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The ovarian hormone estradiol plays a crucial role in the control of food intake in females

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Abstract

Despite a strong male bias in both basic and clinical research, it is becoming increasingly accepted that the ovarian hormone estradiol plays an important role in the control of food intake in females. Estradiol's feeding inhibitory effect occurs in a variety of species, including women, but the underlying mechanism has been studied most extensively in rats and mice. Accordingly, much of the data reviewed here is derived from the rodent literature. Adult female rats display a robust decrease in food intake during estrus and ovariectomy promotes hyperphagia and weight gain, both of which can be prevented by a physiological regimen of estradiol treatment. Behavioral analyses have demonstrated that the feeding inhibitory effect of estradiol is mediated entirely by a decrease in meal size. In rats, estradiol appears to exert this action indirectly via interactions with peptide and neurotransmitter systems implicated in the direct control of meal size. Here, I summarize research examining the neurobiological mechanism underlying estradiol's anorexigenic effect. Central estrogen receptors (ERs) have been implicated and activation of one ER subtype in particular, ERα, appears both sufficient and necessary for the estrogenic control of food intake. Future studies are necessary to identify the critical brain areas and intracellular signaling pathways responsible for estradiol's anorexigenic effect. A clearer understanding of the estrogenic control of food intake is prerequisite to elucidating the biological factors that contribute to obesity and eating disorders, both of which are more prevalent in women, compared to men.

Keywords

estrous cycle; sex differences; estrogen receptor alpha; meal size

1. Introduction

Most would acknowledge that a strong male bias exists in both basic and applied animal research. Indeed, a recent, comprehensive survey of articles published in representative neuroscience journals revealed that the ratio of single-sex studies involving males versus females is almost 6 to 1 [1]. Similar surveys revealed failures to report the sex of subjects and, when both sexes were studied, failures to analyze and present data in a sex-specific manner [2]. Others have noted that the sex of tissues or cell lines is rarely reported [3]. This male bias and indifference towards sex-based research is difficult to justify in light of the many behavioral traits and physiological responses that are known to be sexually dimorphic.

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An informal survey of the literature suggests that the male bias in neuroscience research extends to the study of ingestive behavior. The relative lack of female-based, feeding research is particularly troubling, given the higher rates of obesity (characterized by a body mass index > 40) and eating disorders in women than in men [4–7]. We and others have argued that an understanding of the normal control of food intake in females is prerequisite to identifying the biological factors contributing to the high prevalence of disordered eating in women. This review summarizes the evidence, derived primarily from rodent models, that the ovarian hormone estradiol plays a critical role in the physiological control of food intake in females. More recent studies designed to investigate the mechanism underlying estradiol's anorexigenic effect are also reviewed.

2. Estradiol is involved in the physiological control of food intake

In addition to its well characterized effects on reproductive behavior, it is now widely accepted that the ovarian hormone estradiol plays an important role in the normal control of food intake in a variety of species [8,9]. In women, fluctuations in daily food intake are correlated with changes in estradiol secretion across the menstrual cycle. The most robust change is a decline in average daily food intake during the peri-ovulatory period, which occurs after the initial (follicular phase) rise in plasma estradiol concentration [10,11]. Others have also reported that average daily food intake is lower during the follicular phase (when plasma estradiol levels are rising), relative to the luteal phase [10,12–14]. As might be expected, these cyclic changes in food intake are not apparent in women experiencing anovulatory menstrual cycles [15,16]. It remains less clear, however, whether food intake increases as a function of the declining estradiol levels in peri-menopausal women. For a more detailed discussion of the literature regarding the ovarian hormonal control of food intake in women, the reader is referred to the following review papers [9,17].

Estradiol's ability to influence food intake is best characterized in the female rat. As described in greater detail below, the pre-ovulatory increase in plasma estradiol concentration is associated with a transient decrease in food intake during estrus in cycling rats [18–22]. Moreover, bilateral ovariectomy produces a rapid (within 1 week) increase in food intake that promotes increases in adiposity and rapid weight gain [18,23–28]. These behavioral and physiological responses to ovariectomy can be normalized by a physiological regimen of estradiol treatment [29,30]. In comparison, a physiological regimen of progesterone treatment alone is not sufficient to attenuate ovariectomy-induced hyperphagia and it fails to alter estradiol's anorexigenic effect in OVX rats [24,31–33]. Thus, it is the decline in circulating estradiol, rather than progesterone, which promotes the rapid increases in food intake and weight gain in OVX rats. It should be noted, however, that OVX rats receiving a high dose of progesterone, in addition to estradiol, consume more food than OVX rats receiving estradiol alone [23,32–34]. Thus, large, pharmacological doses of progesterone can inhibit estradiol's anorexigenic effect.

The gonadal hormone testosterone also influences food intake and body weight. For example, orchiectomized rats display transient decreases in dark-phase meal number, but they are compensated for by increases in light-phase meal size such that daily food intake is not affected by the declining testosterone levels within the first two weeks following orchiectomy [35]. However, beginning about one month following orchiectomy, male rats display a decrease in daily food intake and concomitant weight loss [36,37], both of which can be attenuated by low, physiological doses of testosterone [35,36,38]. Thus, gonadectomy-induced changes in food intake and body weight develop more slowly in male rats, relative to female rats. Physiological doses of testosterone increase food intake in general and protein intake in particular. In comparison, pharmacological doses of testosterone can decrease carbohydrate intake, but this latter effect is likely mediated by

aromatized metabolites of testosterone (e.g., estradiol) since carbohydrate intake is not influenced by the non-aromatizable androgen dihydrotestosterone [39,40].

2.1. Estradiol exerts both phasic and tonic decreases in food intake

The mid-1920s marked the first reports of phasic decreases in food intake during the estrous stage of the female rat's reproductive cycle [41,42]. Interestingly, some have argued that the exclusion of female rats from behavioral experiments may be linked to this and other early reports (e.g., [43]) that locomotor activity fluctuates across the ovarian reproductive cycle (reviewed in [1]). More contemporary studies have shown that this phasic decrease in food intake is correlated with fluctuations in plasma estradiol concentration. In female rats, plasma estradiol concentration begins to rise during diestrus, peaks during the afternoon of proestrus, and then falls rapidly to basal levels at the onset of estrus [44–47]. The feeding rhythm across the ovarian reproductive cycle is just as predictable. Daily food intake typically peaks during diestrus and reaches a nadir during estrus (e.g., [21,22]). Thus, the phasic decrease in food intake during estrus is believed to be mediated by the pre-ovulatory increase in plasma estradiol concentration. An important detail that is often overlooked or misrepresented in published studies is that the decrease in food intake during estrus is temporally associated with low, rather than high, circulating levels of estradiol.

Drewett [22] was the first to recognize that estradiol also exerts a tonic inhibitory effect on food intake. This action of estradiol is revealed in OVX rats, which display increases in daily food intake that exceed that consumed by cycling rats [18,23–28]. This sustained increase in food intake appears sufficient to account for the ovariectomy-induced weight gain (reviewed in [17]), which results primarily from the deposition of adipose tissue [25,48,49]. Interestingly, this tonic inhibitory effect of estradiol on food intake may not be expressed in mice. A recent study by Overton et al [50], demonstrated that ovariectomy-induced increases in the weight gain of C57BL/6 mice was due entirely to a decrease in energy expenditure, manifested as a decrease in both metabolic rate and locomotor activity, with no significant increase in food intake [50]. In this same study, ovariectomy-induced weight gain in Long-Evans rats was due to an increase in food intake and a decrease in locomotor activity, with no change in metabolic rate [50]. Although it remains to be determined whether the phenotype of OVX C57BL/6 mice extends to other strains of mice, the data derived from rat studies indicates that estradiol exerts both tonic and phasic inhibitory effects on food intake that appear critical for the regulation of body weight. That sex differences in food intake are minimized in aged (i.e, reproductively senescent) rats [51], suggests that the phasic and tonic anorexigenic effects of estradiol are critical to the increased daily caloric intake that is often observed in male and female rats of reproductive age.

As would be expected, bilateral ovariectomy abolishes estradiol's phasic inhibitory effect on food intake. Geary and colleagues have, however, developed an acute, physiological regimen of estradiol replacement (a single, subcutaneous, injection of 1–2 μg of estradiol every fourth day) that models the 4-day, cyclic pattern of plasma estradiol concentration and food intake observed in cycling rats. Specifically, this estradiol replacement protocol produces changes in plasma estradiol levels in OVX rats that are similar in both the magnitude and duration to that observed in cycling rats. In addition, food intake is reduced every fourth day, beginning ~ 30 h after injection of estradiol [30]. Rather than relying upon chronic estradiol replacement via silastic implants containing crystalline estradiol, the adoption of this acute (physiologically-relevant) estradiol replacement protocol in OVX rats has greatly influenced our understanding of the mechanism underlying estradiol's phasic inhibitory effect on food intake (e.g., [31,52–57]).

2.2. Estradiol selectively affects the control of meal size

Analysis of the spontaneous feeding patterns of cycling rats reveals that the decrease in food intake during estrus is mediated by a selective decrease in meal size with either no change or, more commonly, a non-compensatory increase in meal frequency [18,19,21]. It appears unlikely that rats eat less during estrus as a result of being diverted from feeding by other competing behaviors, such as the increases in locomotor and sexual activity that occur during estrus. First, increasing the opportunity to exercise fails to alter the magnitude of the estrous-related decrease in meal size in rats with and without access to running wheels [21]. Second, providing opportunities to engage in sexual or other social behaviors do not prevent estrous-related decreases in food intake in a variety of species including cats, cows, and baboons [58–60]. Thus, the decrease in food intake during estrus appears to involve a selective change in the neurobiological controls of meal size. Likewise, it has been shown that the hyperphagia following ovariectomy is also mediated by an increase in meal size [18,30] that can be normalized by acute estradiol treatment [29,30].

According to the theory developed and tested by Smith [61–63], the controls of meal size are either direct or indirect. The direct controls of meal size arise from the sensory stimulation of preabsorptive receptors in the oral cavity and upper digestive tract that are sensitive to the chemical, mechanical, and colligative properties of ingested food. The resulting positive and negative feedback signals, activated by preabsorptive food stimuli, are integrated in the brainstem to provide the direct controls of meal size. The indirect controls of meal size operate independently of these preabsorptive receptors and function to modify the direct controls of meal size. As described in an earlier review [64], estradiol does not fit the definition of a direct control of meal size but rather appears to function as a rhythmic, inhibitory, indirect control of meal size. This theoretical framework has been used with much success in establishing interactions between estradiol and other systems implicated in the direct control of meal size such as cholecystokinin, glucagon, and serotonin. The reader is referred to a number of recent review papers summarizing this research [9,64–66].

3. Mechanism underlying estradiol’s anorexigenic effect

It is becoming increasingly clear that estradiol’s diverse biological activity involves multiple signaling pathways initiated via activation of both intracellular (nuclear) and membrane-associated ERs (nERs and mERs, respectively). Under the direct, genomic-signaling pathway, estradiol binds to the ligand binding domains of the classically-defined nERs, ERα and ERβ. Following translocation to the nucleus, the DNA binding domains of newly formed homo- or hetero-dimeric nER complexes bind to estrogen response elements located in the promoter regions of estradiol-responsive, target genes to initiate gene transcription [67–69]. In addition, an indirect mechanism exists for nERs to regulate gene transcription in estradiol-responsive target cells that lack an estrogen response element. In this case, gene transcription is initiated via nER complexes that are tethered through protein-protein interactions with other DNA-bound transcriptions factors including activator protein-1 (AP-1) and specificity protein-1 (SP-1) [70,71].

Estradiol can also exert rapid biological effects that do not require nuclear targeting of ERs. Such signaling is mediated by activation of mERs capable of triggering electrophysiological and intracellular signaling events that can produce rapid alterations in neuronal activity including changes in gene expression. mERs include ERα and possibility ERβ, as well as the G-protein coupled receptors Gq-mER and GPR30 (e.g., [72–75]). Multiple groups have made significant progress in recent years towards elucidating the specific signaling pathways of G-protein coupled mERs. For example, binding of estradiol or the synthetic ER agonist STX to Gq-mER promotes rapid activation of the phospholipase C – protein kinase C – protein kinase A pathway, which desensitizes μ-opioid and $GABA_B$ receptors resulting

in depolarization of proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus in guinea pigs [72,76,77].

Behavioral studies in rodents have shown that estradiol's anorexigenic effect is typically expressed after a delay. For example, there is a time lag of 36 – 40 h between the initial rise in plasma estradiol concentration during early diestrus and the decline in food intake during estrus [64]. Additionally, OVX rats do not display a decrease in food intake until at least 24 h after peripheral (subcutaneous) administration of physiologically-relevant doses of estradiol [29,30]. While the long latencies of this and other actions of estradiol have often been linked to nERs, and more rapid behavioral and physiological effects of estradiol have often been linked to mERs, the prevailing literature suggests that while this may be a useful metric it does not hold up under all conditions [75]. Indeed, there is a growing literature that estradiol may decrease food intake over a more rapid time course than described above. For example, Kelly and colleagues [76–79] demonstrated that estradiol can cause rapid depolarization of hypothalamic POMC neurons through nontranscriptional events involving Gq-mER-dependent signaling both in guinea pigs and in mice with null mutations of ERα and/or ERβ. While food intake was not assessed in their studies, increases in POMC signaling typically promote decreased feeding [80]. The authors did report, however, that activation of Gq-mER-dependent signaling was effective in reducing ovariectomy-induced weight gain in guinea pigs, however, the degree to which this was mediated via a decrease in food intake is currently unknown.

3.1. Central ERs mediate estradiol's inhibitory effect on food intake

Although ERs are expressed in many peripheral tissues [81–84] and throughout the brain [85–88], there is a growing consensus that estradiol's anorexigenic effect is mediated via activation of central ERs. For example, acute infusions of either dilute crystalline or water-soluble estradiol into specific brain areas (e.g., nucleus of the solitary tract, medial preoptic area, dorsal raphe nucleus, and arcuate nucleus) have been reported to decrease food intake in OVX rats [89–94], but see [89,95]. This action of estradiol is selective since similar activation of ERs in other hypothalamic areas (e.g., paraventricular and ventromedial nuclei) does not inhibit food intake. Taken together, these studies suggest that the activation of central ERs, within a subset of brain nuclei tested to date, is *sufficient* for estradiol's anorexigenic effect.

Considerably less progress has been made towards identifying the sites of the ERs that are *necessary* for estradiol's anorexigenic effect. A primary obstacle is the difficulty in developing pharmaceutical agents that function exclusively as ER antagonists (i.e., exert antiestrogenic effects). Rather, most compounds designed to target ERs function as selective ER modulators (SERMs) in that they exert tissue-specific, mixed agonist/antagonist effects [96]. For example, while the SERMs raloxifene and tamoxifen block estradiol's proliferative effects on breast tissue [96,97], they mimic estradiol's effect on food intake [37,98–100]. In other words, they activate, rather than block, the critical ERs responsible for estradiol's anorexigenic effect. There is, however, a steroidal compound, ICI 182,780, that has been classified as a pure antiestrogen [101–103] with a binding affinity that is similar to estradiol in ER competition assays involving homogenized brain tissue [104]. ICI 182,780 prevents estradiol signaling by impairing ER dimerization [105], disrupting ER nuclear localization [106,107], and causing ER degradation [108]. In rodents, peripheral administration of ICI 182,780 prevents the uptake of tritiated estradiol in peripheral, but not hypothalamic, tissue [104,109], and peripheral, but not central, administration of ICI 182,780 blocks estradiol's uterotrophic effect in OVX rats [110]. Taken together, these studies provide strong evidence that ICI 182,780 fails to cross the blood-brain barrier. Thus, unlike SERMs, ICI 182,780 has the potential to not only identify the ERs necessary for estradiol's anorexigenic effect but

also to test directly the relative contribution of peripheral versus central ERs to this action of estradiol.

Wade and colleagues were the first to demonstrate that peripheral administration of ICI 182,780 failed to attenuate the anorexia associated with chronic (4 weeks), systemic, estradiol treatment in OVX rats and hamsters [104,109]. Assuming adequate blockade of ERs by ICI 182,780, these findings suggest that activation of peripheral ERs is not necessary for estradiol's anorexigenic effect. However, the necessity of either peripheral or central ERs in mediating the anorexia associated with a more physiologically-relevant (i.e., acute) regimen of estradiol treatment was not examined in either study.

To address this question, we examined estradiol's anorexigenic effect in OVX rats pretreated with either peripherally- or centrally-administered ICI 182,780 [111]. Estradiol was administered acutely via subcutaneous injection of a dose of estradiol shown previously to model the changes in plasma estradiol concentration observed in cycling rats [29,30]. While peripheral blockade of ERs by ICI 182,780 did not influence the anorexia induced by acute estradiol treatment, it was sufficient to block estradiol's acute uterotrophic effect (Fig1), suggesting adequate blockade of peripheral ERs. Our findings extend previous reports [104,109] by providing the first demonstration that peripheral ERs are not necessary for the expression of estradiol's phasic, anorexigenic effect.

While peripheral administration of ICI 182,780 failed to influence estradiol's phasic inhibitory effect on food intake in our study [111], central infusion of ICI 182,780 directly into the lateral ventricles blocked the anorexia associated with acute, subcutaneous estradiol treatment in OVX rats (Fig 2). Our regimen of centrally-administered ICI 182,780 did not attenuate estradiol's ability to induce cornification of the vaginal epithelium, which represents a peripheral action of estradiol indicative of estrus. This confirms minimal to no leakage of ICI 182,780 across the blood-brain barrier. While our study provides the first demonstration that selective blockade of central ERs can abolish estradiol's phasic, anorexigenic effect, it does not disclose where the critical, central ERs reside. Additional research, involving site-specific administration of ICI 182,780 should prove useful in this regard. Potential central targets include the nucleus of the solitary tract, medial preoptic area, dorsal raphe nucleus, and arcuate nucleus based on recent reports that infusion of estradiol in each of these brain areas is sufficient to decrease food intake in OVX rats [93,94]. This pharmacological approach is limited, however, by ICI 182,780's ability to bind to both ER subtypes, ERα and ERβ, with similar affinity. Thus, ICI 182,780 cannot be used to distinguish the relative involvement of these two ER subtypes in the estrogenic control of food intake.

3.2. Involvement of ERα versus ERβ

The delay between the increase in plasma estradiol concentration and subsequent decreases in food intake in both OVX and cycling rats has led investigators to examine the involvement of nERs, ERα and ERβ, in the estrogenic inhibition of food intake. To date, several approaches have been used. Studies measuring the body weights of mice with null mutations of ERα and/or ERβ (i.e. αERKO, βERKO, and α/βERKO mice) have produced mixed findings. Heine et al [112] reported that male and female αERKO mice display age-related increases in body adiposity, relative to wild-type mice. At about the same time, another group reported similar weight gain in αERKO and αβERKO, but not βERKO, mice [113]. While these findings appear to offer strong support for the necessity of ERα signaling in the regulation of body weight, a cautionary note is warranted. One must consider the fact that female αERKO mice display a 10-fold increase in circulating plasma estradiol, relative to wild-type mice, which could promote increased signaling through ERβ. Although there are no direct tests of the degree to which increased ERβ signaling may impact weight gain,

ovariectomy was reported to decrease weight gain and attenuate body adiposity in αERKO mice [114], thereby providing some evidence for ERβ signaling in the regulation of body adiposity in mice.

To date, only three studies have examined the feeding behavior of αERKO mice. In the first study, daily food intake was found to be similar in male αERKO and wild-type mice [112]. This suggests that the increased accrual of body fat in the absence of ERα signaling is mediated by a decrease in energy expenditure, rather than an increase in energy intake. This interpretation gains support from a recent study in which the weight gain induced by ovariectomy in C57BL/6 mice, the background strain for ERα null mice, was mediated entirely via a reduction in energy expenditure, manifested as reductions in both metabolism and locomotor activity [50]. In the second study, chronic estradiol treatment decreased food intake in OVX wild-type mice but had no effect in OVX αERKO mice [115], suggesting an important role for ERα in mediating estradiol's tonic inhibition of food intake. In the third study, chronic estradiol treatment produced a small but significant increase in food intake in OVX αERKO mice, relative to vehicle-treated OVX αERKO mice, during one week of a three-week feeding test [114]. While a replication of this very limited response to estradiol is warranted in light of the two earlier studies of the feeding behavior of αERKO mice, this initial finding does raise the possibility that ERβ signaling may increase food intake in mice. Taken together, these studies provide a mixed picture regarding the relative involvement of ERα and ERβ in the estrogenic control of food intake in mice. Conclusions must be tempered, however, by the possible developmental compensation inherent in knockout models, the increased circulating levels of estradiol in ER null mice, and the apparent lack of a feeding phenotype in at least one strain (C57BL/6) of OVX mice with normal ER signaling.

Although the limitations of genetically modified mouse models are avoided in studies involving transient inhibition of ER signaling, such studies have also produced mixed results. For example, estradiol's ability to decrease food intake and weight gain was blocked in OVX rats receiving intracerebroventricular infusions of antisense oligodeoxynucleotides targeting ERβ. In contrast, estradiol was fully efficacious in reducing food intake in OVX rats receiving antisense oligodeoxynucleotides targeting ERα [116]. In another study, site-specific, adeno-associated, viral vectors silencing the expression of ERα in the ventromedial nucleus of the hypothalamus decreased energy expenditure and promoted weight gain in mice and rats. Estradiol's anorexigenic effect was not, however, attenuated by this RNA interference technique [117].

3.3. ERαbut not ERβ, is sufficient for estradiol's anorexigenic effect

Our lab has taken a pharmacological approach to discerning the relative roles of ERα and ERβ in mediating the estrogenic control of food intake. In recent years, two potent and highly selective ER agonists, 4,4′,4″-(4-propyl-[1h]-pyrazole-1,3,5-triyl)tris-phenol (PPT) and 2,3-bis(4-hydroxy-phenyl)-propionitrile (DPN), have been developed to target ERα and ERβ, respectively. PPT has a binding affinity that is ~400-fold greater for ERα than ERβ, whereas DPN has a binding affinity that is ~70-fold greater for ERβ than ERα [118,119]. In addition, the binding affinity of PPT for ERα is approximately threefold greater than the binding affinity of DPN for ERβ [118], and both of these compounds have been shown to target central ERs following peripheral administration [120–122].

As a first step towards understanding the relative roles of ERα and ERβ in the estrogenic control of food intake, we compared the acute effects of estradiol benzoate (EB), PPT, and DPN on the spontaneous feeding patterns of OVX rats [123]. While PPT produced dose-dependent decreases in food intake, similar treatment with even larger doses of DPN failed to exert any anorexigenic effect (Fig 3A). This suggests that ERα signaling alone is

sufficient for estradiol's phasic inhibitory effect on food intake. A direct comparison of PPT and EB revealed that the two compounds differed in their latency to decrease food intake. Each was administered 3 h prior to dark onset and food intake was measured at 24-h intervals for 4 days. While PPT decreased food intake during the first 24-h interval, EB did not decrease food intake until the second 24-h interval (i.e., after a 24-h delay). A more detailed look over the 24-h interval revealed an anorexigenic effect of PPT within the first 3 h of treatment (Fig 3B).

While unexpected, this rapid anorexigenic effect of PPT appears robust. Our findings were replicated by Thammacharoen et al [124], who reported a feeding-inhibitory effect of PPT within 4–6 h following treatment in OVX rats. One interpretation of PPT's short latency to decrease food intake is that it may interact with mERα, rather than nERα. One must proceed cautiously with such an interpretation, however, as it is becoming increasingly clear that the actions of mERs cannot simply be defined by a short latency [75]. Thammacharoen et al [124] further demonstrated that PPT decreased food intake in wild-type, but not αERKO, mice. This confirms that activation of nERα and/or mERα is necessary for PPT's anorexigenic effect and that nERβ and the mERs, GPR-30 and Gq-mER, are not sufficient. Another interpretation of the more rapid anorexigenic effect of PPT, relative to estradiol, is that the two compounds may differ in the time each takes to gain access to the critical ERs. However, it will be difficult to test this hypothesis until a clearer understanding of the relative kinetics of estradiol and PPT, at both the systemic and molecular level, is reached. A final interpretation of PPT's rapid anorexigenic effect is that it may be secondary to the induction of an aversive internal state (e.g., malaise). Three additional findings from our study [123] suggest, however, that the rapid inhibition of food intake by PPT is behaviorally specific. First, the duration by which estradiol and PPT decrease food intake (~12 h) is not only similar, but it models the duration of the estrous-related decrease in food in cycling rats [21]. Second, PPT, like estradiol, decreases food intake by a decrease in meal size, not meal number. Third, PPT failed to induce a conditioned taste aversion to a novel saccharin solution.

In summary, the available data suggest that selective activation of ERα is sufficient to decrease food intake and meal size in OVX rats and mice [123,124], and that selective activation of ERβ produces neither of these effects [123]. The novel finding that PPT decreases food intake within 6 h of administration suggests the possible involvement of nERα. Because the feeding inhibitory effect of PPT appears behaviorally specific otherwise, this raises the possibility that the estrogenic control of food intake and regulation of body weight may be mediated by multiple ER-signaling pathways. In support of this notion, Levine et al [125] recently reported that the knockin of a mutant form of ERα, which signals only at the level of the plasma membrane (i.e., nERα), was sufficient to normalize body weight and rescue the metabolic parameters that are dysregulated in αERKO mice.

3.4. ERα is necessary for estradiol's anorexigenic effect

The first step in establishing the necessity of ERα in the estrogenic control of food intake is to determine whether estradiol's anorexigenic effect can be attenuated by selective ERα blockade. As described above, previous attempts to address this question using mice with null mutations of ERα were subject to the inherent limitations of knockout models and the observation that OVX mice, unlike OVX rats, may not display an overt feeding phenotype. The recent development of a novel ERα antagonist, methyl-piperidino-pyrazole (MPP), allowed us to re-examine this problem using a pharmacological approach. MPP is a non-steroidal, pyrazole compound that contains a basic side chain addition that is reported to convert the pyrazole from an ERα agonist to an ERα antagonist [126]. Receptor binding assays revealed that MPP has a 200-fold higher binding affinity for ERα over ERβ [127] and *in vitro* studies have demonstrated that MPP can down regulate estradiol-responsive genes

[128]. While MPP appears to function as an ERα antagonist in cell-based, *in vitro* studies, two *in vivo* studies suggested that MPP may exert some estrogenic activity [129,130]. As a result, we proceeded with caution since the possibility existed that MPP may better be classified as a SERM, rather than an ERα antagonist. Accordingly, we examined MPP's effect on food intake when administered alone and in combination with estradiol and the ERα agonist PPT. When administered alone, MPP produced a dose-related decrease in 24-h food intake and MPP failed to attenuate the feeding inhibitory effects of estradiol and PPT [131]. Thus, our *in vivo* tests of MPP's effects on food intake revealed estrogenic activity, suggesting that MPP functions as a SERM, rather than as an ERα antagonist as originally described.

As might be expected, it was subsequently shown that MPP can undergo metabolic cleavage during *in vivo* tests, returning it to the compound it was derived from, methyl-pyrazole triol (MPT) [126]. That MPT is an ERα agonist with weak to modest activity [126] explains our findings that *in vivo* administration of MPP produced an estrogenic, rather than antiestrogenic, effect on feeding [131]. This discovery prompted the development of a novel MPP analog, called methyl-piperidinopropyl pyrazole (MPrP), which was developed to prevent any metabolic cleavage during tests of its *in vivo* activity [126]. While MPrP was found to be highly selective for ERα in binding affinity assays and it exerted potent ERα antagonist activity in transcription activation assays [126], our group was the first to test its *in vivo* actions. Once again, we used this purported ERα antagonist to test the hypothesis that estradiol's anorexigenic effect requires activation of ERα [132]. After demonstrating that administration of MPrP alone failed to affect food intake in OVX rats (i.e., it exerted no estrogenic activity), we tested MPrP's ability to attenuate the inhibitory effect of estradiol on food intake. In support of our hypothesis, we demonstrated that acute administration of MPrP not only blocked estradiol's ability to decrease food intake in OVX rats, but it also prevented the estrous-related decrease in food intake in cycling rats (Fig 4). These findings clarify and extend the earlier work involving αERKO mice by providing the first evidence that activation of ERα is necessary for the phasic, inhibition of food intake by both exogenous and endogenous estradiol in female rats.

4. Conclusions

Behavioral studies involving rodent models have been very useful in first defining, and then investigating, estradiol's phasic and tonic inhibitory effects on food intake. Moreover, the careful behavioral analysis of the rat's spontaneous feeding patterns was paramount to linking estradiol to the control of meal size. This provided a critical first step towards understanding the mechanism underlying estradiol's feeding inhibitory action. The development of Smith's unified theory of the direct and indirect controls of meal size [61–63] provided a novel framework by which to investigate estradiol's ability to interact with neuropeptide and neurotransmitter systems controlling meal size, as reviewed elsewhere [9,64–66].

Recently, we and others have focused on identifying the critical ERs that mediate estradiol's anorexigenic effect. As a steroid hormone, estradiol may act upon nERs, mERs, or a combination of both. The clearest results come from studies involving ERα- and ERβ-selective agonists and antagonists. This body of work has shown that selective activation of ERα is both sufficient and necessary to decrease food intake in both OVX and cycling rats [123,131,132]. Additionally, the use of another ER antagonist, that does not cross the blood-brain barrier, provided evidence that the critical ERs reside in the brain, rather than the periphery [111]. Future studies must focus on identification of the specific brain areas and ER subtypes involved in mediating estradiol's anorexigenic effect. Site-specific administration of specific ER antagonists or site-directed silencing of ERs via RNA

interference should prove useful in this regard. Another open question that remains is the relative contribution of nERs and mERs to the estrogenic control of food intake. The recent demonstrations that Gq-mER-initiated signaling within a feeding-related circuit attenuates ovariectomy-induced weight gain [77,133] and selective reinstatement of mERα signaling normalizes weight gain and the metabolic disturbances of αERKO mice [125], provide a strong rationale for examining the involvement on mERs in the estrogenic control of food intake. Finally, there is every expectation to believe that female-focused research that provides a clearer understanding of the critical intracellular signaling pathways responsible for the estrogenic control of food intake will provide new insights into the mechanisms responsible for the greater prevalence of obesity and eating disorders in women [4–7], as well as the postmenopausal weight gain and increased risk of metabolic syndrome that occurs in the absence of estradiol replacement [134].

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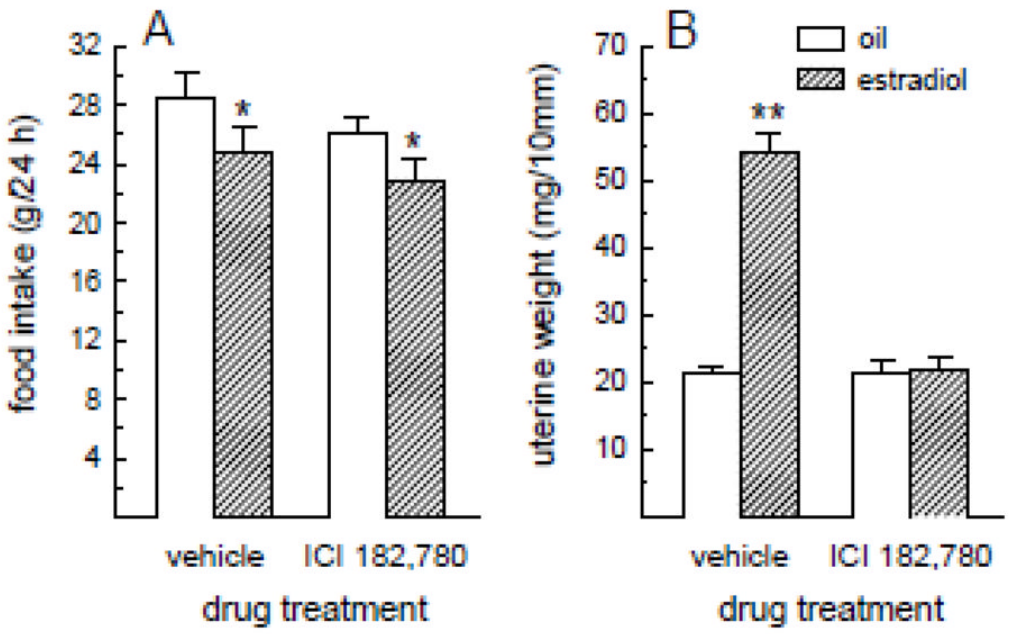

Fig 1.

Estradiol's acute anorexigenic effect is not influenced by peripheral blockade of ERs. Data are means ± SEMs. (A) Acute estradiol treatment produced similar decreases in food intake in rats receiving peripheral (subcutaneous) injections of either vehicle or the ER antagonist ICI 182,780. (B) Estradiol's ability to increase uterine weight was blocked by peripheral administration of ICI, suggesting complete blockade of peripheral ERs. *Estradiol < oil, $p < 0.05$. **Estradiol > oil, $p < 0.05$. Modified from [111].

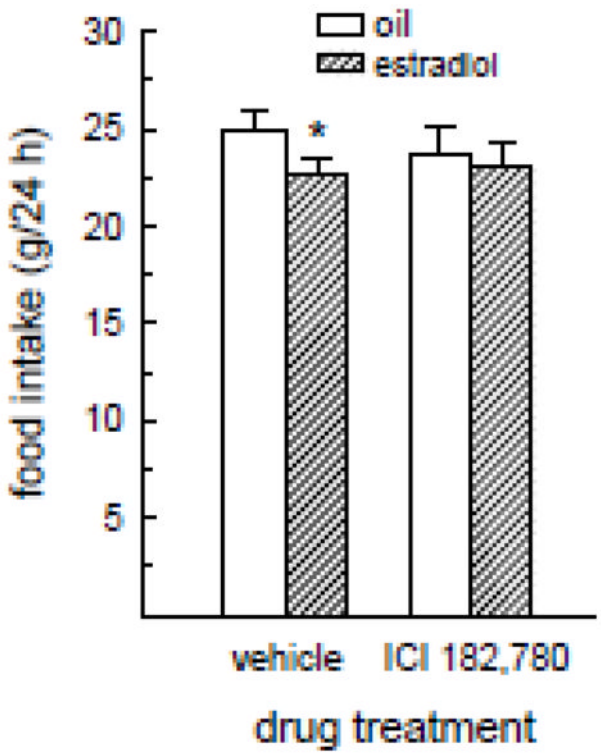

Fig 2.

Estradiol's acute anorexigenic effect is prevented by central blockade of ERs. Data are means ± SEMs. Acute estradiol treatment decreased food intake in rats receiving intracerebroventricular (icv) infusions of vehicle, but not in rats receiving icv infusions of ICI 182,780. *Estradiol < oil, $p < 0.05$. Modified from [111].

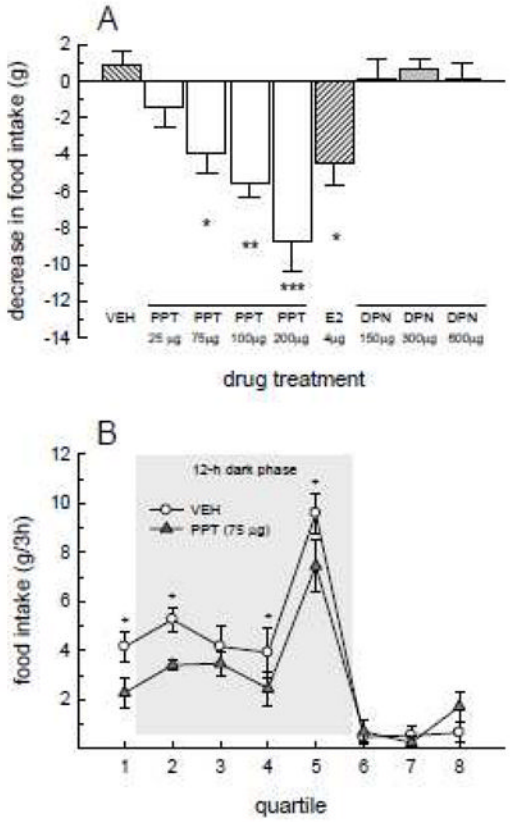

Fig. 3.

Acute activation of ERα, but not ERβ, is sufficient to decrease food intake in OVX rats. Data are means ± SEMs. (A) A dose-related decrease in food intake was observed in OVX rats treated with the ERα agonist PPT. The anorexia observed after 75 µg PPT was similar to a physiological dose of estradiol (E2). Acute administration of the ERβ agonist DPN failed to alter food intake in OVX rats. (B) Activation of ERα by PPT produced a rapid decrease in food intake. Rats received subcutaneous injections of either PPT or vehicle (veh) 3 h prior to dark onset and food intake was monitored at 3-h quartiles for the following 24-h period. PPT decreased food intake during the first 3-h, light-phase quartile, and throughout most of the dark-phase (shaded area). *Greater than vehicle, $p < 0.05$. **Greater than vehicle and 25 µg PPT, $p < 0.05$. ***Greater than vehicle, 25 µg PPT, 75 µg PPT and E2, $p < 0.05$. $^{+}$PPT < vehicle, $p < 0.05$. Modified from [123].

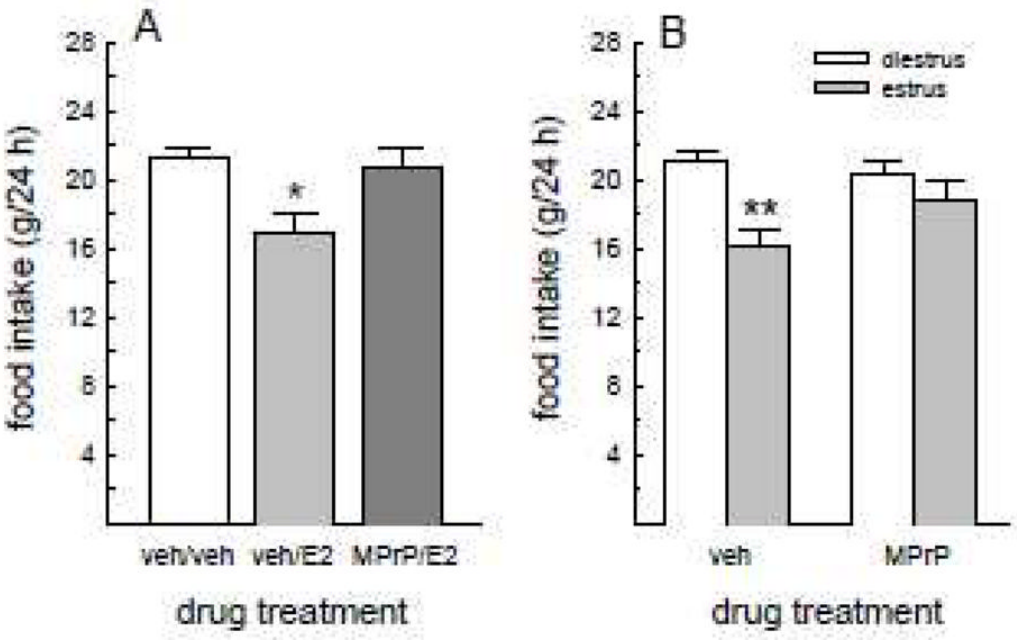

Fig. 4.

Blockade of ERα decreases estradiol's anorexigenic effect in OVX and cycling rats. Data are means ± SEMs. (A) Acute administration of estradiol (E2) decreased food intake in OVX rats. This action of estradiol was blocked by the ERα antagonist MPrP. (B) Food intake was decreased during estrus, relative to diestrus, in vehicle (veh)-treated, cycling rats. This estrous-related decrease in food intake was blocked in rats pretreated with the ERα antagonist MPrP. *Veh/E2< veh/veh and MPrP/E2, $p < 0.05$. **Estrus < diestrus, $p < 0.05$. Modified from [132].