.org/10.1016/j.yhbeh.2018.08.007
Received 15 May 2018; Received in revised form 24 July 2018; Accepted 12 August 2018

1989; Olson et al., 1991) lend further support to the idea that OT plays a key role in the regulation of food intake. For example, male rats given intracerebroventricular (ICV) injections of OT or an OT agonist reduced food intake in a dose dependent manner. Administration of an OT antagonist reversed OT effects, increasing food intake. Benelli et al. (1991) confirmed reductions in food intake by male and female rats after OT administration, but the females were intact and stages of the estrous cycle were not considered (Benelli et al., 1991). Surprisingly, a review of the existing literature revealed no studies investigating the role of OT in the control of food intake in ovariectomized females treated with EB. Thus, the possibility that OT interacts with EB in the control of feeding has not been examined specifically.

Transcription of the rat OT gene is activated by EB in heterologous expression systems (Peter et al., 1990), and later studies demonstrated that the OT promoter region also contains a composite hormone response element (HRE) that can be stimulated by steroid or thyroid hormones (Adan et al., 1993). It seems plausible that EB treatment affects OT expression given the presence of the estrogen response element (ERE) in the promoter region of the OT gene, but several studies show conflicting results (Blyth et al., 1997; Dellovade et al., 1999; Peter et al., 1990; Shughrue et al., 2002). It is noteworthy that all of these experiments utilized different protocols for EB administration (EB concentration range = 2–10 μg; EB treatment time = hours to 10 days). Given the conflicting results for EB upregulation of OT mRNA, it is clear that a time course analysis of OT mRNA expression in response to EB treatment that models the four day estrous cycle of the rat is needed.

Accordingly, we conducted a series of experiments to address the following questions: Does EB increase oxytocin (OT) mRNA in the paraventricular nucleus of the hypothalamus (PVN)? Does EB increase OT protein immunolabeling in the PVN and the nucleus of the solitary tract (NTS)? Does exogenous OT differentially influence food intake and body weight in EB-treated rats? Does OT receptor antagonist differentially influence food intake in EB-treated rats?

2. Materials and methods

2.1. General methods

2.1.1. Animals and housing

All protocols were conducted in accordance with the international standards for animal welfare and approved by the Oklahoma State University – Center for Health Sciences Institutional Animal Use and Care Committee (protocol # 2015-05). Adult female (90 days old) Sprague-Dawley rats (Charles River and OSU-CHS breeding colony) were individually housed and maintained in standard climate-controlled, 12:12 h light:dark (5 am to 5 pm) conditions. Cage bedding was changed once or twice weekly and body weight was monitored throughout the studies. Cage maintenance and weighing took place between 9 am and 11:30 am. Except where noted in the following experiments, rats received ad libitum standard chow (Teklad Diet #2018) and water. EB-replacement decreases food intake in rats whether eating standard chow or a palatable diet (Butera et al., 2010).

2.1.2. Surgeries

All rats were ovariectomized (OVX). Prior to surgery, 0.1–0.2 mL of meloxicam (1.5 mg/mL) was administered orally to reduce inflammation and pain. Bilateral OVX was performed under 2.5% isoflurane anesthesia (Askew et al., 2015).

In some experiments, rats were implanted with a cannula into the right lateral cerebral ventricle. For these experiments, intracerebroventricular (ICV) cannulation and ovariectomy were performed on the same day. Briefly, cannulae (Plastics One, Inc.) were placed into the right lateral ventricle using a stereotaxic device at the following coordinates from bregma (x = −1.7 mm, y = −1.2 mm, z = 5 mm). Cannulae were secured to the skull using dental cement and screws. Rats were allowed to recover for one week before experimentation. At the end of these experiments proper placement of cannulae was verified by ICV injection of 10 μL of methylene blue dye into the cannulae to determine that the dye was dispersed throughout the lateral ventricles. At the conclusion of most of the experiments, CO_2 and decapitation were used to terminate the rats. In experiments that required brain tissue for immunohistochemistry, rats were deeply anesthetized with 0.5–1 mL of sodium pentobarbital (50 mg/mL) and perfused (see Immunohistochemistry section below).

2.1.3. Estrogen treatment protocol

Subcutaneous (SC) injections of either 10 μg 17β-estradiol benzoate (EB) in 0.1 mL oil or 0.1 mL of the oil vehicle (Oil) were administered on days one and two of a four-day protocol. This protocol is frequently used in studies as a means of mimicking the pattern of estrogen fluctuations throughout the estrus cycle of intact rats (Curtis, 2015; Woolley and McEwen, 1994). Food intake studies show that maximum effects of EB on food intake occur on day 4 (72 h) after EB injection; thus, we performed most experiments on day four. In addition, since some physiological effects of EB manifest prior to day four (Graves et al., 2011), mRNA expression was evaluated at additional times prior to day four. The effect of EB treatment was verified by comparing uterine weight in EB- and Oil-treated rats (Graves et al., 2011).

2.1.4. Statistics

Data are presented as means ± S.E.M. Excel was used to conduct *t*-tests and Statistica software was used to conduct two-way factorial ANOVA and two-way and three-way repeated measures (rm) ANOVA. Significant interactions were investigated using Fisher's LSD test; planned comparisons were made using Bonferroni correction. Effect sizes for *t*-tests were calculated using Cohen's d and for ANOVA using partial eta squared (η_p^2). Specific analyses are described in each section.

2.2. Experiment 1. EB effects on OT mRNA in the PVN

2.2.1. Tissue collection

To assess the time course of EB effects on OT mRNA expression, rats were OVX and treated with EB or Oil as described in *General Methods*. Separate groups of rats were used to examine OT mRNA expression at 90 min, 12 h, 24 h, 36 h, or 72 h after the first EB/Oil injection. Preliminary analysis of data showed no differences in OT mRNA expression at the earliest time points. Therefore, to increase the power of the analysis, data from rats terminated at the 90 min and 12 h time points were combined to form the *Baseline* groups (EB, N = 8; Oil, N = 7), and data from rats terminated at the 24 and 36 h time points were combined to form the *24–36 h* group (EB, N = 7; Oil, N = 6). The third group was *72 h* (EB, N = 6; Oil, N = 6) after injection with EB or Oil.

Immediately upon termination, brains were extracted, frozen in liquid nitrogen, then stored at −80 °C until tissue punches were collected. For tissue punches, a stereotaxic atlas (Paxinos and Watson, 1998) was used to identify regions of interest. Coronal sections on either side of the hypothalamus were made, and using a 1 mm wide × 3 mm deep tissue punch (Ted Pella, Inc.#15099), bilateral punches (one punch per side) were taken from the PVN, immediately lateral to the third ventricle. Tissue punches were stored in 100 μL of RNA*later* (Ambion # AM7020) at −80 °C until processed for mRNA.

2.2.2. RNA isolation and primer design

A standard, commercially available kit (BioRad #732-6820) was used to isolate RNA. OT mRNA expression was assessed using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). Sequence-specific OT primers (see Table 1) were designed using the National Center for Biotechnology Information (NCBI)/Primer-BLAST tool. Primers to amplify the reference gene, β-actin, were selected based on a previous report (Zhao et al., 2008). β-actin was