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Activation of ERα is necessary for estradiol's anorexigenic effect in female rats

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Abstract

While there is considerable evidence that the ovarian hormone estradiol reduces food intake in female rats, it is unclear which estrogen receptor (ER) subtype, ERα or ERβ, mediates this effect. While several studies have demonstrated that activation of ERα, but not ERβ, is sufficient to reduce food intake in ovariectomized (OVX) rats, there are limited data regarding which receptor subtype is necessary. Here we used the selective ERα and ERβ antagonists, MPrP and PHTPP, respectively, to investigate this question. We found that antagonism of ERα, but not ERβ, prevented the decrease in food intake following acute administration of estradiol in OVX rats. In addition, antagonism of ERα prevented the estrous-related, phasic reduction in food intake that occurs in response to the rise in circulating levels of estradiol in cycling rats. We conclude that activation of ERα is necessary for the anorexigenic effects of exogenous and endogenous estradiol in female rats.

Keywords

Estradiol; Food Intake; Estrous Cycle; Estrogen Receptor; MPrP; PHTPP

Introduction

The ovarian hormone estradiol appears to play a physiological role in the control of food intake in female rats. The best evidence in support of this statement comes from studies in which ovariectomy has been shown to cause hyperphagia and weight gain (Wade and Gray, 1979), both of which can be prevented by estradiol treatment alone (Asarian and Geary, 2002). Additionally, the pre-ovulatory rise in circulating estrogens in cycling rats promotes a reduction in food intake during the estrous stage of the ovarian reproductive cycle (Drewett, 1973; Blaustein and Wade, 1976; Eckel *et al.*, 2000). This estrous-related decrease in food intake appears to be mediated by estradiol, with minimal involvement of estriol or estrone, since

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estradiol treatment alone can reinstate this cyclic reduction in food intake in OVX rats (Asarian and Geary, 2002).

Many of the behavioral effects of estradiol are not apparent until hours or days following the rise in circulating estradiol in female rats. For example, the decrease in food intake observed in estrous rats does not occur until about 60 h after the initial rise in circulating estradiol (Becker *et al.*, 2005) and treatment with exogenous estradiol takes ~ 36 h before any behavioral change in food intake is detected in ovariectomized (OVX) rats (Asarian and Geary, 2002). Thus, the anorexigenic effect of estradiol likely involves a genomic mechanism that is initiated by activation of one or both of the nuclear estrogen receptors (ERs), ERα and ERβ.

Studies involving either activation or blockade of ERα/ERβ signaling have been used to investigate the relative contribution of each ER subtype to estradiol's anorexigenic effect. Multiple groups have shown that treatment with an ERα agonist decreases food intake in OVX rats and mice (Roesch, 2006; Santollo *et al.*, 2007; Thammacharoen *et al.*, 2009). In comparison, treatment with an ERβ agonist fails to alter either food intake or the ability of an ERα agonist to reduce food intake (Roesch, 2006; Santollo *et al.*, 2007). While these studies suggest that activation of ERα alone is sufficient to reduce food intake in OVX rats, other studies involving disruptions in ERα/ERβ signaling have provided equivocal evidence regarding the necessity of each ER subtype in mediating estradiol's anorexigenic effect. For example, an examination of the feeding behavior of female mice with a null mutation of the ERα subtype (i.e., αERKO mice) revealed that they were insensitive to the effects of estradiol treatment on several feeding-related measures (Geary *et al.*, 2001). This finding suggests that estradiol's anorexigenic effect requires ERα and extends previous demonstrations that ERβ alone is not sufficient. In another study, however, the anorexigenic effect of estradiol was blocked by intracerebroventricular administration of an ERβ-selective, but not an ERα-selective, antisense oligodeoxynucleotide in the OVX rat (Liang *et al.*, 2002). This finding suggests that estradiol's anorexigenic effect in the rat requires a functional ERβ.

Progress in determining the relative necessity of ERα and/or ERβ signaling in mediating estradiol's anorexigenic effect has been further hampered by the lack of suitable ER-selective antagonists. For example, methyl-piperidino-pyrazole (MPP), a non-steroidal, pyrazole compound (Sun *et al.*, 2002), was originally classified as an ERα antagonist based on *in vitro* tests of its ability to antagonize estrogen-regulated genes (Harrington *et al.*, 2003). However, in subsequent tests of its *in vivo* actions, MPP increased uterine weight in mice and rats (Davis *et al.*, 2006; Santollo and Eckel, 2009), reduced food intake in OVX rats (Santollo and Eckel, 2009), and failed to attenuate the anorexigenic effects of estradiol and an ERα agonist in OVX rats (Santollo and Eckel, 2009). Despite these multiple, estradiol-like effects, MPP did attenuate the estrous-related decrease in food intake in cycling rats (Santollo and Eckel, 2009). Taken together, these studies suggest that MPP acts as an ERα antagonist following *in vitro* applications, but exerts mixed ERα agonist/antagonist actions following *in vivo* applications. Thus, MPP, which was initially categorized as an ERα antagonist, better resembles a selective ER modulator (SERM; a compound that exerts mixed agonist/antagonist activities at ERs). Indeed, MPP's structure, which is comprised of a core, non-steroidal ER ligand with a basic side chain, is similar to the structure of most SERMs. Moreover, it appears that under certain conditions MPP's basic side chain may be metabolically cleaved resulting in a compound with agonist, rather than antagonist, qualities (Zhou *et al.*, 2008). In light of these findings, Katzenellenbogen's group developed a novel MPP analog, called methyl-piperidinopropyl pyrazole (MPrP), which contains a basic side chain that cannot undergo metabolic cleavage. While this novel compound is highly selective for ERα in binding affinity assays and exerts potent ER antagonist activity in transcription activation assays (Zhou *et al.*, 2008), its *in vivo* actions have yet to be evaluated.

Currently, there is no evidence that ERβ-selective compounds, like the ERβ antagonist 4-[2-phenyl-5,7-bis(trifluoromethyl) pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP), exert the mixed agonist/antagonist properties that are often seen in ERα-selective compounds. For example, PHTPP displays 36-fold selectivity for ERβ over ERα and displays complete antagonism for ERβ in reporter gene assays in co-transfected endometrial cells (Compton *et al.*, 2004). The goal of the present study was to use these ER-selective antagonists (MPrP and PHTPP) to evaluate the relative necessity of ERα- and ERβ-activation in mediating estradiol's anorexigenic effect in OVX and cycling rats.

Materials and methods

Animals and Housing

Female, Long-Evans rats (Charles River Breeding Laboratory, Raleigh, NC), weighing ~250 g at study onset, were individually housed in custom, shoebox cages. Each cage contained a spill-proof food cup, a water bottle, and a sleeping niche. Rats had free access to powdered chow (Purina 5001) and tap water. The colony room was maintained at 20°C with a 12:12 h 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.

Surgery

Rats that underwent ovariectomy surgery (Experiments 1 and 2), were anesthetized by intraperitoneal (i.p.) injections of a mixture of ketamine (50 mg/kg; Ketaset, Fort Dodge Animal Health, IA) and xylazine (4.5 mg/ml; Rompun, Mobay, Shawnee, KS) and then bilaterally ovariectomized (OVX) using an intra-abdominal approach. Immediately following surgery, rats were given single, i.p. injections of butorphanol (0.5 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and gentamicin (10 mg/ml; Pro Labs Ltd, St. Joseph, MO) to minimize post-surgical pain and the risk of infection, respectively. Rats were given 10 days to recover from surgery and then behavioral testing commenced once stable levels of food intake were observed.

Experiment 1: Does acute administration of either MPrP or PHTPP alter food intake in OVX rats?

It is becoming increasingly clear that pharmacological compounds designed to antagonize ERs can sometimes function as SERMs and exert mixed agonist/antagonist actions following *in vivo* administration (Wade and Heller, 1993; Bryant and Dere, 1998; Santollo and Eckel, 2009). As a first step in our efforts to determine the effects of selective ERα/β antagonism on food intake in the female rat, we investigated whether acute administration of either MPrP or PHTPP produces any estrogenic effects in OVX rats. Four h prior to dark onset, rats (n = 6) received randomized, subcutaneous (s.c.) injections of either 0 or 25 μg of the ERα antagonist MPrP (synthesized by J.A. Katzenellenbogen, University of Illinois (Zhou *et al.*, 2008)) dissolved in DMSO vehicle at 4-day intervals. A second group of rats (n = 8) received randomized, s.c. injections of 0, 25, or 50 μg of the ERβ antagonist PHTPP (Tocris) dissolved in DMSO vehicle at 4-day intervals. The dose of MPrP was chosen based on work involving MPP, the compound from which MPrP was derived. Previously, we demonstrated that a single injection of 25 μg MPP does not exert an estrogenic effect on food intake in OVX rats (Santollo and Eckel, 2009). In addition, this dose of MPP was sufficient to attenuate the estrous-related decrease in food intake that occurs in cycling rats (Santollo and Eckel, 2009). These findings, together with the fact that MPrP has greater ERα binding selectivity than MPP in a competitive, radiometric, binding assay (Zhou *et al.*, 2008), suggested that a similar dose of MPrP (25 μg) was an appropriate starting point. We choose our doses of PHTPP (25 and 50 μg) based on previous *in vitro* studies (Chen *et al.*, 2008; Ben-Jonathan *et al.*, 2009). Although food intake was measured daily throughout the experiment, we were particularly interested in the 24-h

period following drug treatment since this interval corresponds to the time in which SERMS such as MPP and tamoxifen decrease overnight food intake (Santollo and Eckel, 2009; Wade and Heller, 1993).

Experiment 2: Does either MPrP or PHTPP attenuate the anorexigenic effect of exogenous estradiol?

Rats (n = 6) received randomized, s.c. injections of vehicle, 2 μg estradiol benzoate (EB; Sigma), or 25 μg MPrP followed immediately by 2 μg EB. This yielded three treatment conditions: vehicle, EB and MPrP/EB. This series of injections was administered in random order 4 h prior to dark onset at 4-day intervals over a period of 12 days. A second group of rats (n = 8) received a similar series of drug injections but 50 μg PHTPP was administered in place of 25 μg MPrP. This yielded three treatment conditions: vehicle, EB, and PHTPP/EB. We choose to administer drug treatments at 4-day intervals based on a previous demonstration that injection of 2 μg EB every fourth day mimics the changes in endogenous estradiol secretion observed in cycling rats and, beginning approximately 24-h after injection, models the cyclic reduction in 24-h food intake that is observed during estrus in cycling rats (Asarian *et al.*, 2002). In order to coincide with the period that models estrus, food intake was monitored for 24 h commencing at the start of the second dark phase following drug treatment.

Experiment 3: Does MPrP attenuate the anorexigenic effect of endogenous estradiol?

The estrous cycles of 8 female rats were monitored by examining the appearance and abundance of cells within vaginal cytology samples as previously described (Becker *et al.*, 2005). Cycle stage labels were assigned to the previous 24-h period ending at the time of sampling. With this method, the light-phase peak in estradiol and luteinizing hormone secretion occurred during proestrus and estrus included the following dark phase when female rats display estrous-related decreases in food intake (Becker *et al.*, 2005). Data collection did not begin until all rats displayed two consecutive 4-day, estrous cycles. Rats received s.c. injections of either 0 or 25 μg MPrP dissolved in DMSO vehicle just prior to the dark phase of proestrus. Twelve h later, a second s.c. injection of either vehicle or 37.5 μg MPrP was administered. Drug treatment was administered in a counterbalanced manner across two consecutive estrous cycles and food intake was monitored daily throughout this time. Food intake during estrus (i.e., the cycle stage that is preceded by high plasma estradiol levels) was compared with food intake during diestrus 2 (i.e., the cycle stage that is preceded by low plasma estradiol levels). This regimen of MPrP treatment was chosen based on a past study in which MPP given during these time points attenuated the phasic reduction of food intake in estrous rats (Santollo and Eckel, 2009).

Data analysis

Data are presented as the means ± SEMs. Group differences in food intake in Experiments 1 and 2 were analyzed using either a dependent t-test or repeated-measures ANOVAs as appropriate. Group differences in food intake in Experiment 3 were assessed using a two-factor, repeated-measures ANOVA (cycle stage × drug treatment). Newman Keul's post-hoc test was used to determine individual group differences following significant main or interactive ANOVA effects ($p < 0.05$).

Results

Experiment 1

Food intake during the 24-h period following drug treatment was not influenced by acute administration of 25 μg MPrP in OVX rats, $t(5) = 1.29$, n.s. (Fig. 1A). During this same period, neither dose of PHTPP influenced food intake in OVX rats, $F(2,7) = 1.85$, n.s. (Fig. 1B). In

addition, food intake was not influenced by either drug during the second and third days following drug treatment (data not shown).

Experiment 2

The anorexigenic effect of EB was blocked in OVX rats that were treated with the ERα antagonist MPrP, $F(2,5) = 8.06$, $P < 0.01$ (Fig. 2A). As expected, EB treatment reduced food intake in OVX rats, $P < 0.01$. This action of EB was blocked by concurrent treatment with MPrP (Fig. 2A). In contrast, the anorexigenic effect of EB was not influenced in OVX rats treated with the ERβ antagonist PHTPP, $F(2,7) = 32.37$, $P < 0.01$ (Fig. 2B). Once again, EB treatment reduced food intake in OVX rats, $P < 0.01$. However, this action of EB was not influenced by concurrent treatment with PHTPP. Both EB treatment alone and in combination with PHTPP produced similar reductions in food intake, relative to that observed following vehicle treatment, $Ps < 0.01$.

Because there is little information available about the half life of PHTPP, it is possible that the PHTPP may have been degraded more rapidly than EB. Such an occurrence would have limited PHTPP's ability to antagonize ERβ. Therefore, we repeated Experiment 2B with a subset of animals that received a second dose of 50 μg PHTPP 24 h after the first dose. This regimen of drug treatment also failed to attenuate the anorexigenic effect of EB (data not shown).

Experiment 3

Treatment with the ERα antagonist MPrP blocked the estrous-related decrease in food intake in cycling rats, $F(1,7) = 5.04$, $P < 0.05$ (Fig. 3). As expected, food intake was significantly reduced during estrus, relative to diestrus 2, in vehicle-treated rats, $P < 0.01$. This estrous-related decrease in food intake, observed following vehicle treatment, was blocked by MPrP treatment.

Discussion

Studies involving disruptions in ERα/ERβ signaling have provided equivocal evidence regarding the necessity of each ER subtype in mediating estradiol's anorexigenic effect (e.g., (Geary *et al.*, 2001; Liang *et al.*, 2002). Progress in answering this question has also been hampered by the limited availability of selective ER antagonists that are devoid of tissue- or species-specific estrogenic activity (Wade and Heller, 1993; Davis *et al.*, 2006; Santollo and Eckel, 2009). The goal of the present study was to investigate the estrogenic inhibition of food intake in female rats treated with either a newly developed selective ERα antagonist, MPrP (Zhou *et al.*, 2008), or a selective ERβ antagonist, PHTPP (Compton *et al.*, 2004). After demonstrating that neither compound exerts an estrogenic effect on food intake, we tested the hypothesis that selective blockade of either ERα or ERβ would attenuate estradiol's anorexigenic effect in OVX rats. Acute administration of MPrP, but not PHTPP, blocked estradiol's ability to decrease food intake in OVX rats. This provides clear evidence that exogenous estradiol decreases food intake via selective activation of ERα. Finally, we extended this finding by demonstrating that selective blockade of ERα prevents the estrous-related decrease in food intake in cycling rats. Taken together, these findings provide the first report that *in vivo* administration of the newly developed ERα antagonist MPrP blocks the anorexigenic effects of both exogenous and endogenous estradiol in female rats.

Compounds designed to selectively target ERs often function as SERMs in that they produce mixed agonist/antagonist effects. For example, while tamoxifen exerts an estrogenic effect on feeding (Wade *et al.*, 1993; Wade and Heller, 1993), presumably via activation of ERα, it exerts an antiestrogenic effect on reproductive behavior (Etgen, 1979), presumably by blocking the activation of ERα (Ogawa *et al.*, 1998). Thus, tamoxifen appears capable of producing both

estrogenic and antiestrogenic effects at the same ER subtype. The mechanism underlying this complicated behavioral response to tamoxifen is unclear, however, it may be explained by the fact that the critical ERs are located in different tissues. For example, ER-dependent changes in feeding behavior appear to be mediated by activation of ERα in the brain (Rivera and Eckel, 2005), whereas ER-dependent changes in reproductive behaviors appear to be mediated by activation of ERα in peripheral tissues and in the brain (Gardener and Clark, 2001; Wade *et al*., 1993). Such tissue-specific, mixed agonist/antagonist effects are common features of SERMs, like tamoxifen, and have limited our progress in understanding the relative necessity of ERα versus ERβ to the estrogenic control of feeding.

MPP represents another SERM that was originally classified as an ER antagonist based on *in vitro* assays (Sun *et al.*, 2002; Harrington *et al.*, 2003), but was subsequently shown to mimic estradiol's effects on food intake and uterine tissue (Davis *et al.*, 2008; Santollo and Eckel, 2009). Therefore, before testing the abilities of MPrP and PHTPP to block the feeding inhibitory effect of estradiol, we first measured food intake following acute administration of each compound in OVX rats to determine whether they produce any estrogenic effects on food intake. Neither MPrP nor PHTPP produced any change in food intake in OVX rats. This suggests that, at the doses administered in the current study, neither compound mimics estradiol's inhibitory effect on food intake. Thus, this first experiment allowed us to identify doses of MPrP and PHTPP that do not show any estrogenic effects on food intake and, as a result, could be used to investigate the relative necessity of ERα and ERβ activation in mediating estradiol's anorexigenic effect.

Previous studies involving acute and chronic administration of ERα/β-selective agonists have shown that activation of ERα, but not ERβ, is sufficient to decrease food intake in OVX rats (Roesch, 2006; Santollo *et al.*, 2007; Thammacharoen *et al.*, 2009). Here, we extend these findings by demonstrating that selective activation of ERα is necessary for the estrogenic inhibition of food intake in the OVX rat. In the present study, the feeding inhibitory effect of a single injection of estradiol was blocked by MPrP, an ERα antagonist. Similar treatment with an ERβ antagonist, PHTPP, failed to alter estradiol's anorexigenic effect. Taken together, these findings in OVX rats demonstrate that activation of ERα is necessary for estradiol's anorexigenic effect. While blockade of ERβ with PHTPP failed to attenuate the inhibitory effect of estradiol, it is premature to rule out the possibility that ERβ is involved in mediating the estrogenic inhibition of food intake. Further validation that our doses and treatment regimen of PHTPP are sufficient to antagonize estrogen-dependent behavioral responses thought to be mediated by ERβ is necessary to rule out ERβ's involvement in estradiol's anorexigenic action. Such a study has not yet been performed. The lack of data in this area is likely related to the fact that there are limited estradiol-dependent behavioral responses that are thought to be mediated exclusively by ERβ. However, it has been shown that administration of ERβ-selective agonists elicit antianxiety behaviors in OVX rats (Walf and Frye, 2005). Thus, it will be important for future studies to determine whether the ERβ-selective antagonist PHTPP, within the dose range used here, can block estradiol's antianxiety effects. Such a finding would strengthen the conclusion that ERβ is not necessary for estradiol's anorexigenic effect.

Our findings are consistent with a previous study that utilized targeted RNA interference of ERα in the ventromedial nucleus of the hypothalamus (VMH) of OVX mice and rats. One week following ovariectomy, a 15% increase in daily food intake was reported in the ERα deficient mice, relative to controls (Musatov *et al.*, 2007). This suggests that the hyperphagia and associated weight gain resulting from estradiol withdrawal post-ovariectomy surgery is mediated, in part, by activation of ER in the VMH. This same group reported that mice with and without functional ERα in the VMH were equally responsive to a subcutaneous pellet containing estradiol (Musatov *et al.*, 2007). This finding is consistent with previous reports that site-specific administration of estradiol into the VMH is not sufficient to reduce food intake

in OVX rats (Butera and Beikirch, 1989). Finally, our findings are inconsistent with a report that the inhibitory effect of estradiol on food intake was blocked in OVX rats following ventricular infusion of antisense oligodeoxynucleotides targeting ERβ, but not by antisense oligodeoxynucleotides targeting ERα (Liang *et al.*, 2002). At this time, we are unable to reconcile this sole report that ERβ is necessary for estradiol's anorexigenic effect with our current findings or previous findings from multiple groups providing converging evidence that ERα is both sufficient and necessary for estradiol's anorexigenic effect in the rat (Roesch, 2006; Musatov *et al.*, 2007; Santollo *et al.*, 2007; Thammacharoen *et al.*, 2009).

Studies involving ERα null (i.e., αERKO) mice have provided another approach to investigate the relative necessity of ERα and ERβ activation to estradiol's anorexigenic effect. For example, Geary and colleagues demonstrated that estradiol's anorexigenic effect, present in OVX wild-type mice was absent in OVX αERKO mice (Geary *et al.*, 2001). Consistent with the current findings, these results in mice suggest that activation of ERα is necessary for estradiol's anorexigenic effect. However, in another study, three weeks of chronic estradiol treatment normalized the food intake of OVX αERKO mice to that of ovarian-intact αERKO mice during one of the three weeks (Naaz *et al.*, 2002). While this limited response to estradiol treatment suggests some involvement of ERβ in the maintenance of daily food intake in the mouse, it may be specific to knockout models because αERKO show a 10-fold increase in estradiol levels that could lead to changes in signaling through ERβ (Naaz *et al.*, 2002). In addition to this type of developmental compensation, the interpretation of studies involving ER null mice is limited by a recent report that the increase in body weight in OVX, C57BL/6 mice, the background strain for the ERα deletion, is mediated via reductions in energy expenditure, not by increases in food intake (Witte *et al.*, 2010). This suggests that rats may provide a better model organism than mice by which to study estradiol's anorexigenic effect. Here, our use of transient, pharmacological ER blockade in adult rats avoided these possible limitations of previous studies investigating the relative involvement of ERα and ERβ in the feeding behavior of mice.

Because blockade of ERα, but not ERβ, was capable of blocking the anorexigenic effect of exogenous estradiol, we then tested whether the ERα antagonist MPrP could attenuate the estrous-related, phasic reduction in food intake in cycling female rats. MPrP was administered immediately before and after the 12-h dark phase of proestrus. These time points were chosen because they correspond to the initial rise and then the subsequent peak in estradiol secretion in cycling rats (Becker *et al.*, 2005). Here, using MPrP, we showed a complete blockade of the phasic reduction in food intake in cycling rats. This provides the first demonstration that blockade of ERα prevents the estrous-related decrease in food intake observed in cycling rats. In addition to showing that ERα is necessary for the estrous-related decrease in food intake, these data provide evidence that the changes in estradiol secretion, rather than other hormones which are elevated during the peri-ovulatory period, underlie the estrous-related decrease in food intake.

In summary, the results of the current study provide the first evidence that activation of ERα is necessary for estradiol's anorexigenic effects in both OVX and cycling rats. We have also demonstrated that, under our testing conditions, MPrP and PHTPP do not possess ER agonist properties. As such, they can still be considered presumptive ER antagonists, rather than being classified as SERMs. Future studies will be necessary to rule out the possibility that, under other conditions, in different species, or at different doses, these putative ER antagonists are capable of exerting estrogenic effects since SERMs can possess tissue- and species-specific effects (Jordan and Robinson, 1987). However, under our test conditions, MPrP and PHTPP have proven to be useful tools in determining that estradiol's anorexigenic effect requires activation of ERα.

Research Highlights

- estradiol decreases food intake in female rats
- estradiol's anorexigenic effect is blocked by the ERα antagonist, MPrP
- estradiol's anorexigenic effect was not influenced by the ERβ antagonist, PHTPP
- activation of ERα is necessary for estradiol's anorexigenic effect

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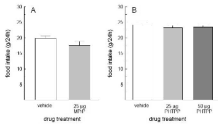

Figure 1.

Food intake in OVX rats was not influenced by blockade of either ERα or ERβ Treatment with either MPrP, an ERα antagonist (A) or PHTTP, an ERβ antagonist (B), failed to alter food intake during the 24-h period following drug treatment.

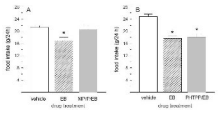

Figure 2.

Blockade of ERα, but not ERβ, prevented the anorexigenic effect of estradiol benzoate (EB) in OVX rats. Food intake was monitored for 24 h beginning 24 h following treatment with vehicle, EB, or EB co-administered with either MPrP, an ERα antagonist, or PHTPP, an ERβ antagonist. (A) The EB-induced reduction in food intake was blocked by MPrP. (B) Administration of EB alone, and EB in combination with PHTPP, produced similar decreases in food intake, relative to that observed following vehicle treatment. *Less than vehicle, $P < 0.01$.

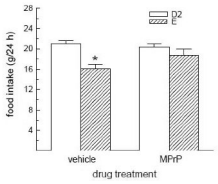

Figure 3.

Blockade of ERα prevented the estrous-related decrease in food intake in cycling rats. Female rats received one injection of either vehicle or MPrP just prior to the dark phase of proestrous and a second injection of the same compound 12 h later. Drug treatment was administered in a counterbalanced manner across two consecutive estrous cycles. Following vehicle treatment, rats displayed a phasic reduction in food during estrus (E) compared to diestrus 2 (D2). This E-related decrease in food intake was blocked in rats pre-treated with MPrP, an ERα antagonist. *Less than all other groups, $P < 0.01$.