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Acute activation of ER α decreases food intake, meal size, and body weight in ovariectomized rats

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Santollo J, Wiley MD, Eckel LA. Acute activation of ER α decreases food intake, meal size, and body weight in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol* 293: R2194–R2201, 2007. First published October 17, 2007; doi:10.1152/ajpregu.00385.2007.—Estradiol exerts many of its actions by coupling with two nuclear estrogen receptor (ER) proteins, ER α , and ER β . While the acute, anorexigenic effect of estradiol appears to involve such a mechanism, the relative contributions of ER α and ER β are equivocal. To address this problem, food intake was monitored in ovariectomized (OVX) rats following acute administration of a selective ER α agonist (4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol, PPT; dose range = 0–200 μ g), a selective ER β agonist (2,3-bis(4-hydroxyphenyl)-propionitrile, DPN; dose range = 0–600 μ g), and a physiological (4 μ g) dose of estradiol benzoate (EB). While PPT-treated rats displayed dose-dependent decreases in daily food intake and body weight, neither of these measures was influenced by any dose of DPN. In addition, DPN failed to modulate the anorexigenic effect of PPT when the two ER agonists were coadministered. Meal pattern analysis revealed that the anorexigenic effect of 75 μ g PPT (a dose of PPT that produced a similar decrease in daily food intake as 4 μ g EB) was mediated by a decrease in meal size, not meal number. Thus, PPT, like EB and endogenous estradiol, decreases food intake by selectively affecting the controls of meal size. The finding that acute administration of 75 μ g PPT failed to induce a conditioned taste aversion suggests that the anorexigenic effect of this dose of PPT is not secondary to malaise. Taken together, our findings demonstrate that selective activation of ER α decreases food intake, body weight, and meal size in the ovariectomized rat.

4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol; 2,3-bis(4-hydroxyphenyl)-propionitrile; food intake; estradiol

THE OVARIAN HORMONE ESTRADIOL exerts an inhibitory effect on food intake that is particularly well characterized in the rat. For example, the periovulatory increase in estradiol secretion in female rats is associated with a phasic decrease in food intake during estrus (4, 8, 12), and bilateral ovariectomy promotes sustained hyperphagia (33). Available data involving estradiol benzoate (EB) or progesterone replacement alone and in combination provide evidence that the hyperphagia displayed by ovariectomized (OVX) rats is mediated solely by the postsurgical decline in estradiol secretion (2, 17).

Food intake is defined by the product of meal size and meal number over a given period of time. Accordingly, any change in food intake must involve a change in one or both of these parameters. Because meal size and meal number are independently controlled in the rat (32), an important first step in identifying the mechanism underlying estradiol's anorexigenic effect involved a detailed analysis of the spontaneous feeding patterns of cycling and EB-treated OVX rats. Such studies

revealed that estradiol decreases food intake by decreasing meal size, not meal number (4, 12, 20). Recently, estradiol has been identified as an indirect control of meal size (9). That is, estradiol decreases meal size by increasing the strength of anorexigenic compounds (e.g., cholecystokinin and serotonin) that signal meal termination and by decreasing the strength of orexigenic compounds (e.g., melanin-concentrating hormone and ghrelin) that sustain a meal (5, 17, 23, 29). Although it is apparent that estradiol decreases food intake by interacting at the cellular level with key elements of the system controlling meal size, the molecular mechanism underlying this action of estradiol is poorly understood.

As a steroid hormone, estradiol exerts many of its actions by coupling with nuclear estrogen receptor (ER) proteins, ER α , and ER β (31). At present, there are limited data concerning which ER subtype underlies estradiol's anorexigenic effect, and the data that are available are equivocal. Recent studies involving mice with null mutations of either ER α or ER β have generated some support for the involvement of both ER subtypes (16, 19, 27). In the OVX rat, ventricular infusion of antisense oligodeoxynucleotides targeting ER β , but not ER α , blocked estradiol's anorexigenic effect (22), whereas chronic, peripheral administration of an ER α agonist, but not an ER β agonist, promoted hypophagia (30). In each of these studies, rats received chronic administration of either EB or ER α / β -selective agonists. Because these regimens of drug treatment fail to model the fluctuations in estradiol secretion in the cycling rat, very little is known about the relative contributions of ER α and ER β to the acute, anorexigenic effect of estradiol. In the present study, we addressed this issue by monitoring food intake, body weight, and meal patterns in OVX rats following acute administration of 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT), a specific ER α receptor agonist, and/or 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), a specific ER β receptor agonist.

METHODS

Animals and surgery. Female Long-Evans rats (Charles River Breeding Laboratory, Raleigh, NC; $n = 50$), weighing 225–250 g at the onset of each experiment, were housed individually in Plexiglas, shoebox cages. Rats were given free access to chow (Purina 5001) and tap water, unless otherwise noted. The testing rooms were maintained at $20 \pm 2^\circ\text{C}$ under a reverse 12:12-h light-dark cycle (dark onset = 1300). Animal usage and all procedures were approved by the Florida State University Institutional Animal Care and Use Committee.

At the onset of each experiment, rats were anesthetized by intraperitoneal injection of a mixture of ketamine (50 mg/kg; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (4.5 mg/ml;

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Rompun, Mobay, Shawnee, KS) and then bilaterally OVX using an intra-abdominal approach. Following surgery, each rat received an intraperitoneal injection of butorphanol (0.5 mg/kg; Fort Dodge Animal Health) and a subcutaneous injection of gentamicin (10 mg/ml; Pro Labs, St. Joseph, MO) to minimize postsurgical pain and the risk of infection, respectively. Rats were given 1 wk to recover from surgery before being transferred to custom-designed cages, as described below.

Experiment 1: Comparison of the acute effects of EB, PPT, and DPN on food intake and body weight. OVX rats were housed in cages equipped with feeding niches that provided access to spill-resistant food cups containing powdered chow. Each day at 0900, the rats' body weights were recorded, and food cups were weighed (± 0.1 g). Behavioral testing did not commence until stable levels of food intake were observed. Using a within-subject, randomized design, one group of OVX rats ($n = 8$) received acute, subcutaneous injections (0.1 ml) of one of the following treatments each Wednesday morning at 1000: DMSO vehicle (Sigma, St. Louis, MO), 4 μ g EB (Sigma), 25, 75, 100, or 200 μ g PPT (Tocris, Ellisville, MO). A second group of rats ($n = 8$) received the same schedule of acute, subcutaneous injections of DMSO, 4 μ g EB, 150, 300, or 600 μ g DPN (Tocris). PPT and DPN were administered peripherally to be consistent with the typical (subcutaneous) route of EB administration. Available evidence, however, suggests that peripherally administered PPT and DPN reach the rat's central nervous system. For example, subcutaneous administration of DPN produced antianxiety behavior, which is believed to be mediated in the brain (34). In addition, subcutaneous administration of DPN produced an increase in striatal dopamine transporter binding that was similar to that observed following subcutaneous administration of EB (21). Finally, subcutaneous administration of PPT increased progesterone receptor mRNA expression in the arcuate nucleus and ventromedial hypothalamus (18). Taken together, these studies provide compelling, indirect evidence that PPT and DPN cross the blood-brain barrier.

Throughout the 5-wk (DPN) and 6-wk (PPT) testing protocols, food intake and body weight were monitored each Monday through Thursday. This schedule of acute drug injections and cyclic, 4-day behavioral assessments was based on a previous report that an acute injection of EB on Wednesday morning (or *day 3* of a 4-day cycle) models the changes in estradiol secretion observed across the 4-day estrous cycle in ovarian-intact rats, and it decreases food intake on Thursday evening (or *day 4* of a 4-day cycle; the day that models behavioral estrus) (2). The dose ranges of PPT and DPN were selected based on two previous studies involving *in vivo* administration of these compounds in OVX rats (18, 30), and a previous *in vitro* study, in which the binding affinity of PPT for ER α was approximately threefold greater than the binding affinity of DPN for ER β (24).

Experiment 2: time course of PPT's anorexigenic effect. OVX rats ($n = 8$) were housed in cages that facilitated the analysis of spontaneous feeding patterns (DiLog Instruments, Tallahassee FL). The cages were equipped with feeding niches that provided access to spill-resistant food cups mounted on weight-sensitive load beams. Infrared beams, located on either side of the feeding niches and centered above the feeding cups, were also used to signal the occurrence of feeding behavior. Any food spillage was collected on a platform surrounding the food cup. Outputs from the load and photo beams were fed via an interface into a computer located in an adjacent room. Custom-designed software (ESP 500; R. Henderson, Florida State University, Tallahassee FL) recorded the weight of each load beam (± 0.001 g) and the activity of each photo beam at 30-s intervals. Additional software (Meal Weight Analysis; R. Henderson) was used to assess daily food intake at 1-h intervals. Using a within-subject, crossover design, half of the OVX rats received subcutaneous injections of 75 μ g PPT, while the other half received DMSO vehicle. Four days later, the treatment conditions were reversed. The dose of PPT was chosen because it exerted a similar decrease in food intake and body weight as that observed following acute administration of 4 μ g

EB in *experiment 1*. On testing days, injections were administered at 1000, and food intake was monitored continuously for 2 days.

Experiment 3: acute effects of PPT and DPN on feeding patterns. OVX rats ($n = 8$) were housed in the same cages as described in *experiment 2*. Using a within-subject, randomized design, OVX rats received subcutaneous injections of 75 μ g PPT, a combination of 75 μ g PPT and 150 μ g DPN, or DMSO vehicle at 1000 on each of 3 test days occurring at 4-day intervals. On test days, food intake and meal patterns were monitored using our automated system. Custom software (meal weight analysis; R. Henderson) was then used to convert individual bouts of ingestive behavior into meals. A meal was defined as any feeding bout of at least 0.35 g that was separated from other feeding bouts by at least 15 min. In previous studies, these criteria accounted for 97–99% of daily food intake (e.g., 12).

Experiment 4: effect of PPT on preference for a 0.1% saccharin solution. OVX rats ($n = 18$) were housed in cages that provided access to two drip-resistant drinking bottles. Rats were adapted to a 22-h water deprivation schedule (daily access from 1100 to 1300) over a 5-day period. On *day 6*, rats were given 2-h access to a novel 0.1% saccharin solution instead of water. Immediately following saccharin access, rats received subcutaneous injections of either 0, 75, or 200 μ g PPT ($n = 6$ per group), dissolved in DMSO vehicle. Preference for the saccharin solution was assessed on *day 7* by providing 2-h access to one bottle containing water and one bottle containing 0.1% saccharin. To minimize the development of a drinking side preference, the position of the water and saccharin bottles was reversed during the second hour of the test. Throughout the experiment, the amount of fluid (water and/or 0.1% saccharin) consumed was measured by weighing drinking bottles (± 0.1 g) before and after fluid access.

Data analyses. In *experiment 1*, repeated-measures ANOVAs (drug dose \times day) were used to compare the acute effects of PPT, DPN, and EB on daily food intake across the 4-day test cycle. These initial analyses revealed that acute administration of PPT decreased food intake on *day 3*, whereas the same regimen of EB treatment decreased food intake on *day 4* (i.e., there was a 24-h delay before the anorexigenic effect of EB was apparent). In previous studies involving similar EB replacement protocols (e.g., 1, 11), the anorexigenic effect of EB has been assessed by calculating the differences in food intake and body weight on *day 2*, the day that models diestrus 2, and *day 4*, the day that models estrus. This model was based on a well-established method of comparing the changes in food intake and body weight from the diestrous to the estrous stage of the ovarian reproductive cycle in ovarian-intact rats (e.g., 3, 10, 13). Because of the rapid, anorexigenic effect of PPT, a modification to this model was necessary to quantify the inhibitory effects of our ER-selective agonists on food intake and body weight. Although changes in food intake and body weight were assessed from *day 2* to *day 4* in EB-treated rats (i.e., consistent with the established model), changes in food intake and body weight were assessed from *day 1* to *day 3* in PPT- and DPN-treated rats. The resulting change scores were analyzed using repeated-measures ANOVAs. To determine the time course of PPT's anorexigenic effect (*experiment 2*), noncumulative food intake was monitored hourly for 2 days following drug treatment. The resulting data, presented in 3-h intervals, were analyzed using repeated-measures ANOVAs (drug \times time). Meal pattern data (*experiment 3*) were analyzed using repeated-measures ANOVAs. In addition, the effect of PPT on average meal size during each quartile of the first dark phase following drug treatment was analyzed using a two-factor, repeated-measures ANOVA (drug \times quartile). Finally, preference for the 0.1% saccharin solution was determined by the ratio of 0.1% saccharin consumed to the total amount of fluid consumed by each rat during the two-bottle preference test (*experiment 4*). The effect of PPT treatment on the 0.1% saccharin preference ratio was analyzed using a one-way ANOVA (0, 75, or 200 μ g PPT).

RESULTS

Experiment 1: comparison of the acute effects of EB, PPT, and DPN on food intake and body weight. During the 4-day testing period, acute administration of EB produced a reliable decrease in food intake, $F(3,21) = 7.02$, $P < 0.005$ (Fig. 1A). As expected, the anorexigenic effect of EB was not observed until the second 24-h interval following drug treatment (i.e., on day 4). At this time, EB-treated rats consumed less food than DMSO-treated rats, $P < 0.05$. No group differences were observed at any other time points. Acute administration of PPT also produced a reliable decrease in food intake, $F(12,84) = 4.72$, $P < 0.0001$ (Fig. 1B). Unlike that observed following EB

treatment, the anorexigenic effect of PPT was observed during the first 24-h interval following drug treatment (i.e., on day 3). At this time, the three largest doses of PPT (75–200 μg) induced dose-dependent decreases in food intake, relative to that observed following DMSO treatment, $P < 0.05$. The anorexigenic effect of PPT was limited to 24 h, as no group differences in food intake were observed on day 4. In contrast to that observed following EB and PPT treatment, neither a main, nor an interactive, effect of DPN on food intake was detected, $F(3,21) = 0.71$ and $F(9,63) = 0.86$, respectively, not significant (ns) (Fig. 1C).

The changes in food intake and body weight from day 2 to day 4 (EB-treated rats) and from day 1 to day 3 (DMSO-, PPT-, and DPN-treated rats) were determined to make direct comparisons between the potencies of our three compounds. The mean change in food intake in the PPT-treated group was influenced by drug treatment, $F(5,35) = 9.79$, $P < 0.0001$ (Fig. 2A). The three highest doses of PPT (75, 100, and 200 μg) and 4 μg EB decreased food intake, relative to that observed in DMSO-treated rats, $P < 0.05$. While the decrease in food intake following 75 μg PPT was similar in magnitude to that observed following EB treatment (~ 4 g), the highest dose of PPT (200 μg) produced a decrease in food intake that was more than twice (~ 9 g) that observed following EB treatment, $P < 0.05$. In contrast, the mean change in food intake was not influenced by DPN treatment, $F(3,21) = 0.11$, ns (Fig. 2B). As would be expected, the mean change in body weight in the PPT-treated group was influenced by drug treatment, $F(5,35) = 22.79$, $P < 0.00001$ (Fig. 2C). The three highest doses of PPT (75, 100, and 200 μg) and 4 μg EB decreased body weight, relative to that observed in DMSO-treated rats, $P < 0.05$. The decrease in body weight following the highest dose of PPT (200 μg) was greater than that observed in rats treated with 75 μg PPT, $P < 0.05$. Body weight was not influenced by DPN treatment, $F(3,21) = 0.21$, ns (Fig. 2D).

Experiment 2: time course of PPT's anorexigenic effect. An initial analysis of hourly, noncumulative food intake revealed that the anorexigenic effect of PPT was first detected during the last hour prior to dark onset (i.e., beginning 2 h following drug treatment). During this time, DMSO-treated rats consumed 1.9 ± 0.5 g and PPT-treated rats consumed 0.8 ± 0.4 g, $P <$

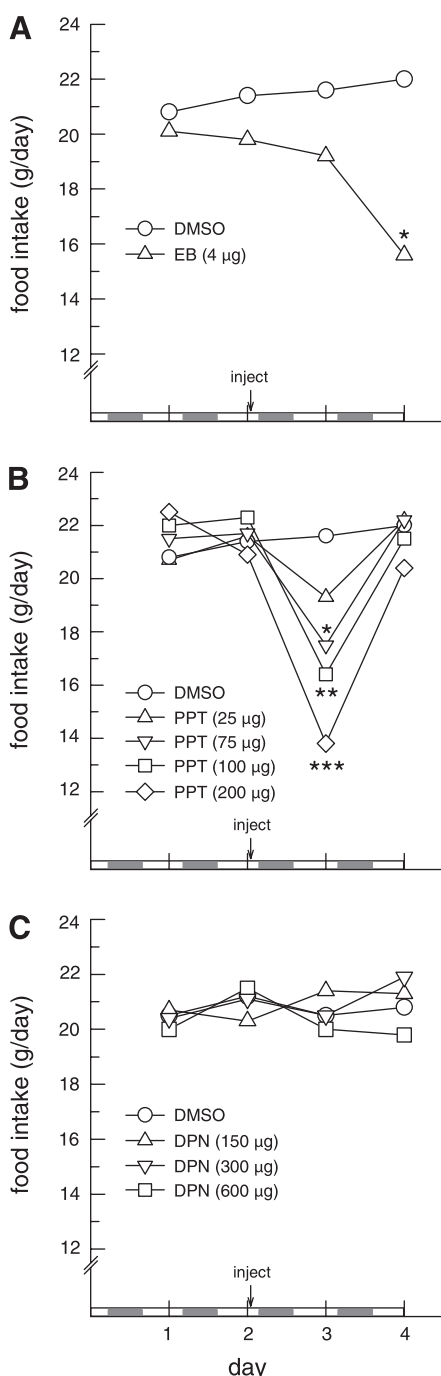


Fig. 1. Acute administration of 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT), but not 2,3-Bis(4-hydroxyphenyl)-propionitrile (DPN), produced a dose-dependent decrease in food intake in OVX rats. In *experiment 1*, daily food intake was monitored across multiple 4-day testing periods, during which one group of rats received subcutaneous injections of varying doses of PPT, 4 μg estradiol benzoate (EB), and DMSO vehicle ($n = 8$), while a second group of rats received subcutaneous injections of varying doses of DPN, 4 μg EB, and DMSO vehicle ($n = 8$). Injections were administered once weekly, at 1000 on day 3 (i.e., 3 h before dark onset). Dark shading along the x-axis depicts the 12-h dark phases of the light cycle. To clarify data presentation, error bars have been omitted and EB data are presented independent of PPT and DPN data. A: acute administration of EB produced a reliable decrease in daily food intake. Consistent with previous studies (2, 11), the anorexigenic effect of EB was not apparent until 24 h after drug treatment (i.e., on day 4). EB produced similar decreases in food intake in the PPT group (data shown) and the DPN group (data not shown). B: acute administration of PPT produced a rapid, dose-dependent decrease in daily food intake. Unlike that observed following EB treatment, the anorexigenic effect of PPT was limited to the first 24 h following drug treatment (i.e., day 3). C: no dose of DPN influenced food intake throughout the 4-day test period. *Less than DMSO, $P < 0.05$. **Less than DMSO and 25 μg PPT, $P < 0.05$. ***Less than DMSO, 25 μg PPT, and 75 μg PPT, $P < 0.05$.

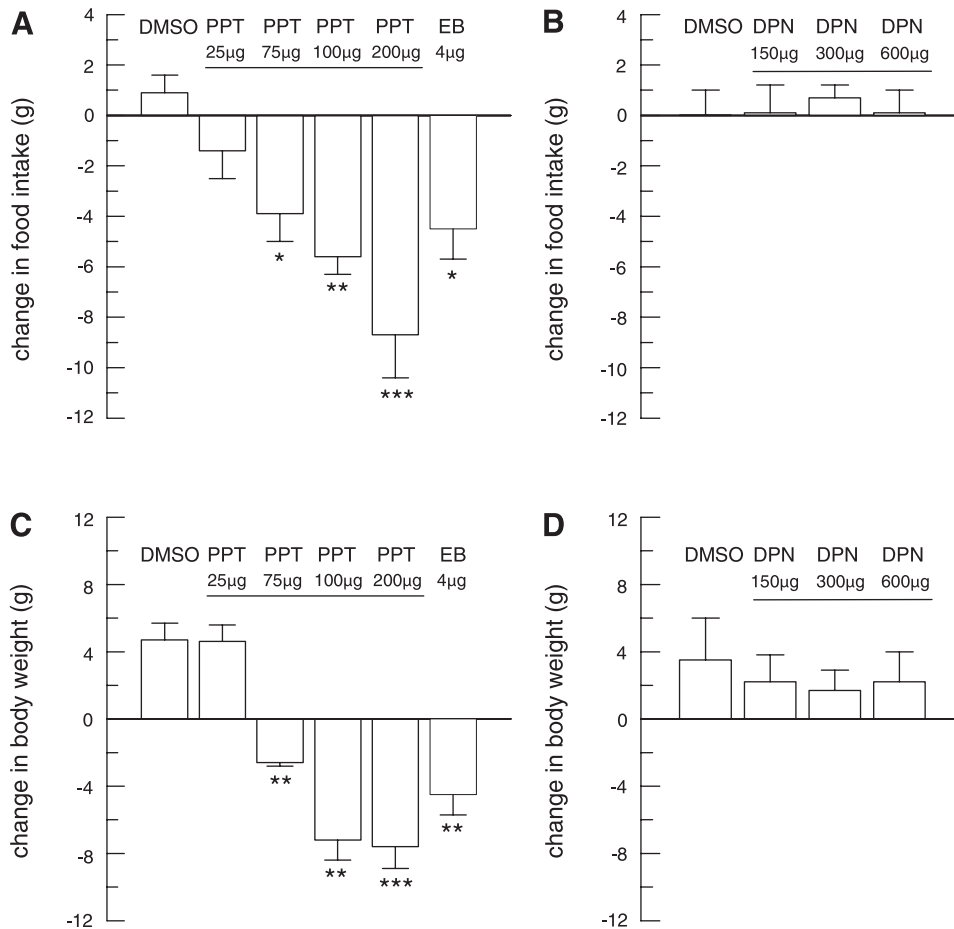


Fig. 2. PPT induces dose-dependent decreases in food intake and body weight in OVX rats. Data illustrate means \pm SE changes in food intake and body weight between *day 1* and *day 3* in DMSO-, PPT-, and DPN-treated rats, and between *day 2* and *day 4* in EB-treated rats. (A, C) PPT induced dose-dependent decreases in food intake and body weight on *day 3*. Analysis of this dose-response curve revealed that the threshold anorexigenic dose of PPT was 75 μ g. At this dose, PPT produced decreases in food intake and body weight that was similar to that observed following EB treatment (\sim 4 g). (B, D) No dose of DPN produced a reliable decrease in either food intake or body weight. *Less than DMSO, $P < 0.05$. **Less than DMSO and 25 μ g PPT, $P < 0.05$. ***Less than DMSO, 25 μ g PPT, 75 μ g PPT, and EB, $P < 0.05$.

0.05. Subsequent analysis of the time course of PPT's anorexigenic effect was conducted at 3-h quartiles. During the first day following drug treatment, noncumulative food intake was influenced by an interactive effect of drug and quartile, $F(7,49) = 3.16$, $P < 0.005$ (Fig. 3). PPT decreased food intake by the end of the first 3-h interval of the light phase and during three of the four 3-h intervals of the dark phase, $P < 0.05$. No

further effect of PPT was detected during the latter part of the light phase. In both groups, food intake was greater throughout the 3-h interval preceding dark onset and the dark phase (i.e., intervals 1–5), relative to the latter part of the light phase (intervals 6–8), $P < 0.05$. No group differences in food intake were detected during the second day following drug treatment (Fig. 3). Thus, the anorexigenic effect of this dose of PPT

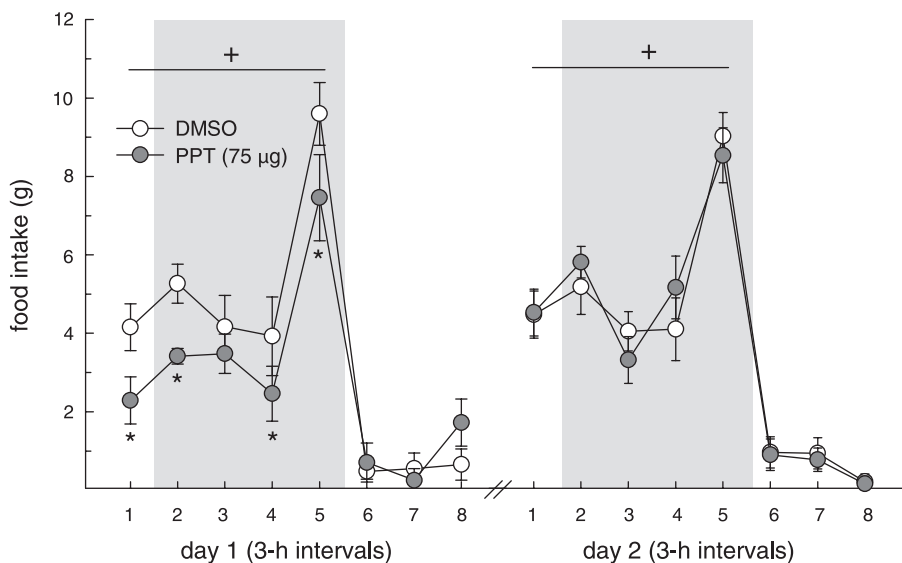


Fig. 3. Time course of PPT's anorexigenic effect. Using a cross-over design, rats were injected with 75 μ g PPT or DMSO vehicle at 1000 (3 h prior to dark onset), and food intake was monitored for the next 2 days. Data are expressed as means \pm SE, noncumulative food intakes at 3-h intervals across two consecutive light-dark phases. Shading depicts the 12-h dark phase. An anorexigenic effect of PPT was detected by the end of the first 3-h interval, and this effect persisted throughout the first dark phase following injection. No group differences in food intake were observed on *day 2*. *Less than DMSO, $P < 0.05$. +Greater than intervals 6–8, $P < 0.05$.

persists for ~15 h (Fig. 3). Consistent with that observed during *day 1*, a circadian rhythm in food intake was observed on *day 2*, $F(7,49) = 42.84$, $P < 0.0001$. Both groups displayed greater food intake during *intervals 1–5*, relative to *intervals 6–8*, $P < 0.05$.

Experiment 3: acute effects of PPT and DPN on feeding patterns. Daily food intake was influenced by our regimen of drug treatment, $F(2,12) = 12.83$, $P < 0.001$ (Fig. 4A). As expected, a decrease in 24-h food intake was observed in PPT-treated rats, relative to DMSO-treated rats, $P < 0.05$. There was no evidence that the anorexigenic effect of PPT was modulated by coadministration of DPN, since food intake was similar in PPT- and PPT/DPN-treated rats. Meal pattern analysis revealed that the anorexigenic effect of PPT was mediated by a decrease in meal size, $F(2,12) = 6.24$, $P < 0.05$ (Fig. 4B).

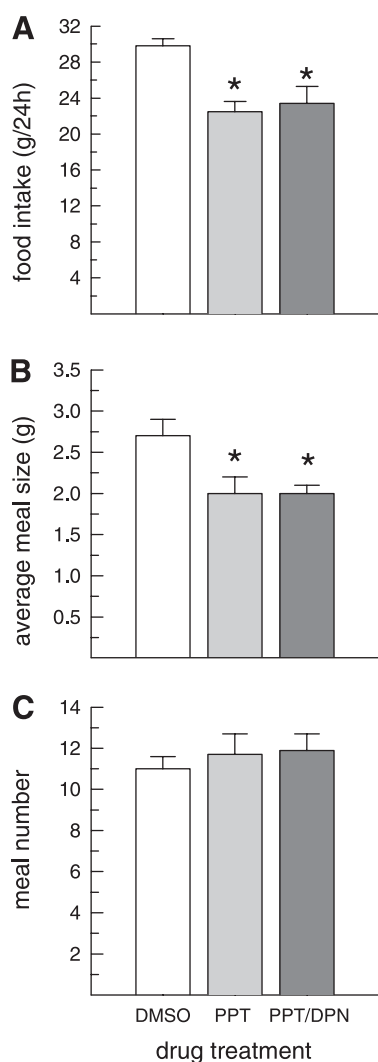


Fig. 4. The anorexigenic effect of PPT, which is mediated by a selective decrease in meal size, is not influenced by coadministration of DPN. Data are means \pm SE. **A:** acute administration of PPT produced a reliable decrease in 24 h food intake, relative to DMSO-treated rats. This action of PPT was not modulated by coadministration of DPN. **B:** Acute administration of PPT produced a decrease in average daily meal size, relative to DMSO-treated rats. Again, DPN failed to modulate PPT's ability to decrease average meal size. **C:** PPT treatment alone, and in combination with DPN, failed to influence the number of meals consumed during the first day following drug treatment. *Less than DMSO, $P < 0.05$.

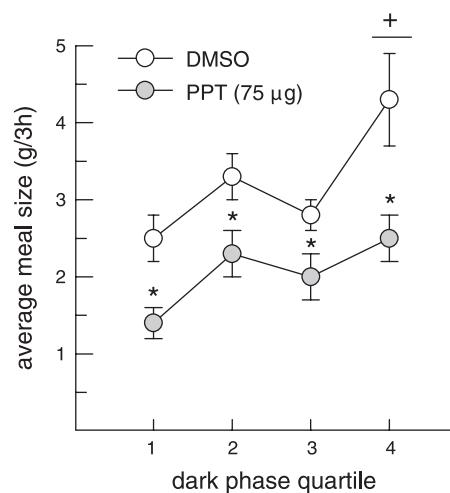


Fig. 5. Acute administration of PPT decreased meal size throughout the dark phase. Data are expressed as means \pm SE. Average meal size was inhibited by PPT during each quartile of the dark phase. *Less than DMSO, $P < 0.05$. †Greater than quartile 1, $P < 0.05$.

Average meal size throughout the 24-h interval following drug treatment was decreased by PPT treatment, $P < 0.05$. This action of PPT was not influenced by coadministration of DPN. While PPT induced a potent inhibition of meal size, it failed to influence the number of meals consumed throughout the first 24 h following drug treatment, $F(2,12) = 0.48$, ns (Fig. 4C).

An assessment of the time course of PPT's inhibitory effect on meal size throughout the dark phase following drug treatment revealed main effects of drug, $F(1,7) = 23.42$, $P < 0.005$, and time, $F(3,21) = 5.88$, $P < 0.01$ (Fig. 5). PPT decreased average meal size throughout each quartile of the dark phase, $P < 0.05$. In both groups, average meal size increased throughout the night such that food intake during *quartile 4* was greater than food intake during *quartile 1*, $P < 0.05$.

Experiment 4: effect of PPT on preference for a 0.1% saccharin solution. Prior to drug treatment, all rats avidly consumed the 0.1% saccharin solution on the conditioning day (mean intake = 26.9 ± 2.3 g/2 h). On the following conditioned taste aversion (CTA) test day, preference for the 0.1% saccharin solution was influenced by drug treatment, $F(2,15) = 8.91$, $P < 0.005$ (Fig. 6). Rats treated with either DMSO or 75 µg PPT displayed a strong preference for the saccharin solution during the two-bottle saccharin preference test. However, rats treated with 200 µg PPT displayed a clear avoidance of the 0.1% saccharin solution, $P < 0.05$.

DISCUSSION

While the mechanism underlying estradiol's anorexigenic effect is poorly understood, available data are compatible with the idea that estradiol acts at nuclear ERs (ER α and/or ER β) to alter the transcription of various genes implicated in the control of food intake (9). Here, we used selective ER agonists, PPT and DPN, to begin to determine the relative contribution of ER α and ER β , respectively, in mediating estradiol's acute, anorexigenic effect. Dose-dependent decreases in food intake and body weight were observed only in PPT-treated rats. Analysis of the rats' spontaneous meal patterns revealed that PPT, like EB, decreases food intake by a selective decrease in meal size. Finally, the anorexigenic effect of a dose of PPT that

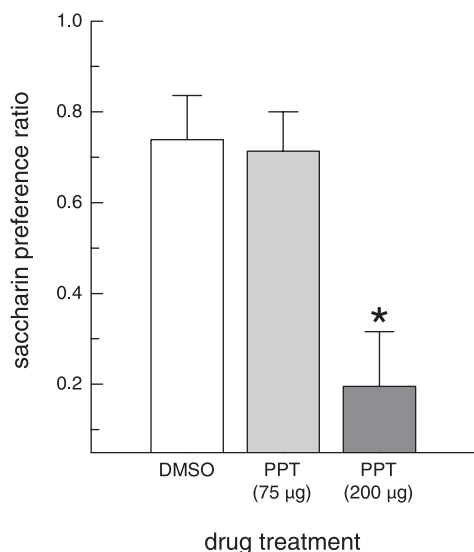


Fig. 6. Acute injection of a large dose of PPT (200 μ g) induced a conditioned taste aversion (CTA) in OVX rats. Following adaptation to a 22-h water deprivation schedule, rats were given 2-h access to a novel 0.1% saccharin solution. The following day, preference for the 0.1% saccharin solution was assessed by dividing the amount of 0.1% saccharin consumed by the total amount of fluid (saccharin plus water) consumed. During the two-bottle test, rats that received acute injections of either 75 μ g PPT or DMSO displayed a strong preference for the 0.1% saccharin solution. In contrast, acute administration of a larger dose of PPT (200 μ g) produced a robust aversion to the 0.1% saccharin solution. *Less than DMSO and 75 μ g PPT, $P < 0.05$.

produced a similar decrease in food intake as a physiological dose of EB failed to induce a CTA. Taken together, our data demonstrate that acute activation of ER α alone is sufficient to decrease food intake, meal size, and body weight in OVX rats.

In *experiment 1*, we compared the acute effects of PPT, DPN, and EB on daily food intake and body weight in OVX rats. PPT produced dose-dependent decreases in food intake and body weight during the 24-h period following drug treatment (i.e., on *day 3*). As expected, acute administration of a physiological dose of EB also decreased food intake and body weight. Unlike PPT, but consistent with previous studies (2, 11), this action of EB was not observed until 24 h after drug treatment (i.e., on *day 4*). At present, it is difficult to resolve this difference in the latency of PPT's vs. EB's ability to decrease food intake since the relative kinetics of PPT vs. EB, at either the systemic pharmacokinetic or the molecular level, are poorly understood. For example, it is possible that the transcriptional efficiency of PPT may exceed that of EB. It is also possible that PPT may cross the blood-brain barrier more quickly than EB and, as a result, gain more rapid access to central ERs. Additional studies are necessary to test these hypotheses.

In contrast to that observed following PPT and EB treatment, neither food intake nor body weight was influenced by any dose of DPN. In addition, DPN failed to modulate the anorexigenic effect of PPT when both ER agonists were coadministered. Because DPN was administered peripherally, it is possible that our negative findings may be explained by the drug's inability to reach the brain. This seems unlikely, however, based on available, indirect evidence that DPN crosses the blood-brain barrier (21, 34). Thus, it is doubtful that DPN failed to influence food intake due to its inability to reach

central ERs. Rather, our findings suggest that selective activation of ER β is not sufficient to inhibit food intake in the OVX rat.

Here, we identified a dose of PPT (75 μ g) that produced a decrease in daily food intake that was similar in magnitude (\sim 4 g) as that observed in rats treated with a physiological (4 μ g) dose of EB. A more detailed time-course analysis of the anorexigenic effect of this dose of PPT revealed a decrease in food intake that was first evident at 3 h after injection (i.e., during the 1-h interval preceding dark onset) and, like that observed in EB-treated rats (2), persisted throughout the 12-h dark phase. No further anorexigenic effect of PPT was observed during the subsequent light phase or the following 24-h period (Fig. 3). While PPT and EB differ in their latency to decrease food intake, the duration of the anorexigenic effect of both compounds is not only similar but it also models that observed in the estrous rat. That is, cycling rats display a transient decrease in food intake that is expressed throughout the 12-h dark phase of estrus (12). While this provides some evidence for the behavioral specificity of PPT, caution is needed since the half-life of PPT has not been established in the rat. As a result, we cannot rule out the possibility that the pharmacokinetic properties of PPT may mimic the duration of estradiol's anorexigenic effect, but in a behaviorally nonspecific manner.

The effect of selective ER α / β activation on food intake and body weight was examined in a previous study in which OVX rats received chronic, daily injections of PPT, DPN, or EB (30). Consistent with our findings, decreases in cumulative, 14- and 21-day food intake and weight gain were observed only in rats receiving chronic PPT treatment. The present findings extend this report by providing the first demonstration that acute administration of PPT is sufficient to decrease food intake and body weight in OVX rats. Moreover, we have identified a dose of PPT (75 μ g) that produces a decrease in food intake that is similar in magnitude and duration as that observed in rats treated with a physiological dose of EB, chosen for its ability to mimic the changes in estradiol secretion across the rat's 4-day estrous cycle. Thus, our findings appear to have revealed a dose of PPT that could be used in subsequent studies to further assess the role of ER α in mediating estradiol's anorexigenic effect.

Studies of mice with null mutations of ER α (α ERKO), ER β (β ERKO), or both ER subtypes (α / β ERKO) have produced equivocal findings regarding the relative contribution of ER α vs. ER β in mediating the inhibitory effects of estradiol on energy homeostasis. Heine et al. (19) were the first to report that α ERKO mice display age-related increases in body adiposity, relative to wild-type litter mates. This finding was confirmed and extended in a subsequent report of elevated body adiposity in α ERKO and α / β ERKO mice, but not in β ERKO mice (28). While these studies suggest that ER α signaling alone is necessary for the normal regulation of adipose tissue in mice, one must also consider the fact that female α ERKO mice display a 10-fold increase in circulating plasma estradiol that could promote increased estradiol signaling through ER β (6). This, in turn, could also contribute to the obesity that develops in α ERKO mice. Indeed, ovariectomy has been shown to attenuate the enhanced body adiposity of α ERKO mice, a finding that provides some evidence for the involvement of ER β in the regulation of body adiposity (27).

Currently, only two studies have examined the feeding behavior of α ERKO mice. In the first study, daily food intake was similar in male α ERKO and wild-type mice (19). This is not surprising, however, since the increased body adiposity of α ERKO mice appears to be mediated primarily via a decrease in energy expenditure (19). In a subsequent study, chronic estradiol treatment failed to reduce food intake in OVX α ERKO mice (16). Taken together, these limited studies of ER null mice generally suggest a greater involvement of ER α over ER β in mediating estradiol's inhibitory effects on energy homeostasis. However, the findings are somewhat equivocal, and conclusions must be tempered by the possible developmental compensation inherent in knockout models.

The issue of developmental compensation in ER knockout models was avoided in a recent study that used site-specific adeno-associated viral vectors designed to silence the expression of ER α (26). Using this RNA interference technique, selective silencing of ER α expression within the ventromedial nucleus (VMN) of the hypothalamus of OVX mice and rats promoted an obesity phenotype characterized primarily by reduced energy expenditure. Interestingly, the silencing of ER α in the VMN failed to attenuate the anorexigenic effect of EB. This suggests that expression of ER α in the VMN is not necessary for this action of estradiol. While this study provides compelling evidence for ER α involvement in estradiol's ability to regulate energy expenditure and body adiposity, another study suggests selective involvement of ER β . Liang et al. (22) reported that the inhibitory effects of estradiol on food intake and body weight were blocked in OVX rats by ventricular infusion of antisense oligodeoxynucleotides targeting ER β , whereas antisense oligodeoxynucleotides targeting ER α were without effect. Our current findings, which implicate ER α in the acute anorexigenic effect of estradiol, are consistent with the former, but not the latter, study. Additional studies involving either selective ER silencing techniques or site-specific administration of selective ER agonists, both of which are devoid of possible developmental compensation inherent in ER knockout models, are necessary to clearly differentiate the relative involvements of ER α and ER β in mediating the acute anorexigenic effect of estradiol.

Behavioral analyses reveal that estradiol's inhibitory effect on food intake is mediated by a decrease in meal size, not meal number (4, 12, 20). Here, we demonstrated that PPT's inhibitory effect on food intake is also mediated by a selective decrease in meal size that persists throughout the entire dark phase following drug treatment. In addition, we also demonstrated that DPN failed to modulate PPT's ability to inhibit meal size. This provides further evidence that selective activation of ER α mimics the behavioral changes that follow acute EB treatment in OVX rats and the rise in estradiol secretion in cycling rats.

In the current study, PPT produced a robust decrease in food intake at the higher end of the dose-response curve. For example, a 40% reduction in food intake was observed in rats treated with 200 μ g PPT. In comparison, a physiological (4 μ g) dose of EB produced only a 25% decrease in food intake. It is possible; therefore, that the profound anorexigenic effect of 200 μ g PPT may be secondary to a shift in taste preference or the induction of an aversive internal state. To test this hypothesis, we examined whether PPT could induce a CTA to a novel saccharin solution. On the conditioning day, fluid-

restricted rats were given 2-h access to a 0.1% saccharin solution. Immediately following saccharin access, rats were injected with either DMSO vehicle, 75 μ g PPT, or 200 μ g PPT. On the following day, rats treated previously with 200 μ g PPT displayed a strong aversion to the saccharin solution during a sensitive two-bottle preference test. In comparison, rats treated with either DMSO or 75 μ g PPT displayed a strong preference for saccharin over water. This suggests that PPT, administered in a dose that models the anorexigenic effect of a physiological dose of EB, decreases food intake without inducing an aversive internal state capable of conditioning a taste aversion. Once again, we find that the behavioral response to PPT and EB is similar. At physiological doses (1–10 μ g), acute administration of EB induces a transient decrease in food intake that is behaviorally specific (14). At larger, pharmacological doses (50–400 μ g/kg), acute administration of EB induces a decrease in food intake that is accompanied by a shift in taste preference and the expression of a CTA (7, 15, 25).

In summary, our results show that acute administration of PPT produces dose-dependent decreases in food intake and body weight in OVX rats. Like EB, PPT decreases food intake by selectively affecting the controls of meal size. In contrast, acute administration of DPN did not modulate food intake or body weight in OVX rats, nor did it modulate the anorexigenic effect of PPT. Taken together, our findings demonstrate that selective activation of ER α alone is sufficient to decrease food intake, meal size, and body weight in OVX rats. Future studies involving strategies to selectively block ER α will be important to determine whether ER α signaling is both sufficient and necessary to decrease food intake, meal size, and body weight in the OVX rat. It will also be critical to determine whether selective ER α blockade is both sufficient and necessary to attenuate the estrous-related decrease in food intake observed in cycling rats. Such studies have the potential to provide compelling evidence that estradiol's anorexigenic effect is mediated via selective activation of ER α .

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