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Research report

ERα, but not ERβ, mediates the expression of sexual behavior in the female rat

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ABSTRACT

Estrogen has well known effects on sexual behavior, however the role of the estrogen receptors (ER) α and β on sexual behavior remains to be fully determined. This study investigated the individual and co-operative involvement of ERα and β on sexual behaviors in the adult female rat. Subtype selective ER agonists, propyl-pyrazole triol (PPT; ERα agonist) and diarylpropionitrile (DPN; ERβ agonist) were utilized to examine each receptor subtype's contribution, individual and co-operative, for both receptive (lordosis) and proceptive (hopping/darting, 'ear wiggling') female sexual behaviors. Ovariectomized female rats received subcutaneous injections of either: sesame oil (OIL), dimethylsulfoxide (DMSO), estradiol benzoate (EB; 10 μg/0.1 ml OIL), one of three doses of the ERα agonist PPT (1.25 mg, 2.5 mg or 5.0 mg/0.1 ml DMSO), one of three doses of the ERβ agonist DPN (1.25 mg, 2.5 mg or 5.0 mg/0.1 ml DMSO) or a combination dose of PPT and DPN (2.5 mg PPT + 2.5 mg DPN/0.1 ml DMSO) for two consecutive days, 48 and 24 h prior to testing followed by a progesterone injection (500 μg/0.1 ml OIL) 4 h prior to testing in order to elicit sexual behavior. The ERα agonist PPT, but not the ERβ agonist DPN, elicited both proceptive and receptive behavior. PPT at doses of 2.5 and 5.0 mg significantly elicited lordosis and proceptive behavior ('ear wiggling', hopping and darting). Intriguingly, the administration of both agonists together at the 2.5 mg dose resulted in reduced levels of proceptivity and receptivity, suggesting that ERβ modulates ERα's ability to elicit receptive and proceptive sexual behavior.

1. Introduction

Estradiol has profound effects on brain and behavior [45]. There are two identified estrogen receptors (ER), ERα [11] and ERβ [29], that act as ligand-inducible transcription factors. The majority of estradiol's identified transcriptional actions in mammals are mediated by the classical receptor ERα [11] and the more recently cloned ERβ [10,29,37,56]. Since the discovery of ERβ, investigating the mechanisms of action behind estrogens and related synthetic drugs has become increasingly complex. For example, the synthetic estrogenic agent tamoxifen has both agonist/antagonist properties depending on the tissue in which it is examined; it serves as an estrogen-receptor agonist in bone [36,57] but acts as an estrogen receptor antagonist in breast tissue [23] and in the brain [19]. Furthermore, the mode of interaction of estrogenic agents also depends on the ER subtype involved; for instance, tamoxifen is both an agonist and antagonist of ERα and a pure antagonist of ERβ [3,6,32,61]. Therefore, understanding the differential effects of estradiol requires the consideration of cell context along with receptor subtype involvement.

Many studies examining estrogen receptor subtype neural activity and behavior use estrogen receptor knockout mice and the results have demonstrated the importance of ERα for the expression of sexual behavior [28,40,41,49]. However, there are various experimental caveats to consider when working with knockout models including pleotropic effects, developmental failures, the production of truncated proteins that have uncertain activity in vivo and potential compensatory effects such as overexpression of a related gene. The use of ER subtype-selective ligands [35,47,60] and antisense oligodeoxynucleotide for ERα and ERβ [59] provide an alternative, complementary approach to the use of receptor knockout mice to substantiate these important findings on female sexual behavior and allow us to determine the relative contribution of these receptors to adult behavior. The present study utilized recently available ER subtype agonists, propyl-pyrazole triol (PPT), an ERα agonist, and diarylpropionitrile (DPN), an ERβ agonist, to examine the individual physiological roles of the two receptors and

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their potential co-modulatory effects in mediating female sexual behavior. PPT is a potent ERα agonist [55], whereas DPN is a full ERβ agonist [34]. These agonists are valuable tools for elucidating the biological activity of ER subtypes and understanding estradiol's diverse effects on brain and behavior.

ERα and ERβ are expressed in the neural sites implicated in female sexual behavior [45–47] such as the ventromedial hypothalamus (VMH) [33,50], and the medial preoptic area (mPOA) [20,24]. Additionally, ERα mRNA and ERβ mRNA have been found to overlap within cells of the caudal VMH [22] and the mPOA [15] suggesting the two subtypes are present within the same cells. Previous work has shown that estradiol acts within the VMH to facilitate receptivity, defined as the lordosis response [33,50], while the mPOA seems to be important for both proceptive and receptive behavior [20,24]. While both receptor subtypes are expressed in areas involved in female sexual behavior, studies have found that ERα is more critical for the expression of female receptivity. For example, in studies utilizing ERα knockout mice the lordosis reflex was eradicated, while studies using ERβ knockout mice maintained receptivity with some subtle changes, indicating that ERα is crucial for female sexual behavior [28,41,49]. Although knockout studies suggest the exclusive involvement of ERα in female rodent receptivity [41,49], ERβ knockout females display significantly higher receptivity than wild-type (WT) females on the day after behavioral estrus [40]. These findings suggest that female receptivity, an ERα activated behavior, may be modulated by ERβ, perhaps by means of fine-tuning the expression of receptivity. Along with the VMH and mPOA, ERβ mRNA and ERβ-immunoreactive (ir) neurons have been found in the spinal cord and the amygdala, two other areas implicated in female sexual behavior further suggesting that ERβ may play a role in the expression of female sexual behavior [15,58]. In addition, mating stimulation induces Fos expression in the mPOA in cells co-expressing ERα and ERβ, and the medial amygdala in cells co-expressing only ERβ [15], further suggesting a modulatory role for ERβ on female sexual behavior. With novel agonists currently available, this investigation provides a complementary study to solidify behavioral alterations observed in genetically modified mice.

Very little work has investigated the role of SERMs (selective estrogen receptor modulators) on female sexual behavior [35,47,59,60]. Recently Frye and her colleagues have shown that ERα is important for lordosis in the rat [47,60]. They, and others, have demonstrated that administration of estradiol or the ERα agonist, PPT, enhanced lordosis ratings whereas administration of the ERβ agonist, DPN, did not elicit lordosis [35,47,60]. However, to our knowledge no study has investigated the combined contribution of both ERs on sexual behavior, the role of ERs on both proceptive and receptive female sexual behaviors, and doses necessary to elicit an effect [35,47,60]. Therefore our investigation provides an exclusive approach by employing ER agonists individually and in combination to determine their relative contributions towards proceptive and receptive behavior. A plausible hypothesis is that estradiol is mediating sexual behavior through the action of both ERα and ERβ to elicit proceptive and receptive behaviors in adult female rats.

2. Materials and methods

All experiments were conducted and approved in accordance with the policies established by the University of British Columbia and the Canadian Council on Animal Care regarding the ethical treatment of animals used for research.

2.1. Subjects

Thirty-six adult female Sprague–Dawley (250–300 g) rats were obtained from Charles Rivers Laboratories (Quebec, Canada). All rats were housed singly in polyurethane cages with access to food (Purina Lab Diet 5012, Richmond, Indiana, USA) and water *ad libitum*. Female rats were maintained on a reversed 12:12 h light dark cycle with lights off at 09:00 h in order to schedule testing during their active cycle. Twenty sexually experienced Long–Evan male rats (450–500 g) served as stud males for sexual behavior testing.

Table 1
Number of female rats in each group and the order of testing per group

Group	1st	2nd	3rd	Total
OIL	3	2	1	6
DMSO	3	2	1	6
EB	2	2	2	6
PPT 1.25	5	2	1	8
PPT 2.5	6	1	1	8
PPT 5.0	2	2	3	7
DPN 1.25	2	4	2	8
DPN 2.5	2	3	3	8
DPN 5.0	2	3	2	7
PPT + DPN	3	1	5	9

2.2. Surgery

Approximately 1–2 weeks after arrival, all females were bi-laterally ovariectomized using aseptic technique. Rats were placed in a chamber to which halothane was delivered at an induction flow rate of 4% (flow rate of O_2 was 2%) and maintained on a flow rate of 1–3% to maintain a stable respiratory rate during surgery. Rats were given 7 days to recover prior to the commencement of experimental manipulation.

2.3. Drug treatment

Ovariectomized female rats were randomly assigned to 1 of 10 groups (n = 6–9 per group) and received subcutaneous (s.c.) 0.1 ml injections of either: sesame oil (OIL), dimethylsulfoxide (DMSO), estradiol benzoate (EB; 10 μg/0.1 ml OIL), one of three doses of the ERα agonist propyl-pyrazole triol (PPT; 1.25 mg, 2.5 mg or 5.0 mg/0.1 ml DMSO), one of three doses of the ERβ agonist diarylpropionitrile (DPN; 1.25 mg, 2.5 mg or 5.0 mg/0.1 ml DMSO) or a combination dose of PPT and DPN (2.5 mg PPT + 2.5 mg DPN/0.1 ml DMSO) for two consecutive days, 48 h and 24 h prior to testing followed by a progesterone injection (500 μg/0.1 ml OIL) which was administered 4 h prior to testing in accordance with [17]. Twenty-two of the female rats were tested three times to account for all treatment groups and groups were counterbalanced with re-testing occurring at least 10 days apart in order for the treatment to fully dissipate before the next treatment condition. Table 1 shows the number of rats in each group and the number of rats per group in each session that was tested.

2.4. Drug preparation

The ERα agonist, propyl-pyrazole triol (PPT; Tocris, Ellisville, MO, USA) and the ERβ agonist, diarylpropionitrile (DPN; Tocris, Ellisville, MO, USA) were dissolved in dimethylsulfoxide (DMSO; Sigma–Aldrich, Oakville, Ontario, Canada). 17β-Estradiol benzoate (Sigma–Aldrich) was prepared to obtain a concentration of 10 μg EB per 0.1 ml sesame oil and was stored in a light insensitive container. Progesterone (Sigma–Aldrich) was prepared to obtain a concentration of 500 μg progesterone per 0.1 ml sesame oil and stored in a light insensitive container.

2.5. Testing procedure and sexual behavior measurements

Following post-operative recovery (7 days), each animal was handled for 5 min on two occasions. Following handling, all animals were habituated to the sex testing apparatus, a bi-level chamber (described below), for 10 min on two occasions.

The sex-testing chambers were narrow in width (width × length; 7 in. × 24 in.), which maintains an optimal sideway orientation of the animal to the experimenter and consisted of two levels (height; 30 in.). The rats had easy access to both levels by ramps at the ends of the chamber. These chambers have been used previously to examine the relationship of appetitive and consummatory sexual behaviors in male and female rats, and allow for female pacing behavior [46]. Testing was done within 4–6 h after progesterone administration, with the female rat placed on the top level of the bi-level chamber and a sexually vigorous male rat placed on the bottom level. Behaviors were video recorded for 10 min for analysis. All males were pre-screened to confirm their sexual motivation and the data was excluded from any male that did not show mounting behavior during the session. The female behavior measurements evaluated include: (1) level changes, (2) 'ear wiggling', (3) hops and darts, (4) lordosis quotient, (5) lordosis rating, and (6) rejection quotient. In addition, the number of mounts and mount attempts by the male rat was recorded to evaluate their sexual aggressiveness and mount latency was recorded to examine attractivity of the female rats. Distinct proceptive behaviors of female rats were 'ear wiggling' (rapid oscillatory movement of the females ears due to rapid head movements which is consequent to a high degree of tension in the axial muscles) and hops and darts (jump and scatter directly in front of the male). The receptive

behavior was the lordosis response; a postural reflex with a dorsiflexion of the vertebral column [18]. Two receptivity measurements were determined by examining the lordosis response; the proportion of time the female rat exhibited lordosis in response to a sexual contact (lordosis quotient; LQ = # of lordosis response scores of 2 or 3/# of mounts) and the intensity of lordosis responses (lordosis rating; LR = sum of 0, 1, 2, or 3 response scores/# mounts) [5]. Defensive behaviors were measured by addition of three rejection responses (fending, kicking and rolling) with each response receiving one point (rejection quotient; RQ = total number of rejection scores/#mounts).

2.6. Statistical analyses

The dependent variables (level changes, 'ear wiggling', hopping and darting, lordosis quotient, lordosis rating, rejection quotient, mount attempts and mount latency) were each analyzed using an analysis of variance (ANOVA) with treatment group (OIL, EB, DMSO, PPT 1.25, PPT 2.5, PPT 5.0, DPN 1.25, DPN 2.5, DPN 5.0 and PPT and DPN 2.5) and order (1, 2, 3) as the independent variables. *A priori* comparisons used Dunnett's procedure while post hoc comparisons used Neuman-Keul's method unless otherwise specified. All statistical procedures set $\alpha = 0.05$.

3. Results

3.1. Male sexual behavior

To determine whether male response was different according to treatment group and order we examined the number of mounts and mount latency. There was a significant interaction between treatment and order for number of mounts ($F(18, 43) = 1.87$, $p < 0.04$) however, post hoc tests failed to find any significant difference between any groups. There was a main effect of treatment ($F(9, 43) = 2.63$, $p < 0.016$) but no main effect of order ($p < 0.32$), and post hoc tests revealed that the number of mounts was increased in the PPT 1.25 group compared to DMSO controls. There were no other differences between treatment groups and their relative control group (data not shown). To test male motivation we examined mount latency. There was a significant interaction between treatment and order for mount latency ($F(18, 43) = 2.72$, $p < 0.004$) however, post hoc tests failed to find any significant difference between any groups. There was no significant effect of order ($p < 0.12$) but a trend for a main effect of treatment ($p < 0.054$). *A priori* tests on the main effect of treatment found that mount latency was significantly higher in the DMSO group compared to the PPT 1.25 and DPN 5.0 ($p < 0.019$; $p < 0.025$, respectively, although with a Bonferroni correction these would no longer be significant). Thus this suggests that all males were *initially* equally sexually vigorous and motivated to perform sexual behavior irrespective of female treatment.

For females, regardless of treatment or order of testing gross overall motor activity was not effected [main effect of treatment $p \leq 0.83$; main effect of order $p < 0.52$, interaction $p < 0.30$], indicating that any changes due to agonist administration were not due to overall differences in locomotor activity.

3.2. The ERα agonist, but not the ERβ agonist, treatment induces proceptive behavior

There was a main effect of treatment on 'ear-wiggling' ($F(9, 43) = 5.71$, $p < 0.001$) but no other significant effects (order: $p < 0.27$; interaction: $p < 0.95$). Post hoc analysis revealed that EB and PPT 5.0 females showed significantly more 'ear wiggling' relative to their respective vehicles ($p \leq 0.0002$; $p \leq 0.011$, respectively; Fig. 1A). There were no other significant differences between treatment groups. The concomitant administration of PPT and DPN dose produced very little effect on 'ear wiggling'; specifically, only two females (2/9) from this treatment group exhibited 'ear wiggling'.

There was a significant interaction between treatment and order for hopping and darting ($F(18, 43) = 3.10$, $p < 0.002$). Post hoc analysis indicated that PPT 2.5 rats tested during session 3 expressed a greater number of hops and darts than the other 2 sessions and PPT 5.0 rats tested during session 1 exhibited greater number of hops and darts than the other 2 sessions. Post hoc analysis on the main effect of treatment revealed that PPT 5.0 and PPT 2.5 treated groups demonstrated significantly more hopping and darting relative to their DMSO vehicles ($p \leq 0.04$; $p \leq 0.01$, respectively; $F(9, 43) = 5.67$, $p < 0.0001$; Fig. 1B). All other groups were not significantly different relative to their vehicle (all p's > 0.1). The concomitant administration of PPT and DPN dose produced no significant effect on hops and darts but four females (4/9) from this treatment group exhibited hops and darts.

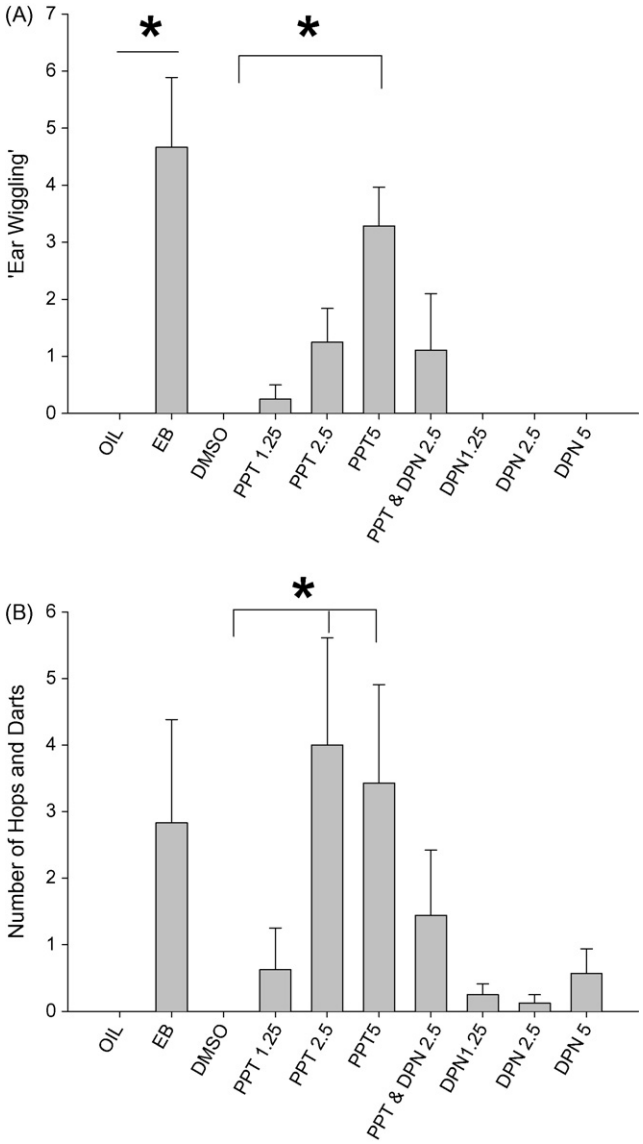

Fig. 1. (A) Frequency of 'ear wiggling' as a function of treatment in female rats. The EB and PPT 5.0 treated rats showed significantly more 'ear wiggling' relative to their respective vehicles ($p \leq 0.0002$; $p \leq 0.011$, respectively; main effect of treatment on 'ear-wiggling' ($F(9, 43) = 5.71$, $p < 0.001$)). 'Ear wiggling' of the female rats administered the combination agonist treatment (2.5 mg PPT + 2.5 mg DPN/0.1 ml DMSO) did not statistically differ from vehicle suggesting that dual receptor subtype activation does not mimic the effect of estradiol alone. (B) Number of hops and darts as a function of treatment in female rats. PPT 5.0 and PPT 2.5 treated rats demonstrated significantly more hopping and darting relative to DMSO ($p \leq 0.04$; $p \leq 0.01$, respectively). The combination agonist treatment (PPT and DPN 2.5 mg/0.1 ml DMSO) did not statistically differ from vehicle. Error bars indicate standard error of mean (S.E.M.; $n = 6$–9). *Significantly different from control group (OIL or DMSO) ($p < 0.05$).

3.3. The ERα agonist, but not the ERβ agonist, elicits receptive behavior in a dose-dependent manner

There was a significant main effect of treatment on lordosis quotient ($F(9, 43) = 10.90$, $p < 0.0001$) but no main effect of order or interaction ($p \leq 0.34$; $p \leq 0.27$, respectively). Post hoc analysis demonstrated that EB, PPT 2.5 and PPT 5.0 groups demonstrated a significantly greater lordosis quotient relative to their respective vehicle ($p \leq 0.0001$, $p \leq 0.04$, $p \leq 0.0001$, respectively; Fig. 2A). There were no other significant differences between groups (all p's > 0.2). The concomitant administration of PPT and DPN dose produced very little effect on lordosis quotient; specifically, only two females (2/9) from this treatment group exhibited a lordosis quotient greater than zero. There was a significant main effect of treatment on lordosis rating ($F(9, 43) = 11.76$, $p < 0.0001$) but no main effect of order or interaction ($p \leq 0.56$; $p \leq 0.13$, respectively). Post hoc tests revealed that EB, PPT 1.25, PPT 2.5 and PPT 5.0 treated females demonstrated a significantly higher lordosis rating (LR) relative to their vehicles (EB $p \leq 0.0001$; PPT 1.25 $p \leq 0.04$; PPT 2.5, $p \leq 0.02$; PPT 5.0, $p \leq 0.0001$; Fig. 2B). There were no other significant differences between groups (all p's > 0.8).

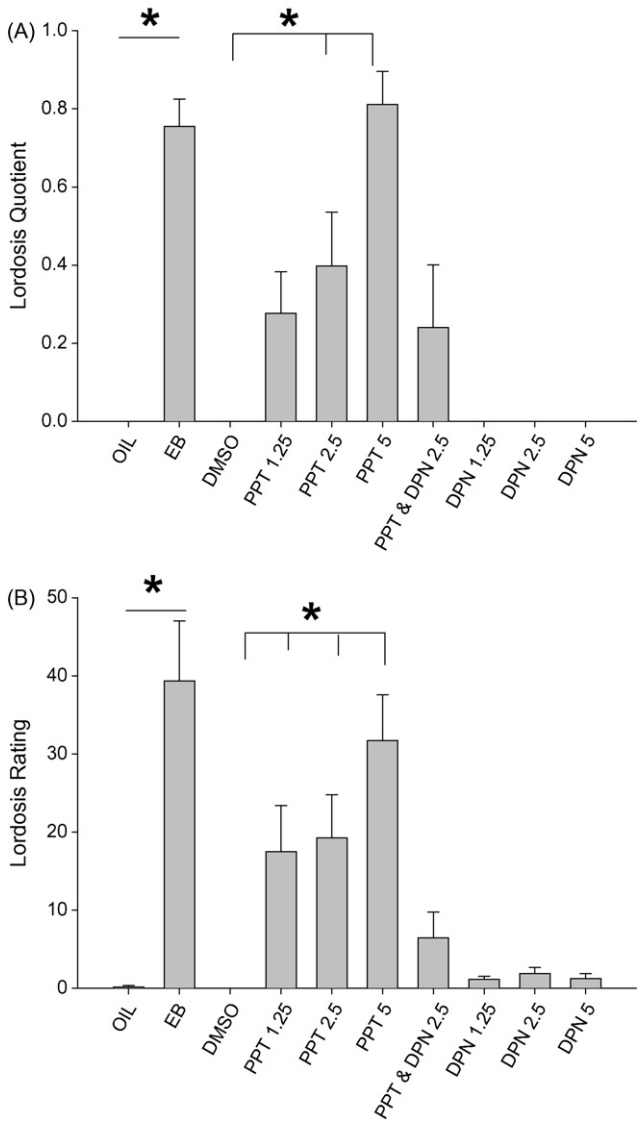

Fig. 2. (A) Lordosis quotient (LQ) as a function of treatment in female rats. EB, PPT 2.5 and PPT 5.0 treated rats demonstrated a significantly higher LQ relative to their respective vehicle ($p \leq 0.0001$, $p \leq 0.04$, $p \leq 0.0001$, respectively; main effect of treatment on lordosis quotient ($F(9, 43) = 10.90$, $p < 0.0001$)). Combined agonist treatment group (P and D 2.5) did not statistically differ from vehicle. DPN treated females did not display the lordosis posture. (B) Lordosis rating (LR) as a function of treatment in female rats. EB, PPT 5.0, PPT 2.5 and PPT 1.25 treated females displayed a significant lordosis rating relative to their vehicles (EB $p \leq 0.0001$; PPT 5.0, $p \leq 0.0001$; PPT 2.5, $p \leq 0.02$; PPT 1.25, $p \leq 0.04$; main effect of treatment on lordosis rating ($F(9, 43) = 11.76$, $p < 0.0001$)). Error bars indicate S.E.M. ($n = 6$–9). *Significantly different from control group (OIL or DMSO) ($p < 0.05$).

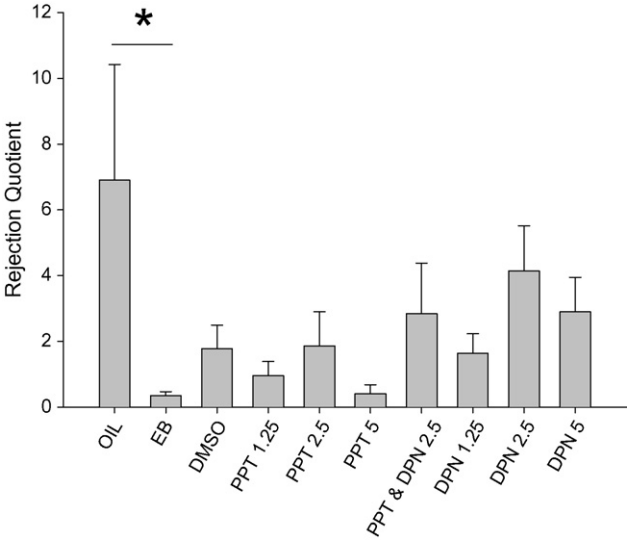

Fig. 3. Rejection quotient as a function of treatment in female rats. EB treated animals demonstrated significantly less rejection behavior relative to vehicle ($p \leq 0.02$; main effect of treatment on rejection quotient ($F(9, 43) = 2.12$, $p < 0.04$)). All PPT doses (PPT 1.25, PPT 2.5 and PPT 5.0) and combined agonist (PPT and DPN 2.5) treated rats demonstrated no significant differences relative to vehicle (all p's > 0.5). There was no significant differences in rejection quotient for all DPN treated rats relative to vehicle (all p's > 0.5). Error bars indicate S.E.M. ($n = 6$–9). *Significantly different from control group (OIL or DMSO) ($p < 0.05$).

There was a significant main effect of treatment on rejection quotient ($F(9, 43) = 2.12$, $p < 0.04$), a trend for a main effect of order ($p \leq 0.056$) but not an interaction ($p \leq 0.44$). Post hoc analyses revealed that EB treated animals demonstrated significantly less rejection behavior relative to vehicle ($p \leq 0.02$; Fig. 3). There were no other significant differences between groups (all p's > 0.6). Due to the trend for a main effect of order, we used an *a priori* with a Bonferroni correction and did not find any significant differences between order, however, the direction of the means indicated that there were greater rejection quotient scores during the first testing session compared to the second and third testing sessions.

4. Discussion

The results from the present experiment clearly show a disassociation in function between ERα and ERβ using the ERα and ERβ selective agonists, propyl-pyrazole triol (PPT) and diarylpropionitrile (DPN). The ERα agonist, PPT, but not the ERβ agonist, DPN, when administered in isolation, elicited sexual proceptivity and receptivity in the female rat. Therefore, our findings indicate ERα, but not ERβ, is involved in both proceptive and receptive female sexual behavior, consistent with prior findings using knockout mice [41,47,49,59]. Furthermore we found that DPN eliminated the PPT-induced increase in expression of both receptive and proceptive female sexual behavior, when co-administered with PPT. This suggests that although the ERβ agonist DPN on its own does not elicit receptive or proceptive sexual behavior, it can modulate the ERα agonist's degree of expression on female sexual behavior.

Previous studies on the role of ERs on female sexual behavior have not directly investigated the expression of proceptive behaviors in the female [35,46,59,60] such as 'ear wiggling' and hops and darts [4]. The results from the present study suggest that ERβ activation is not essential for eliciting proceptive behaviors, as DPN alone failed to elicit proceptive behaviors such as 'ear wiggling' or hopping and darting. However evidence from the βERKO mice suggests that female reproduction is altered as βERKO females have reduced fertility and decreased litter size, likely due to a decrease in the number of oocyctes released [27]. In addition, the neural areas important for proceptive behavior such as the VMH [1] and medial preoptic area (mPOA [20,24]), both contain ERα and ERβ [51–53], suggesting that ERβ can directly modulate ERα elicited proceptive behaviors as was found in the present study.

In agreement with previous work of Frye and colleagues [47,59,60] and Miller et al. [35], our results demonstrate that ERβ is not essential to elicit receptive behavior, as PPT, but not DPN, elicited high lordosis ratings and increased lordosis quotient (LQ) to a similar level as estradiol-treated rats [35,47,59,60]. In the present study we also showed a dose response curve with dose of PPT of 2.5 and 5.0 mg eliciting significantly higher LQs than controls and all doses of PPT tested eliciting significantly higher lordosis ratings than controls. To the best of our knowledge, no study has examined a dose response curve for female sexual behavior using these agonists. Intriguingly, two studies found that a dose of 10 μg of PPT elicited LQs to similar levels as estradiol-treated rats [47,60], while Miller et al. [35] found that a dose of approximately 3 mg of PPT elicited LQ levels to the same level as estradiol-treated rats [35]. These results coupled with the present study suggest that there may be a sinusoidal dose relationship between PPT and LQ with lower and higher doses eliciting high LQ levels. Furthermore it should be noted that the size and shape of the testing chamber can profoundly affect the expression of sexual behavior especially in female rats [42]. Paced mating is more rewarding to female rats and the use of the bi-level chamber, as was used in the present study, allows for more paced behavior on the part of the female [46]. In addition, experience plays a role in the activation of neural areas involved in sexual behavior in females [8]. Although in the present experiment we did not have large enough sample size to examine experience, there were some subtle session differences particularly in rejection quotient across sessions, suggesting that experience may play a role in the expression of sexual behavior in the female rat.

Our results demonstrate the ERα is important for both proceptive and receptive behaviors, and that ERβ has a modulatory role on sexual proceptivity. This is not surprising as this ER subtype is present in neural areas implicated in proceptive and receptive behavior [51,53]. For example, a recent study found that after females experienced mounting, Fos-ir was expressed in ERα-containing cells in the mPOA, however after intromissions Fos-ir cells containing both ERα and ERβ were expressed in the mPOA [15]. These findings suggest that specific mating stimuli activates cells that express ERβ in concert with ERα or alone, further suggesting a modulatory role of ERβ on ERα for the expression of female sexual behavior.

Rejection quotient was significantly decreased only in estradiol-treated females, although PPT 5.0-treated females also showed reduced rejection quotient scores that were not significantly different compared to their control. Intriguingly, rejection quotients were non-significantly elevated in DPN injected rats (except for DPN 1.25), while PPT injected females showed reduced rejection scores. This result further supports a dichotomy between the actions of ERα and ERβ on affiliative and aggressive behaviors, consistent with previous literature [7,38,39,54]. Intriguingly, ERβKO male mice show more aggressive behaviors than WT or ERαKO male mice, suggesting that ERβ activation attenuates aggressive behaviors in male mice [38,39]. Male macaque monkeys fed a high soy isoflavone-rich diet, which has a greater affinity for ERβ, elicited more aggression than monkeys fed a control diet [54], suggesting that ERβ may have aggressive-promoting tendencies. Future studies should aim to investigate the potential differences between ERβ and ERα in stimulating rejection behaviors and/or aggression in female rodents.

Our observations using an ERβ selective agonist coupled with previous studies using transgenic mice provide additional evidence that ERβ is not essential for the expression of female rodent sexual behavior [41,47,49,59]. However recent studies investigating the organizational influences of ERβ have found ERβ agonists inhibit female sexual behavior if administered during development [25,26]. Furthermore, in our study, the combined dose of PPT and DPN did not enhance female sexual behavior, and in fact, DPN eliminated the PPT-induced facilitation of sexual behavior. These results complement the finding in knockout mice showing that ERβKO female mice exhibit extended receptivity further suggesting a modulatory role of ERβ. βERKO females demonstrate significantly higher receptivity than WT females the day after behavioral estrus and exhibit a proceptive/still posture throughout the cycle [40]. These findings indicate a modulatory role of ERβ, perhaps to regulate the expression of receptivity, specifically the switching-off phase during the estrous cycle. Because each receptor subtype is present in the neural areas implicated in proceptive and receptive behavior [51,53], ERβ function lends itself to a modulatory role. ERβ's subtle role in the regulation of sexual behavior in adulthood may be due to acting as a regulatory of ERα transcriptional activity. Indeed, ERβ has been demonstrated to have both an enhancing and inhibitory effect on ERα activity [12,16]. These findings suggest that ERβ is inhibiting ERα activity when each receptor subtype is bound by the complementary agonist. ERβ's inhibitory role is further supported by work in mammalian cells [16,43] and in ER knockout mice [9,44] which report that ERα mediates the proliferative effects, while ERβ favors the suppression, of gene expression. Furthermore each ER subtype has the ability to regulate different genes and have separate or shared functional roles at the cellular and/or behavioral level when both expressed in the same neural area. For instance, ERα in the hippocampus has been suggested to influence neuronal morphology by stimulating dendritic branching [2] whereas ERα [13] and ERβ [47,48] have been suggested to play a role in hippocampus-dependent spatial learning and neurogenesis in the hippocampus [30]. Evidence demonstrating ERβ's ability to regulate ERα's activity is beginning to elucidate how estradiol can regulate a plethora of distinct effects in a variety of cellular and physiological contexts.

Although ERα is the predominant estrogen receptor in the VMH and is found in neurons throughout the rostrocaudal extent of this brain region [52,53], studies have also found ERβ localized in the VMH [51,53]. Additionally, ERα mRNA and ERβ mRNA have been found to overlap within cells of the caudal VMH [22] and the mPOA [15] suggesting the two subtypes are present within the same cell. The suggestion of ERα and ERβ cellular co-expression invites the possibility of three types of dimers (ERβ- and ERα-homodimers and ERα–β heterodimers) that might play different roles in this hormone-regulated brain function or behavior via a complex regulatory mechanism [31]. Recent data support the hypothesis that ERβ expression is regulated via the ERα pathway [14] and that both ERα and ERβ regulate unique subsets of downstream genes within a given cell type [21]. Considering the location, expression and activity of each receptor subtype this deliberation invites a variety of possibilities of mechanistic action in order to mediate the desired brain function and/or behavioral outcome.

5. Conclusion

In the present study we have shown that ERα, but not ERβ, is primarily involved in eliciting both proceptive and receptive female sexual behavior. Understanding how estradiol and its receptors dynamically regulate various physiological processes is of vital importance to further evaluate its potential therapeutic role for various conditions and disease states. Estradiol is a network chemical communicator within the mammalian system that will continue to demand a thorough approach for clearer understanding of its mechanisms and molecular chaperoned pathways.

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References

[1] Aou S, Oomura Y, Yoshimatsu H. Neuron activity of the ventromedial hypothalamus and the medial preoptic area of the female monkey during sexual behavior. Brain Res 1988;455:65–71.
[2] Audesirk T, Cabell L, Kern M, Audesirk G. Beta-estradiol influences differentiation of hippocampal neurons in vitro through an estrogen receptor-mediated process. Neuroscience 2003;121:927–34.
[3] Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J, Nilsson S. Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. Mol Pharmacol 1998;54:105–12.
[4] Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 1976;7:105–38.
[5] Brandling-Bennett EM, Blasberg ME, Clark AS. Paced mating behavior in female rats in response to different hormone priming regimens. Horm Behav 1999;35:144–54.
[6] Buteau-Lozano H, Ancelin M, Lardeux B, Milanini J, Perrot-Applanat M. Transcriptional regulation of vascular endothelial growth factor by estradiol and tamoxifen in breast cancer cells: a complex interplay between estrogen receptors alpha and beta. Cancer Res 2002;62:4977–84.
[7] Choleris E, Ogawa S, Kavaliers M, Gustafsson JA, Korach KS, Muglia LJ, et al. Involvement of estrogen receptor alpha, beta and oxytocin in social discrimination: a detailed behavioral analysis with knockout female mice. Genes Brain Behav 2006;5:528–39.
[8] Coolen LM, Peters HJ, Veening JG. Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison. Brain Res 1996;738:67–82.
[9] Couse JF, Dixon D, Yates M, Moore AB, Ma L, Maas R, et al. Estrogen receptor-alpha knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. Dev Biol 2001;238:224–38.
[10] Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, et al. Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. J Clin Endocrinol Metab 1997;82:4258–65.
[11] Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988;240:889–95.
[12] Frasor J, Barnett DH, Danes JM, Hess R, Parlow AF, Katzenellenbogen BS. Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) alpha activity by ERbeta in the uterus. Endocrinology 2003;144:3159–66.
[13] Fugger HN, Cunningham SG, Rissman EF, Foster TC. Sex differences in the activational effect of ERalpha on spatial learning. Horm Behav 1998;34:163–70.
[14] Glaeser M, Niederacher D, Djahansouzi S, Hanstein B, Dittrich R, Beckmann MW, et al. Effects of the antiestrogens tamoxifen and raloxifene on the estrogen receptor transactivation machinery. Anticancer Res 2006;26:735–44.
[15] Greco B, Blasberg ME, Kosinski EC, Blaustein JD. Response of ERalpha-IR and ERbeta-IR cells in the forebrain of female rats to mating stimuli. Horm Behav 2003;43:444–53.
[16] Hall JM, McDonnell DP. The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 1999;140:5566–78.
[17] Hansen S, Harthon C, Wallin E, Lofberg L, Svensson K. Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness. Behav Neurosci 1991;105:588–98.
[18] Hardy DF, Debold JF. Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. Physiol Behav 1971;7:643–5.
[19] Hilton GD, Ndubuizu AN, McCarthy MM. Neuroprotective effects of estradiol in newborn female rat hippocampus. Brain Res Dev Brain Res 2004;150:191–8.
[20] Hoshina Y, Takeo T, Nakano K, Sato T, Sakuma Y. Axon-sparing lesion of the preoptic area enhances receptivity and diminishes proceptivity among components of female rat sexual behavior. Behav Brain Res 1994;61:197–204.
[21] Hurst AG, Goad DW, Mohan M, Malayer JR. Independent downstream gene expression profiles in the presence of estrogen receptor alpha or beta. Biol Reprod 2004;71:1252–61.
[22] Ikeda Y, Nagai A, Ikeda MA, Hayashi S. Sexually dimorphic and estrogen-dependent expression of estrogen receptor beta in the ventromedial hypothalamus during rat postnatal development. Endocrinology 2003;144:5098–104.
[23] Jordan VC. Alternate antiestrogens and approaches to the prevention of breast cancer. J Cell Biochem Suppl 1995;22:51–7.
[24] Kato A, Sakuma Y. Neuronal activity in female rat preoptic area associated with sexually motivated behavior. Brain Res 2000;862:90–102.
[25] Kouki T, Kishitake M, Okamoto M, Oosuka I, Takebe M, Yamanouchi K. Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. Horm Behav 2003;44:140–5.
[26] Kouki T, Okamoto M, Wada S, Kishitake M, Yamanouchi K. Suppressive effect of neonatal treatment with a phytoestrogen, coumestrol, on lordosis and estrous cycle in female rats. Brain Res Bull 2005;64:449–54.
[27] Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, et al. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc Natl Acad Sci USA 1998;95:15677–82.
[28] Kudwa AE, Rissman EF. Double oestrogen receptor alpha and beta knockout mice reveal differences in neural oestrogen-mediated progestin receptor induction and female sexual behavior. J Neuroendocrinol 2003;15:978–83.
[29] Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996;93:5925–30.
[30] Mazzucco CA, Lieblich SE, Bingham BI, Williamson MA, Viau V, Galea LA. Both estrogen receptor alpha and estrogen receptor beta agonists enhance cell proliferation in the dentate gyrus of adult female rats. Neuroscience 2006;141:1793–800.
[31] McDonnell DP, Connor CE, Wijayaratne A, Chang CY, Norris JD. Definition of the molecular and cellular mechanisms underlying the tissue-selective agonist/antagonist activities of selective estrogen receptor modulators. Recent Prog Horm Res 2002;57:295–316.
[32] McInerney EM, Katzenellenbogen BS. Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. J Biol Chem 1996;271:24172–8.
[33] Meisel RL, Dohanich GP, McEwen BS, Pfaff DW. Antagonism of sexual behavior in female rats by ventromedial hypothalamic implants of antiestrogen. Neuroendocrinology 1987;45:201–7.
[34] Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, Katzenellenbogen JA. Estrogen receptor-beta potency-selective ligands: structure–activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. J Med Chem 2001;44:4230–51.
[35] Miller NR, Jover T, Cohen HW, Zukin RS, Etgen AM. Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. Endocrinology 2005;146:3070–9.
[36] Moon LY, Wakley GK, Turner RT. Dose-dependent effects of tamoxifen on long bones in growing rats: influence of ovarian status. Endocrinology 1991;129:1568–74.
[37] Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996;392:49–53.
[38] Nomura M, Andersson S, Korach KS, Gustafsson JA, Pfaff DW, Ogawa S. Estrogen receptor-beta gene disruption potentiates estrogen-inducible aggression but not sexual behaviour in male mice. Eur J Neurosci 2006;23:1860–8.
[39] Nomura M, Durbak L, Chan J, Smithies O, Gustafsson JA, Korach KS, et al. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor beta knockout (betaERKO) male mice. Horm Behav 2002;41:288–96.
[40] Ogawa S, Chan J, Chester AE, Gustafsson JA, Korach KS, Pfaff DW. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc Natl Acad Sci USA 1999;96:12887–92.
[41] Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. Endocrinology 1998;139:5070–81.
[42] Paredes RG, Vazquez B. What do female rats like about sex? Paced mating. Behav Brain Res 1999;105:117–27.
[43] Pettersson K, Delaunay F, Gustafsson JA. Estrogen receptor beta acts as a dominant regulator of estrogen signaling. Oncogene 2000;19:4970–8.
[44] Pettersson K, Gustafsson JA. Role of estrogen receptor beta in estrogen action. Annu Rev Physiol 2001;63:165–92.
[45] Pfaff DW, Vasudevan N, Kia HK, Zhu YS, Chan J, Garey J, et al. Estrogens, brain and behavior: studies in fundamental neurobiology and observations related to women's health. J Steroid Biochem Mol Biol 2000;74:365–73.
[46] Pfaus JG, Smith WJ, Coopersmith CB. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers. I. A correlational and factor analysis and the effects of ovarian hormones. Horm Behav 1999;35:224–40.
[47] Rhodes ME, Frye CA. ERbeta-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. Neurobiol Learn Mem 2006;85:183–91.
[48] Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA. Disruption of estrogen receptor beta gene impairs spatial learning in female mice. Proc Natl Acad Sci USA 2002;99:3996–4001.

[49] Rissman EF, Wersinger SR, Fugger HN, Foster TC. Sex with knockout models: behavioral studies of estrogen receptor alpha. Brain Res 1999;835:80–90.
[50] Rubin BS, Barfield RJ. Priming of estrous responsiveness by implants of 17 beta-estradiol in the ventromedial hypothalamic nucleus of female rats. Endocrinology 1980;106:504–9.
[51] Shima N, Yamaguchi Y, Yuri K. Distribution of estrogen receptor beta mRNA-containing cells in ovariectomized and estrogen-treated female rat brain. Anat Sci Int 2003;78:85–97.
[52] Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol 1997;388:507–25.
[53] Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. J Comp Neurol 2001;436:64–81.
[54] Simon NG, Kaplan JR, Hu S, Register TC, Adams MR. Increased aggressive behavior and decreased affiliative behavior in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones. Horm Behav 2004;45:278–84.
[55] Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, et al. Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. J Med Chem 2000;43:4934–47.
[56] Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, et al. Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. Mol Endocrinol 1997;11:353–65.
[57] Turner RT, Wakley GK, Hannon KS, Bell NH. Tamoxifen prevents the skeletal effects of ovarian hormone deficiency in rats. J Bone Miner Res 1987;2:449–56.
[58] Vanderhorst VG, Gustafsson JA, Ulfhake B. Estrogen receptor-alpha and -beta immunoreactive neurons in the brainstem and spinal cord of male and female mice: relationships to monoaminergic, cholinergic, and spinal projection systems. J Comp Neurol 2005;488:152–79.
[59] Walf AA, Ciriza I, Garcia-Segura LM, Frye CA. Antisense oligodeoxynucleotides for estrogen receptor-beta and alpha attenuate Estradiol's modulation of affective and sexual behavior, respectively. Neuropsychopharmacology 2008;33:431–40.
[60] Walf AA, Frye CA. ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. Neuropsychopharmacology 2005;30:1598–609.
[61] Zou A, Marschke KB, Arnold KE, Berger EM, Fitzgerald P, Mais DE, et al. Estrogen receptor beta activates the human retinoic acid receptor alpha-1 promoter in response to tamoxifen and other estrogen receptor antagonists, but not in response to estrogen. Mol Endocrinol 1999;13:418–30.