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Selective activation of estrogen receptors, ERα and GPER-1, rapidly decreases food intake in female rats

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ABSTRACT

Many of estradiol's behavioral effects are mediated, at least partially, via extra-nuclear estradiol signaling. Here, we investigated whether two estrogen receptor (ER) agonists, targeting ERα and G protein-coupled ER-1 (GPER-1), can promote rapid anorexigenic effects. Food intake was measured in ovariectomized (OVX) rats at 1, 2, 4, and 22 h following subcutaneous (s.c.) injection of an ERα agonist (PPT; 0–200 μg/kg), a GPER-1 agonist (G-1; 0–1600 μg/kg), and a GPER-1 antagonist (G-36; 0–80 μg/kg). To investigate possible cross-talk between ERα and GPER-1, we examined whether GPER-1 blockade affects the anorexigenic effect of PPT. Feeding was monitored in OVX rats that received s.c. injections of vehicle or 40 μg/kg G-36 followed 30 min later by s.c. injections of vehicle or 200 μg/kg PPT. Selective activation of ERα and GPER-1 alone decreased food intake within 1 h of drug treatment, and feeding remained suppressed for 22 h following PPT treatment and 4 h following G-1 treatment. Acute administration of G-36 alone did not suppress feeding at any time point. Blockade of GPER-1 attenuated PPT's rapid (within 1 h) anorexigenic effect, but did not modulate PPT's ability to suppress food intake at 2, 4 and 22 h. These findings demonstrate that selective activation of ERα produces a rapid (within 1 h) decrease in food intake that is best explained by a non-genomic signaling pathway and thus implicates the involvement of extra-nuclear ERα. Our findings also provide evidence that activation of GPER-1 is both sufficient to suppress feeding and necessary for PPT's rapid anorexigenic effect.

1. Introduction

Estradiol decreases food intake in many species, including humans (Lyons et al., 1989), and loss-of-function studies show that deficits in estradiol signaling promote overeating and weight gain in both sexes (Binh et al., 2011; Chen et al., 2009; Lovejoy et al., 2008). While this provides compelling evidence that estradiol plays a critical role in controlling food intake, the underlying cellular and molecular mechanisms are poorly understood and will remain so until the specific estrogen receptors (ERs) and downstream signaling events are identified.

Estradiol was once thought to exert its diverse effects solely through two members of the nuclear steroid hormone receptor superfamily, ERα and ERβ, which regulate transcription of estradiol-responsive genes (Nilsson et al., 2001). In addition to this genomic signaling pathway, it is now well established that estradiol interacts with extra-nuclear ERs, including cytosolic ERα and ERβ, palmitoylated forms of ERα and ERβ that are trafficked to the plasma membrane (Pedram et al., 2007), and the de novo membrane-associated ER (mER), GPER-1 (originally called GPR30), a G protein-coupled receptor that is structurally unrelated to ERα and ERβ (Carmeci et al., 1997). Ligand-bound, extra-nuclear ERs promote rapid alterations in cell signaling by interacting with effector proteins that activate kinase cascades and other second messenger systems. As a result, extra-nuclear ERs transduce estradiol signals into more rapid changes in cellular activity, and thus behavior, than the canonical nuclear ERs, which require hours to days to manifest a change in behavior (Balthazart et al., 2018). It should be noted, however, that extra-nuclear ER-initiated signaling can also affect gene expression via targeted interactions with downstream transcription factors (Vasudevan et al., 2005). Thus, while extra-nuclear ERs alone transduce rapid cellular responses, including changes in membrane excitability, synaptic plasticity, and cell survival (Levin, 2009), both nuclear and extra-nuclear ERs modulate gene transcription. Taken together, these recent advances in our understanding of rapid estradiol signaling have led to the growing acceptance that extra-nuclear ERs contribute to many of estradiol's actions that were once believed to be mediated solely by nuclear ERs (Levin, 2009).

Various approaches have been used to investigate the specific ERs that mediate estradiol's anorexigenic effect. Transgenic studies have shown that a null mutation of ERα, but not ERβ, promotes obesity in

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mice (Heine et al., 2000; Ohlsson et al., 2000), but it is unclear whether the weight gain is due to changes in energy intake or expenditure (Eckel, 2011). Pharmacological studies provide clearer evidence in support of a role for ERα in the estrogenic control of food intake. Administration of the ERα agonist 4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT), but not the ERβ agonist 2,3-bis(4-Hydroxyphenyl)-propionitrile (DPN), decreases food intake in ovariectomized (OVX) rats (Santollo et al., 2007; Thammacharoen et al., 2009; Wegorzewska et al., 2007). Unlike the non-specific ER agonist estradiol benzoate (EB), which suppresses feeding with a latency of ~24 h (e.g., Asarian and Geary, 2002; Santollo et al., 2007), PPT decreases food intake within 3–6 h of treatment (Santollo et al., 2007; Thammacharoen et al., 2009). This suggests that PPT may preferentially target extra-nuclear ERα or increase trafficking of ERα to the membrane. Because PPT's anorexigenic effect has not been examined until at least 3 h post-treatment (Santollo et al., 2007; Thammacharoen et al., 2009), a more detailed time-course analysis, particularly within the first hour after treatment, is needed to determine whether PPT suppresses food intake with a sufficiently short latency that would preclude the involvement of nuclear ERα and thus indirectly implicate extra-nuclear ERα.

The involvement of extra-nuclear ERs in the estrogenic control of food intake is further supported by a study in which central administration of a membrane-delimited form of estradiol (E2-BSA; filtered through a 3-kDA cutoff filter to remove any free estradiol that could trigger intracellular effects) decreased food intake in OVX rats (Santollo et al., 2013). While this provides evidence that mER-initiated signaling is sufficient to decrease food intake, it does not reveal which mERs are involved. One possible candidate is mERα, since activation of ERα by PPT suppresses feeding within 3 h (Santollo et al., 2007). Another possible candidate is GPER-1. Imaging studies confirm GPER-1 expression in feeding-related brain areas (Brailoiu et al., 2007; Spary et al., 2013), and some pharmacological studies report an anorexigenic effect of GPER-1. For example, acute administration of the GPER-1 agonist (±)-1-[(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[*c*]quinolin-8-yl]-ethanone (G-1) decreased daily food intake in OVX guinea pigs (Washburn et al., 2013), but failed to decrease 24-h food intake in OVX rats (Santollo and Daniels, 2015). These discrepant findings, together with emerging reports of functional cross talk between ERα and GPER-1 in cultured cells (Vivacqua et al., 2009) and dopaminergic neurons in mice (Bourque et al., 2015), highlight the need for further studies investigating both the independent and interactive involvement of ERα and GPER-1 in the estrogenic control of food intake.

The current study investigated the time course over which activation of ERα and GPER-1 suppresses feeding in female rats. First, we tested the hypothesis that PPT, which targets both nuclear and extra-nuclear ERα, decreases food intake with a short latency that is best explained by the more rapid signaling actions of extra-nuclear ERα. We also examined the acute effects of the GPER-1 agonist G-1 and the GPER-1 antagonist (±)-(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-8-(1-methylethyl)-3H-cyclopenta[*c*]quinolone (G-36) on food intake. Because some ER antagonists can act as selective estrogen receptor modulators (SERMs) with mixed agonist/antagonist effects (Kuiper et al., 1999), the latter experiment was conducted to rule out the possibility that the GPER-1 antagonist G-36 might suppress feeding, similar to that observed following treatment with the GPER-1 agonist G-1. To investigate whether cross talk between ERα and GPER-1 contributes to the estrogenic control of food intake, we investigated whether GPER-1 blockade attenuates PPT's anorexigenic effect.

2. Methods

2.1. Animals and housing

Female Long-Evans rats (Charles River Breeding Laboratory, Raleigh, NC), weighing 225–250 g at study onset, were housed individually in custom plastic tub cages that provided access to spill-resistant food cups. Throughout the study, rats were given ad libitum access to powdered chow (Purina 5001, St. Louis, MO) and tap water unless otherwise specified. Animal rooms were maintained at 20 ± 2 °C with a 12:12 h reverse light-dark cycle (dark onset = 1300 h). Animal usage and all procedures were approved by the Florida State University Institutional Animal Care and Use Committee.

2.2. Surgery

Animals were anesthetized with 3% isoflurane (Butler Schein Animal Health, Dublin, Ohio), delivered at a rate of 1 L/min, and bilaterally OVX using an intra-abdominal approach. Following surgery, animals received intraperitoneal (i.p.) injections of butorphanol (0.5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) to minimize postoperative pain, and subcutaneous (s.c.) injections of gentamicin (10 mg/kg; Pro Labs Ltd., St. Joseph, MO) to minimize risk of infection. Behavioral testing commenced after two weeks of postoperative recovery.

2.3. Experiment 1: acute effect of the ERα agonist PPT on food intake

A within-subject design was used to assess the anorexigenic effect of varying doses of the ERα agonist PPT (Tocris Bioscience, Minneapolis, MN) in OVX animals (N = 9). We choose PPT because it has a 410-fold selectivity for ERα over ERβ (Harris et al., 2002) and has been used extensively to examine the contribution of ERα to the estrogenic control of food intake. PPT was dissolved in 50% dimethyl sulfoxide (DMSO) vehicle (Sigma-Aldrich, St. Louis, MO; diluted in physiological saline (Teknova, Hollister, CA)), to yield the following doses: 0, 10, 20, 40, 100, or 200 μg/kg PPT. On test days, food was removed from the animals' cages during the last 2 h of the light phase to prevent the consumption of a meal just prior to drug treatment. Within 5 min of dark onset, animals received s.c. injections of a single dose of PPT, administered in random order. Food cups were returned at dark onset and food intake was monitored at 1, 2, 4, and 22 h. Test days were spaced at least 3 days apart (range = 3–5 days), and daily food intake was monitored on non-test days. This 3–5 day wash-out period was based on a previous study that provided a detailed examination of the time course of PPT's anorexigenic effect in female rats (Santollo et al., 2007). In this study, acute administration of PPT (300 μg/kg) decreased food intake for 15 h, with no further decrease in food intake during the 7 days following PPT treatment (Santollo et al., 2007). This suggests that PPT's anorexigenic effect is restricted to the day of injection, as has been reported in other studies (Roesch, 2006; Santollo and Eckel, 2009; Thammacharoen et al., 2009), with no further anorexigenic effect that could reflect a delayed genomic effect. Thus, while our within-subjects design does not allow us to completely rule out the possibility that a delayed genomic effect of one dose of PPT could contribute to the anorexia observed in response to a subsequent dose of PPT, the 24-h restricted time course of PPT's anorexigenic effect suggests that such an outcome is unlikely. The doses of PPT and the time course over which food intake was monitored were chosen to extend previous studies that utilized higher doses of PPT (300–2000 μg/kg) and limited assessment of food intake to the 3–24 h period following drug treatment (Roesch, 2006; Santollo et al., 2007; Thammacharoen et al., 2009).

2.4. Experiment 2: acute effects of the GPER-1 agonist (G-1) and GPER-1 antagonist (G-36) on food intake

A within-subject, randomized design was used to assess the anorexigenic effect of varying doses of the GPER-1 agonist G-1 (Cayman Chemical Company, Ann Arbor, MI) in OVX animals (N = 8). G-1 is a racemic but diastereomerically pure compound with high (nanomolar)