Hormones and Behavior 107 (2019) 20–25

Contents lists available at ScienceDirect

Hormones and Behavior

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

Anorexigenic effects of estradiol in the medial preoptic area occur through membrane-associated estrogen receptors and metabotropic glutamate receptors

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ARTICLE INFO

Keywords:
Food intake
Water intake
Membrane estrogen receptors

ABSTRACT

Activation of membrane-associated estrogen receptors (mER) decreases food and water intake in female rats. Additional studies suggest these effects are mediated, at least in part, by membrane-associated estrogen receptor alpha (ERα). Nevertheless, the critical site of action and the intracellular signaling required for the ingestive effects of ERα remain unclear. Estradiol given to the medial preoptic area (mPOA) decreases ingestive behaviors, and membrane-associated ERα has been shown to affect intracellular signaling through interactions with metabotropic glutamate receptor (mGluR) subtypes, but an involvement of this signaling pathway, in the mPOA, in ingestive behavior remains untested. To address these open questions, we first showed that activation of mER in the mPOA decreased both overnight food and water intake, and did so in a time course consistent with a genomic mechanism of action. Next, we tested the requirement of mGluR1a signaling in the mPOA for the anorexigenic and anti-dipsogenic effects of estradiol. As expected, estradiol in the mPOA decreased food intake, but only in the absence of an mGluR1a antagonist. The same was not true for estradiol effects on water intake, which were unaffected by an mGluR1a antagonist. These results suggest that estrogens require mGluR activation for at least some of their effects on ingestive behaviors, and indicate that the mPOA is a critical site of action. The results also reveal an interesting divergence in the estrogenic control of ingestive behavior by which mGluR signaling in the mPOA plays a role in the control of food intake, but not water intake.

1. Introduction

Daily food and water intakes vary across the estrous cycle in female rodents, with lowest intakes during estrus (Blaustein and Wade, 1976; Drewett, 1973; Eckel et al., 2000; Findlay et al., 1979). Hormone replacement studies demonstrate that the ovarian hormone estradiol (E2) mediates the anorexigenic and anti-dipsogenic effects (Findlay et al., 1979; Geary and Asarian, 1999). This action of E2, from a physiological standpoint, likely protects against excessive body weight gain and perturbations in fluid balance and, from an evolutionary standpoint, provides an opportunity to spend less time searching for food and water and more time finding a mate. Although the exact mechanisms by which E2 inhibits food and water intake are unclear, multiple studies demonstrate that E2 action in the brain mediates these changes in behavior. For example, E2 action in the arcuate nucleus, dorsal raphe, and nucleus of the solitary tract inhibits food intake while E2 action in the lamina terminalis inhibits water intake (Ciriello and Roder, 2013; Santollo et al., 2011; Thammacharoen et al., 2008). In addition, E2 action in the medial preoptic area (mPOA) inhibits both overnight food and stimulated water intake (Jonklaas and Buggy, 1985; Kucharczyk, 1984; Santollo et al., 2011).

Estradiol exerts its behavioral effects through interactions with a variety of estrogen receptors (ER) (Heldring et al., 2007; Micevych and Dominguez, 2009; Santollo and Daniels, 2015b, 2015c). We demonstrated previously that selective activation of membrane-associated ER (mER) decreased overnight food and water intake in ovariectomized (OVX) rats (Santollo et al., 2013). Multiple ER subtypes localize to the plasma membrane, including the traditionally described "nuclear" ERα and ERβ (Micevych and Dominguez, 2009). Previous studies, however, demonstrate that action at ERα, but not at ERβ or GPER-1, is both sufficient and necessary for the anorexigenic effect of E2 (Roesch, 2006; Santollo and Daniels, 2015a; Santollo et al., 2010; Santollo et al., 2007). Furthermore, activation of ERα, but not of ERβ or GPER-1, inhibits both overnight water intake and intake stimulated by angiotensin II (AngII)

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https://doi.org/10.1016/j.yhbeh.2018.11.001
Received 22 August 2018; Received in revised form 1 November 2018; Accepted 3 November 2018
Available online 23 November 2018
0018-506X/

(Santollo and Daniels, 2015a; Santollo et al., 2016). Together this suggests that membrane-associated ERα (mERα) is involved in mediating the anorexigenic and anti-dipsogenic effects of E2.

The delay between E2 exposure and decreased ingestive behaviors suggests that changes in gene expression underlie the anorexigenic and anti-dipsogenic effects of E2. Indeed, we did not observe any rapid changes in food or water intake after activation of mER; changes in intake were not detected until 23 h post drug treatment (Santollo et al., 2013). While mERα can indirectly influence gene expression through activation of CREB phosphorylation and other transcription factors it is still unclear how mERα activates the critical intracellular signaling pathways (Mani et al., 2012). Current evidence suggests there may be a role for interactions with metabotropic glutamate receptor (mGluR) subtypes (Meitzen and Mermelstein, 2011). For example, mGluR-mERα interactions influence lordosis and drug seeking behavior in female rats (Dewing et al., 2007; Martinez et al., 2016). Whether mGluR are involved in mediating the anorexigenic and anti-dipsogenic effects of E2 is an unanswered question. The goal of this study, therefore, was to determine if mGluR signaling is required for the food and fluid intake effects of E2 in OVX rats. To allow for such an investigation, however, we first had to identify an area of the brain where activation of mER inhibits food and water intake. Given that E2 acts in the mPOA to inhibit ingestive behaviors, and mGluR1a is expressed in this area of the brain (Jonklaas and Buggy, 1985; Santollo et al., 2011; Van den Pol, 1994), we tested the hypothesis that activation of mER in the mPOA decreases food and water intake in OVX rats. We then tested the hypothesis that blocking mGluR in the mPOA would prevent the anorexigenic and anti-dipsogenic effects of E2 in OVX rats.

2. Materials and methods

2.1. Subjects and housing

Female Long Evans rats (Harlan Laboratories, Indianapolis, IN) weighing ~225 g at study onset were used throughout. All rats were individually housed in hanging stainless-steel wire mesh cages (Unifab, Kalamazoo, MI) with continuous access to pellet chow (Teklad 2018, Envigo Laboratories) and tap water, unless otherwise noted. The colony room was temperature- and humidity-controlled and maintained on a reverse 12:12 h light-dark cycle (lights off at 1300 h). All experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

2.2. Surgery

One week after arrival at the facility, all rats were OVX and then were implanted with a chronic indwelling cannula into either the right lateral ventricle ($n = 10$) or a unilateral cannula aimed at the medial preoptic areas ($n = 63$). To this end, rats were anesthetized with a mixture of ketamine (80 mg/kg im; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (4.6 mg/kg im; Akorn Inc., Decatur, Il). Rats were bilaterally OVX using an intra-abdominal approach and then secured in a stereotaxic frame. A small hole was drilled in the skull and a 26 gauge guide cannula was implanted using the following coordinates: −0.9 mm AP, 1.4 mm ML, −2.8 DV (from flat skull) for lateral ventricle (LV) cannulation and −0.7 mm AP, −0.5 mm ML, −8.0 DV for mPOA cannulation. The cannula was fixed to the skull with bone screws and dental cement. All rats received a single injection of carprofen (5 mg/kg sc; Pfizer Animal Health, New York, NY) after surgery to minimize pain and a bolus injection of 5 ml sterile isotonic saline. One week later, accurate LV cannula placement was verified by measuring the drinking response to an injection of 10 ng Ang II. Only rats that drank at least 5 ml in 20 min after Ang II treatment were included in the study.

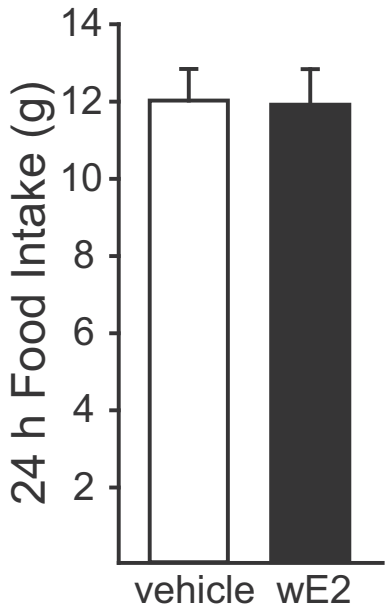

Fig. 1. Intracerebroventricular injection of 9.2 nmol of water-soluble estradiol (wE2) did not affect 24 h food intake. This experiment identifies a dose of wE2 for the following experiments using parenchymal injections such that any effect on intake cannot be attributed to drug leakage from the tissue into the ventricles.

2.2.1. Experiment 1. Effect of mER activation in the mPOA on food and water intake

First, to identify a dose of E2 that would not influence food intake after injection into the LV, rats ($n = 10$) were given a single intracerebroventricular (icv) injection of either 1 µl 2-hydroxypropyl-*b*-cyclodextrin (vehicle; Sigma-Aldrich, St. Louis, MO) or 9.2 nmol β-estradiol-water soluble (wE2; Sigma-Aldrich) dissolved in sterile TBS 30 min before lights off at the speed of 1 µl/30 s, using a hand syringe. The needle remained in the cannula for 1 min after injection. This form of E2 was synthesized with the addition of a carrier molecule, 2-hydroxypropyl-*b*-cyclodextrin, which accounted for 95.31% of the drug dry weight to make the E2 water soluble. The amount of drug was adjusted so that 9.2 nmol (2.5 µg) of E2 was present in the injected solution and the comparable amount of cyclodextrin (45.4 nmol; 51.5 µg) was present in the vehicle injection. The subsequent 24 h food intake was measured by weighing the food hopper at the start and at the end of the test. Plastic transparencies were placed under the cages to collect the spillage. One week later the experiment was repeated with animals receiving the opposite treatment to achieve a repeated measures design.

Next, we determined whether activation of mER in the mPOA is sufficient to reduce overnight food and water intake. OVX rats ($n = 19$) were first screened to determine whether they were responsive to the anorexigenic effect of wE2 in the mPOA. One hour before lights off food and water were removed from the cages. Using a repeated measures counterbalanced design, 30 min before lights off rats received an infusion of cyclodextrin vehicle or 9.2 nmol wE2 dissolved in 200 nl of artificial cerebral spinal fluid (aCSF, Harvard Apparatus, Holliston, MA) into the mPOA at a speed of 200 nl/min, using a hand syringe. The needle remained in the cannula for 3 min after injection. At lights off, food and water were returned to the cages and intakes were measured 24 h later. One week later the experiment was repeated with animals receiving the opposite treatment.

One week later we began testing whether activation of mER in the mPOA is sufficient to reduce overnight food and water intake. Again, food and water were removed from the rat's cages 1 h prior to dark onset. Using a repeated measure counter-balanced design, rats received

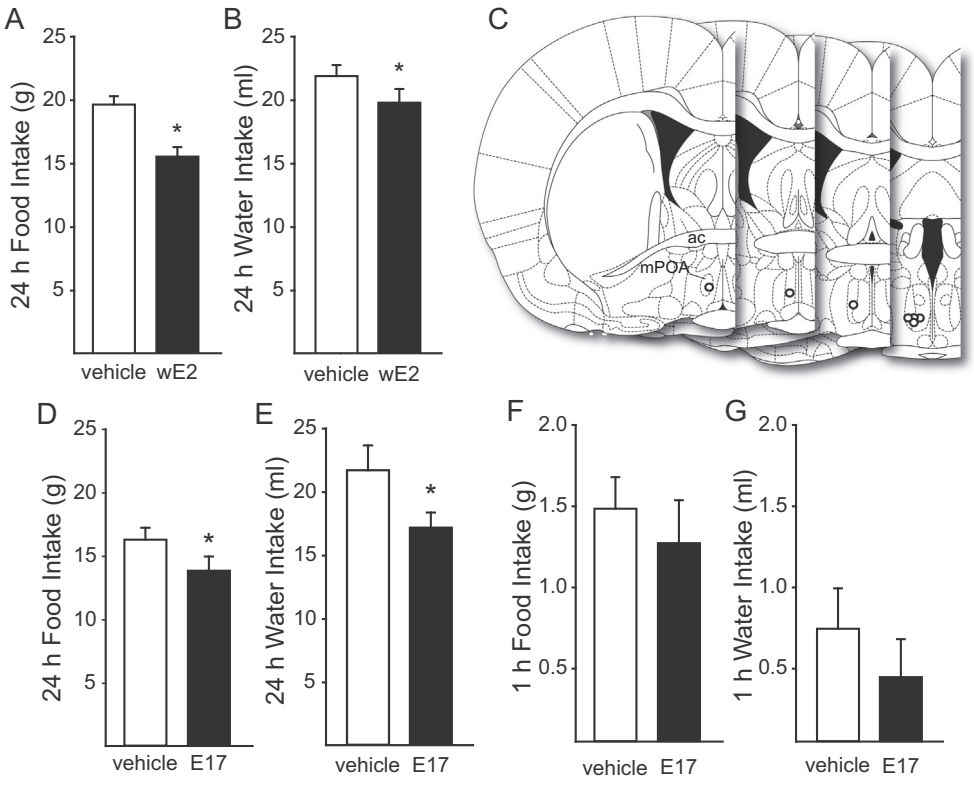

Fig. 2. Activation of membrane-associated estrogen receptors in the medial preoptic area (mPOA) inhibited food and water intake. After treatment with water soluble estradiol (wE2) in the mPOA, rats had significantly lower 24 h food (A) and water (B) intakes, compared to vehicle treatment. (C) Illustration depicting cannula placement in a subset (7) of these rats. When treated with E17-BSA these 11 rats had significantly lower 24 h food (D) and water (E) intakes compared to control treatment. Short-term, 1 h, food (F) and water (G) intakes were not affected by E17-BSA treatment. *Less than vehicle, $p < 0.05$.

injections of 0 or 4.86 pmol 17β-Estradiol 17-Hemisuccinate: BSA (25 nM, E17-BSA, Steraloids Inc., Newport, RI, Batch R282) dissolved in 200 nl of 5% DMSO: 95% aCSF into the mPOA. To reduce the possibility of free E2 in the solution, E17-BSA was filtered immediately before use as described previously (Santollo et al., 2013). This form of E2 was synthesized by the conjugation of 1 BSA molecule with 35 E2 molecules, therefore E2 accounted for ~97% of the total injection or 4.86 pmol of the 5 pmol dose. Injections occurred 30 min before lights off and water and food were returned to the cages at lights off. Food was measured 1 h into the dark phase and 24 h after treatment. Water was measured by weighing the water bottles at the start and end of the experiment. In addition, a contact lickometer was used to determine the number of times the rats licked at the water spout to allow of analysis of drinking during the first h of the testing period. One week later the experiment was repeated with animals receiving the opposite treatment. These drug doses were chosen based on previous research and pilot studies in our laboratory (Santollo et al., 2013).

2.2.2. Experiment 2. Role of mGluR1a on the anorexigenic and anti-dipsogenic effect of E2 in the mPOA

OVX rats ($n = 44$) first were screened to determine whether they were responsive to the anorexigenic effect of wE2 in the mPOA as described above. Infusion of wE2 into the mPOA reduced 24 h food intake in 31 rats whom were then used to determine the role of mGluR1a signaling in mediating the anorexigenic and anti-dipsogenic effect of E2. One hour before lights off food and water were removed and rats ($n = 31$) were treated with 50 nmol LY367385 (mGluR1a antagonist, Tocris; dissolved in 200 nl aCSF) or vehicle (200 nl aCSF). Thirty minutes later, rats in each group were subdivided and given an injection of 9.2 nmol wE2 (in 200 nl aCSF) or cyclodextrin vehicle (51.5 μg 2-hydroxypropyl-*b*-cyclodextrin in 200 nl aCSF). At lights off, pre-weighed food hoppers and water bottles were returned and re-weighed 24 h later. We chose to target the mGluR1a subtype due to its involvement in lordosis behavior in the ARC, another area of the brain involved in the anorexigenic effect of E2 (Dewing et al., 2007). We chose wE2, instead of E17-BSA, for this experiment to more closely mimic the estrogen receptor activation caused by estradiol benzoate in the Dewing et al. (2007) report. The drug doses were chosen based on previous research (Dewing et al., 2007; Santollo et al., 2011).

2.3. Histological verification

After behavioral testing, all rats from Experiment 2 and a subset ($n = 10$) of rats from Experiment 1 were anesthetized by isoflurane, injected with 200 nl India Ink, and decapitated. The brains were dissected rapidly and were frozen at −20 °C. Brains were later cut into 40-μm sections on a cryostat, thaw mounted onto slides, and stained with cresyl violet for histological verification.

2.4. Data analysis

Data are presented as mean ± SEM throughout. Paired *t*-tests were used in Experiment 1 to determine if wE2 or E17-BSA decreased 1 and 24 h food and water intake. Separate ANOVAs (drug X hormone) were used to analyze 24 h food and water intakes in Experiment 2. Newman-Keuls post hoc tests were used to follow up any significant ANOVA result. A significance value of $p < 0.05$ was used throughout. Cohen's d effect size was calculated (*t*-tests) using the Campbell Collaboration Effect Size Calculator and eta squared was calculated for ANOVA analysis as the $SS_{effect}/(SS_{total})$.

3. Results

3.1. Experiment 1. Effect of mER activation in the mPOA on food and water intake

Due to the proximity of the mPOA to the third ventricle, it was important to first identify a dose of wE2 that was ineffective at influencing food intake when injected into the ventricle because injections targeting the mPOA could leak into the ventricle and be distributed to other effective sites. To this end, OVX rats received icv injections of either vehicle or 9.2 nmol wE2 and 24 h food intake was measured. A *t*-

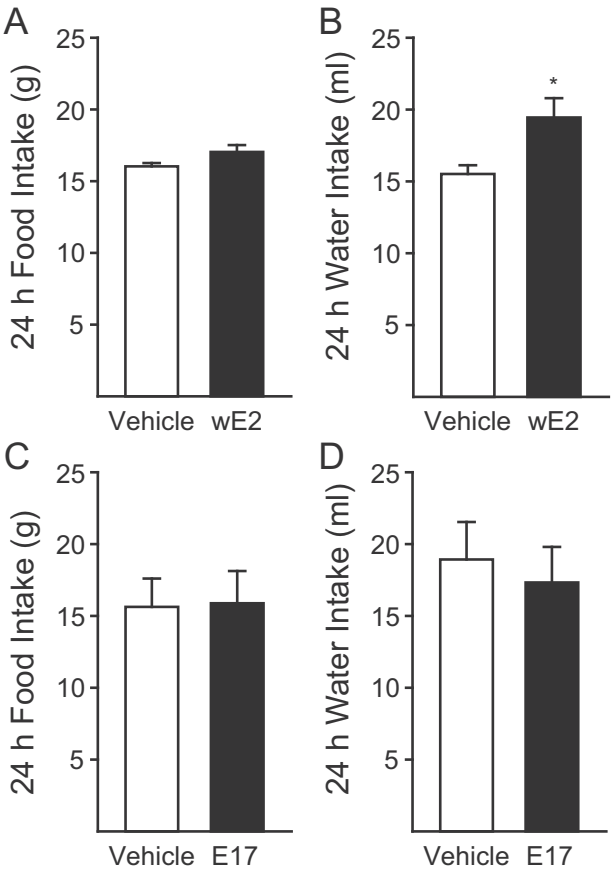

Fig. 3. Estrogen receptor activation had no effect on ingestive behaviors in rats with misplaced cannulas. 24 h food intake in rats with cannula outside of the medial preoptic area were unaffected by wE2 treatment (A). Twenty four-hour water intake was significantly increased after treatment with wE2, compared to intake after control treatment (B). When treated with E17-BSA these rats showed no change in either 24 h food (C) or water (D) intake, compared to intake after control treatment. *Greater than vehicle, $p < 0.05$.

test revealed no effect of wE2 on food intake ($t_{10} = 0.07$, p = n.s., d = 0.0377; Fig. 1).

It has been reported that injections of 9.2 nmol wE2 into the mPOA reduced 24 h food intake (Santollo et al., 2011). Using that information as a guide we first behaviorally verified correct cannula placement by measuring 24 h food and water intake after mPOA injection of 9.2 nmol wE2 or vehicle. Out of 19 rats, 11 animals consumed less food after wE2 than they consumed after vehicle ($t_{10} = 8.02$, $p < 0.001$, d = 1.7453; Fig. 2A). Those 11 rats also had a significant reduction in water intake ($t_{10} = 2.47$, $p < 0.05$, d = 0.6455; Fig. 2B). We then used those 11 rats to test the hypothesis that activation of mER in the mPOA decreases food and water intake. Both 24 h food ($t_{10} = 7.02$, $p < 0.001$, d = 0.7834), and water ($t_{10} = 3.76$, $p < 0.01$, d = 0.8425), intake were significantly less after treatment with E17-BSA than they were after vehicle (Fig. 2D/E). To test for rapid effects on ingestive behaviors, intakes during the first hour of the test were analyzed. Neither 1 h food ($t_{10} = 0.73$, p = n.s., d = 0.2753) nor water ($t_{10} = 0.97$, p = n.s., d = 0.3715), intakes were significantly different after E17-BSA-treatment when compared to intakes after vehicle (Fig. 2F/G). Injection sites for a subset of the rats ($n = 7$) are depicted in Fig. 2C.

Food intake was not affected by wE2 in the mPOA in eight rats ($t_7 = 2.26$, p = n.s., d = 1.138; Fig. 3A). Water intake was, unexpectedly, greater after wE2 treatment ($t_7 = 4.67$, $p < 0.01$, d = 1.3223; Fig. 3B). In animals that showed no anorexigenic effect of E2, E17-BSA treatment in the mPOA also had no effect on either 24 h food ($t_7 = 0.27$, p = n.s., d = 0.39) or water ($t_7 = 0.93$, p = n.s., d = 0.2081) intakes (Fig. 3C/D).

3.2. Experiment 2. Role of mGluR1a on the anorexigenic and anti-dipsogenic effect of E2 in the mPOA

After showing that activation of mER in the mPOA decreased food and water intakes, we tested whether mGluR signaling is necessary for the anorexigenic and anti-dipsogenic effect of E2 in the mPOA. To this end, we first screened rats for proper mPOA cannula placement and for sensitivity to estrogen action in the mPOA. Rats ($n = 44$) were given injections of wE2 or vehicle into the mPOA, and 31 of these ate at least 1 g less than vehicle after infusion of wE2. Next, the rats in which wE2 reduced intake ($n = 31$) were given "treatment" injections of wE2 or vehicle into the mPOA with or without a "pretreatment" of mGluR1a antagonist or vehicle, also given to the mPOA. ANOVA of 24 h food intake revealed a main effect of the treatment ($F_{1,\ 27} = 10.62$, $p < 0.01$, $\eta^2 = 0.237$) and an interaction between the pretreatment and treatment ($F_{1,\ 27} = 5.97$, $p < 0.05$, $\eta^2 = 0.133$), but no main effect of the pretreatment alone ($F_{1,27} = 1.17$, p = n.s., $\eta^2 = 0.026$). Post hoc tests revealed that the interaction effect was driven by intake measured in rats given wE2 in the absence of the antagonist pretreatment, which ate significantly less than during any other condition ($p < 0.05$, Fig. 4A). ANOVA on 24 h water intake revealed a main effect of treatment ($F_{1,27} = 5.89$, $p < 0.05$, $\eta^2 = 0.177$; Fig. 4D) but no effect of pretreatment ($F_{1,27} = 0.41$, p = n.s., $\eta^2 = 0.012$) or an interaction between pretreatment and treatment ($F_{1,27} = 0.06$, p = n.s., $\eta^2 = 0.002$; Fig. 4C). Injection sites are depicted in Fig. 4B.

4. Discussion

The results from this study help elucidate the mechanisms by which E2 acts to decrease overnight food and water intake in female rats. In support of the hypothesis, we found that activation of mER in the mPOA decreased both overnight food and water intakes in OVX female rats. In addition, we found that blocking mGluR1a signaling in the mPOA prevented the anorexigenic effect of E2. Surprisingly, our studies found that blocking mGluR1a signaling in the mPOA did not prevent E2's anti-dipsogenic effect. Together this suggests an interesting divergence in the estrogenic control of ingestive behaviors within the mPOA, by which mGluR signaling is involved in the anorexigenic, but not the anti-dipsogenic, action of E2.

The mPOA is implicated in the control of both food and fluid intake. E2 action in the mPOA, at least partially, mediates its inhibitory effects on ingestive behaviors. For example, E2 infusions into the mPOA decrease short term (2 h) and overnight food intake in female rats (Dagnault and Richard, 1997; Santollo et al., 2011). Furthermore, water intake stimulated by AngII-injection into the mPOA is attenuated on the day of estrus (Kucharczyk, 1984) and E2-injection into the mPOA attenuates water intake stimulated by LV AngII-treatment (Jonklaas and Buggy, 1985). We have extended these findings by demonstrating that acute activation of mER in the mPOA is sufficient to decreases overnight food and water intake. In further support of the specificity of this effect, animals with misplaced cannula that terminated in multiple locations surrounding the mPOA, which did not decrease food intake after E2 treatment, showed no change in food or water intake after treatment with E17-BSA. Furthermore, no change in intake was observed within 1 h of drug treatment, which suggests that these effects are not mediated by rapid non-genomic actions of the mER. Instead, the delayed attenuation in intake suggests these changes are the result of mER indirectly influencing gene expression. We cannot, however, rule out the possibility that the ingestive effects are initiated and maintained through continuous mER signaling that affects neurotransmission, and not gene expression, without additional studies.

While the E17-BSA activated all mER, and ERα, ERβ, and GPER-1 are all expressed in the mPOA (Hazell et al., 2009; Shughrue et al., 1997; Shughrue and Merchenthaler, 2001), the available literature

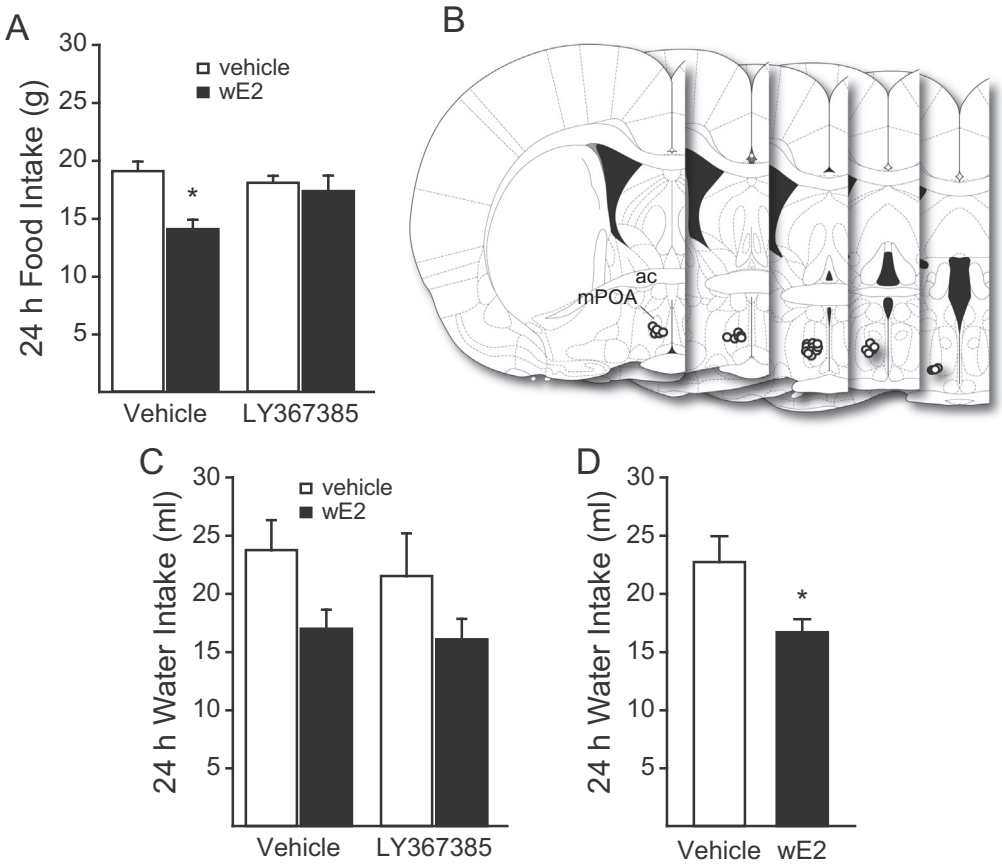

Fig. 4. mGluR1a signaling is necessary for the anorexigenic but not anti-dipsogenic effect of E2. (A) Pretreatment with the mGluR1a antagonist LY367385 prevented the inhibitory effect of E2 on 24 h food intake that was only observed after pretreatment with vehicle. (B) Illustration depicting cannula placement of the 31 rats used in this experiment. (C) Pretreatment with LY367385 had no effect on 24 h water intake. Regardless of pretreatment E2 reduced 24 h water intake (D). *Less than vehicle, $p < 0.05$.

suggests that mERα is the receptor mediating the inhibitory effects of E2. Acute and chronic pharmacological studies have shown that ERα activation reduces food intake in OVX female rats (Roesch, 2006; Santollo et al., 2007). Importantly, antagonizing ERα blocks both the anorexigenic effect of exogenous E2 in OVX rats and the reduction in food intake on the day of estrus in cycling female rats (Santollo et al., 2010). Furthermore, E2 fails to reduce food intake in OVX ERα knockout mice (Geary et al., 2001). Together, this suggests that ERα is the primary mediator of the anorexigenic effect of E2. In addition, pharmacological activation of ERβ or GPER-1 do not affect overnight food intake (Roesch, 2006; Santollo and Daniels, 2015a; Santollo et al., 2007), and blocking ERβ in cycling female rats does not attenuate food intake on estrus (Santollo et al., 2010). Less research has focused on understanding which ER subtypes have anti-dipsogenic effects, however, our group recently demonstrated that acute pharmacological activation of ERα reduced both overnight water intake and water intake stimulated by central AngII, but activation of either ERβ or GPER-1 was ineffective. ERβ or GPER-1 stimulation did, however, affect AngII-stimulated saline intake, but no effect on water intake was observed (Santollo and Daniels, 2015a; Santollo et al., 2016). It is, therefore, plausible to conclude, based on the key role ERα plays in mediating E2's anorexigenic and anti-dipsogenic effects and its expression in the mPOA, that mERα is mediating the inhibitory effect on food and water intake after mER activation reported here. Future studies designed to selectively activate mERα in the mPOA will be necessary to test this conclusion.

Although ERα is traditionally described as a nuclear receptor that directly regulates gene transcription, recent studies demonstrate that it, and ERβ, can associate with the cell membrane and act more like a surface receptor (Micevych and Dominguez, 2009). This membrane association occurs through the posttranslational palmitoylation of the ER and requires interactions with caveolin proteins that anchor the receptors to the cell membrane (Boulware et al., 2007; Christensen and Micevych, 2012). Although mERα can have rapid, non-genomic effects, it can also influence gene transcription via activation of transcription factors such as CREB (Mani et al., 2012). The majority of studies examining the signaling capacity of mERα have focused on the required crosstalk with other receptors. Current evidence suggests that this is accomplished by interactions with mGluR subtypes. For example, in hippocampal neurons, mERα activates mGluR5 and subsequently increases CREB activation, but mERα can also decrease CREB activation by interactions with mGluR3 (Boulware et al., 2005). Within the hippocampus, ERα-mGluR1a interactions activate endocannabinoid signaling which subsequently decreases GABA neurotransmission (Huang and Woolley, 2012). With respect to hypothalamic structures, estradiol's facilitation of lordosis involves actions in the ARC with downstream effects on receptor systems outside of the ARC. Specifically, activation of ARC mER alters neurotransmission that is associated with a rapid internalization of μ-opioid receptors (MOR) in the medial preoptic nucleus. This effect is prevented by an mGluR1a antagonist delivered to the ARC and is mimicked by mGluR1a activation in the ARC (Dewing et al., 2007). Furthermore, this interaction appears critical for the facilitation of reproductive behavior based on the finding that mGluR1a antagonism in the ARC decreases the effect of E2 on lordosis in OVX rats, whereas mGluR1a agonist treatment in the ARC increases lordosis (Dewing et al., 2007). These, and other (Chaban et al., 2011; Grove-Strawser et al., 2010), experiments have provided a crucial understanding of how ERα has a functional role within the cell membrane in spite of an apparent lack of any direct association with intracellular signaling pathways.

Any role of mGluR in mediating the estrogenic inhibition of ingestive behaviors has gone unexplored, until now. We chose to investigate the role of mGluR1a in the ingestive effects of E2 due to its expression within the mPOA (Van den Pol, 1994) and its association with ER in the ARC (Dewing et al., 2007), another key area involved in the anorexigenic effect of E2 (Santollo et al., 2011). As expected, E2

treatment in the mPOA decreased overnight food and water intake. When animals were pre-treated with an mGluR1a antagonist, the inhibitory effect on food intake after E2-treatment was abolished. Overnight water intake, however, was not influenced by mGluR1a antagonism. This suggests that mGluR1a signaling is necessary for the anorexigenic, but not anti-dipsogenic, effect of E2 in the mPOA. This is an interesting divergence in the mechanism by which E2 mediates its inhibitory effect on ingestive behaviors. Future studies examining ER-mGluR interactions in other areas of the brain, such as the lamina terminalis, will be necessary to determine if ER activation can reduce fluid intake without mGluR crosstalk, or if ER subtypes in other brain area require this crosstalk to cause relevant behavioral effects.

Our finding that mGluR1a signaling is necessary for the anorexigenic effect of E2 is a key first step in understanding a role for mER-mGluR1a interactions in the control of food intake. Future studies, however, will be necessary to determine a true interaction between these two proteins. For examine, co-immunoprecipitation studies will be necessary to determine if ERα and mGluR1a physically interact in the mPOA, similar to that reported in the ARC (Mahavongtrakul et al., 2013). It will also be important to determine if mGluR1a activation in the mPOA mimics the anorexigenic effect of E2 and whether any mGluR1a mediated anorexia can be blocked by an ER antagonist.

The mPOA and the control of food intake can now be added to the growing lists of brain areas and behaviors in which mGluR signaling is involved in mediating the effects of E2. While these findings shed new light into the mechanisms underlying E2's anorexigenic effect and also highlight differences between the controls of water and food intake in females, important questions remain unanswered. Future research should focus on identifying the transcription factors that are activated by this ER-mGluR interaction in the mPOA and which genes are being targeted. Furthermore, identifying other brain sites where ER-mGluR interactions are involved in ingestive behaviors will be an important area of research. Ultimately this work helps to elucidate the mechanisms underlying the effects of E2 on ingestive behaviors and can help further our understanding of the biology that may underlie sex differences in obesity and cardiovascular diseases.

Acknowledgments

This work was supported by NIH grants HL091911 and DK107500 to DD and DK098841 to JS. We thank Aniko Marshall and Naomi McKay for technical assistance.

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