Lack of functional estrogen receptor β gene disrupts pubertal male sexual behavior

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Abstract

The estrogen receptor-β (ERβ) mediates estrogen action in the female gonads, reproductive tract, and central nervous system. In addition, in rats and mice, gonadotropin-releasing hormone (GnRH-I) neurons coexpress ERβ. Here we asked if ERβ plays a role in the onset of puberty and in hypothalamic-pituitary-gonadal (HPG) axis function in male mice. We examined mating behavior, testosterone concentrations, steroid negative feedback on gonadotropins, and GnRH-I function in male ERβ knockout (ERβKO) and wild-type (WT) mice. Peripubertal ERβKO males displayed their first ejaculation at a significantly older age than WT littermates. Castrated, adult ERβKO mice had significantly higher plasma luteinizing hormone (LH) than WT counterparts. Estradiol (E2) treatment reduced LH and follicle stimulating hormone (FSH) concentrations to an equivalent degree in castrates of both genotypes. In three different measures of the adult GnRH-I system, no genotypic differences were observed. These data show that ERβ plays an important role in the timing of male sexual behavior at puberty, but does not appear to be involved in adult HPG axis functioning. Furthermore, our data suggest that a primary role of ERβ may be to regulate ejaculatory behavior.

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Introduction

Estrogens have ubiquitous actions on multiple target tissues. The discovery of a second estrogen receptor, estrogen receptor-β (ERβ; Kuiper et al., 1996), offers a new mechanism for estradiol (E2) to act in a tissue-specific manner (Nilsson and Gustafsson, 2002). Yet, ERβ is present in most of the same tissues that contain ERα (Couse et al., 1997; Shughrue et al., 1997a, 1998). ERα and ERβ are both expressed in the hypothalamus, pituitary, and gonads

(Gustafsson, 1999), suggesting that reproduction can be regulated by both receptors, perhaps in a function-specific manner. In addition, it is possible that ERα and ERβ normally work together to affect neural and pituitary responses to estrogen (Gustafsson, 1999; Shughrue et al., 1997a).

Estrogen receptor knockout (ERKO) mice (both ERαKO and ERβKO) have been used to define potential roles of each receptor. Male and female ERαKO mice are infertile (Krege et al., 1998; Lubahn et al., 1993). In both sexes, negative feedback, plasma levels of steroids and gonadotropins, and sexual behavior are disrupted (Lubahn et al., 1993; Wersinger et al., 1997; Wersinger et al., 1999). Female ERβKOs are subfertile and have a reduced response to hormone treatments that promote superovulation (Krege et al., 1998). On the other hand, male ERβKO mice are fertile

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and, thus far, no reproductive deficit has been reported for these mice (Krege et al., 1998; Ogawa et al., 1999).

Recent data indicate that the lack of functional ER\(^{\beta}\) enhances estrogen’s ability to induce progesterone receptor (PR) in the brain (Temple et al., 2001). In addition, the discovery of ER\(^{\beta}\) in GnRH-I neurons in male and female rodents suggests that ER\(^{\beta}\) may play a direct role in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis via actions on GnRH-I (Hrabovszky et al., 2001; Herbison and Pape, 2001; Sharifi et al., 2002). The hypothesis is supported by a recent study showing that ER\(^{\beta}\) is required for E\(_2\)-mediated increases in pCREB in GnRH-I neurons (Abraham et al., 2003). In addition, ER\(^{\beta}\)KO mice show heightened aggressive behavior and elevated plasma testosterone during a short time period that coincides with puberty, suggesting that the ER\(^{\beta}\) may be especially important during that developmental period (Nomura et al., 2002a).

In these studies, we asked if ER\(^{\beta}\) plays a role in male reproduction particularly around the onset of puberty. We used male ER\(^{\beta}\)KO mice and wild-type (WT) littermates and performed copulatory behavior tests beginning prior to puberty to track the onset of behavioral sexual maturation. In young adults, we compared several aspects of the HPG axis regulation, including negative feedback on gonadotropins, proGnRH-immunoreactive (-ir) neuron number, plasma LH levels in response to a GnRH-I challenge, and GnRH-I peptide content.

Materials and methods

General methods

Animals

Mice were generated by mating heterozygous breeders carrying a single copy of the disrupted ER\(^{\beta}\) gene (Kuiper et al., 1996). Offspring were screened by PCR amplification of tail DNA (Temple et al., 2001). The mice were of a mixed 129/SvJ and C57BL/6J background, approximately two generations back-crossed into the C57BL/6J strain for studies conducted in adult males and five generation back-crossed for the pubertal behavior experiment. After weaning (18–20 days of age) mice were individually housed on a 12:12 h light:dark cycle (lights off at 1200 h EDT) and received food (Purina mouse chow no. 5001) and water ad libitum. For prepubertal males, subjects were group housed until 30 days of age and then individually housed for the duration of sexual behavior tests.

Surgery and plasma collection

Castrations were performed while under general anesthesia (ketamine:xylazine, 20 mg:2 mg/ml; 0.1 ml/25 g body wt). Blood was collected by cardiac puncture under general anesthesia or after a lethal injection of sodium pentobarbital (0.1 mg/kg) prior to sacrifice. Plasma was collected and frozen at −70°C until assay. Animal care and surgery were conducted in accordance with the University of Virginia Animal Care and Use Committee guidelines.

Radioimmunoassay

Assays for LH, FSH, and testosterone (T) were conducted using radioimmunoassay (RIA) performed by the University of Virginia Core Ligand and Assay Laboratory [supported by NICHD/NIH through cooperative agreement (U54 HD28934) as part of the Specialized Cooperative Centers Program in Reproduction Research]. For the LH and T assays samples were run in duplicate in a single assay to eliminate interassay variability. The FSH RIA was run with singlicate samples.

Plasma LH was measured by a supersensitive two-site sandwich immunoassay using monoclonal antibodies MAB1 (no. 58187) and TMA (no. 5303, Medix Kauniainen, Finland) against mouse and human respectively. This assay has a sensitivity of 7 pg/tube and the intraassay coefficient of variability of the controls ranged from 1.7 to 10.1%. The assay has been previously validated for use in the mouse (Fallast et al., 1995).

Plasma FSH was measured by RIA using reagents provided by the National Hormone and Pituitary Program. FSH reference prep 1 was used for assay standards and antirat FSH was used as the primary antibody. This assay has a sensitivity of 0.3 ng/ml and <0.5% cross-reactivity with other pituitary hormones. The intraassay coefficient of variability for the controls ranged from 5.5 to 7.2%. The T assay is commercially available (Diagnostic Systems Laboratory; Webster, TX). The detectable range of the assay was from 0.1 to 25.0 ng/ml with an intraassay coefficient of variability of 8.7%.

Statistical analyses

Behavioral data were analyzed by one-way analysis of variance (ANOVA) to detect differences between genotypes. We conducted two-way ANOVAs on the LH, FSH, and T RIA data to assess genotype, hormone treatment, and interactions. Numbers of proGnRH-I cells were subjected to the same type of analyses. A one-way ANOVA was used to examine genotype differences in GnRH-I content and LH concentrations after a GnRH-I challenge. Post hoc comparisons were made using Student-Newman-Keuls tests. The data were considered significantly different when \(P < 0.05\).

Mating behavior

Testing procedure

Starting at 32–33 days of age, 10 gonad-intact WT and 9 ER\(^{\beta}\)KO males were tested for mating behavior with a sexually receptive female. Tests were conducted in the dark (between 1300 h and 1800 h) under red-light illumination. Males were habituated to a Plexiglas test box (18 × 38 cm) for at least 1 h. Test boxes were placed on a mirror stand, which allowed accurate assessment of thrusts and intromissions. In addition to the number of thrusts and intromissions
within a mount event, we also recorded mounts, mounts with thrusts, and mounts with intromission, as well as the latency to perform all these behaviors and the latency to ejaculation. The tests lasted for 30 min if no sexual behavior occurred. If mounting behavior was observed during the 30 min, the male was given an additional 60 min with the female or until an ejaculation occurred, whichever occurred first. Males were tested every other day until they displayed an ejaculation or until they reached 60 days of age. If an ejaculation occurred during a test the male was not retested.

**Stimulus females**

Stimulus females were implanted at the time of ovariec-
tomy (OVX) with a Silastic implant (1.96 mm I.D. × 3.18
mm O.D.) filled with estradiol benzoate (50 µg EB
dissolved in 30 µl sesame oil). Three to five hours prior to
sexual behavior tests, females were injected sc with proges-
terone (500 µg P dissolved in 30 µl sesame oil). Females
were screened for receptivity with a sexually experienced
stimulus male just prior to each behavior test.

**Testosterone measurements**

To assess the possibility that behavioral differences be-
tween WT and ERβKO males were due to differences in T
concentrations, we sacrificed 5 WT and 11 ERβKO males
between the ages of 37 and 44 days. Blood was collected
and seminal vesicles were removed and weighed.

**Gonadotropin responses in adulthood**

**Experimental design**

To examine E2-mediated negative feedback on LH and
FSH, 18 ERβKO and 19 WT male littersmates between the
ages of 55 and 120 days were castrated and implanted with
either a 21-day time-release E2 pellet (0.05 mg/pellet Innova-
tive Research of America, Sarasota, FL) or an inert pellet
placed subcutaneous in the midscapular region. This yielded the following four groups: castrates + inert pellet (WT =
10; ERβKO = 7) and castrates + E2 (WT = 9; ERβKO =
11). The E2 pellet implant (0.05 mg) yields mean plasma E2
levels of 90 pg/ml (Fugger et al., 2000), which is equivalent
to proestrous levels in mice (Bronson and Desjardins, 1974;
Rissman unpublished data). Twenty days after castration
each male was sacrificed, blood was collected and plasma
frozen.

**Analysis of GnRH-I in adulthood**

**Description of GnRH-I neurons**

**Immunocytochemistry.** The same adult males used to study
gonadotropin responses were used for immunocytochemis-
try (10 WT + inert pellet, 9 WT + E2, 7 ERβKO + inert
pellet, and 11 ERβKO + E2). Twenty days after castration
and hormone implantation, mice were deeply anesthetized,
perfused first with heparinized saline (30 ml of 100 units
heparin/1 ml of 0.9% saline) followed immediately by 2%
followed by the addition of 3 M NaHCO₃ and NaOH to bring the pH to 7.0. The homogenate was centrifuged at 2500 rpm for 10 min. GnRH-I content was assessed using a peptide enzyme immunoassay (Peninsula Laboratories, Inc. Belmont, CA, USA). This assay cross-reacts 100% with GnRH-I and has a cross-reactivity of ≤0.1% with all other forms of GnRH-I tested. The detection range of this assay is 0–10 ng/ml. All concentrations measured were within this range. All samples were run in duplicate at the same time using two separate plates each with its own standard curve. The protein content in each sample was assayed using a Bio-Rad Protein Assay for Microtiter Plates (Bio-Rad Laboratories). The linear range of this assay is 0.05 mg/ml–1.5 mg/ml. All GnRH-I values were corrected for the amount of protein present in each sample and reported as pg GnRH-I/μg of protein.

LH responses to GnRH-I challenge

Experimental design. Castrated WT (n = 9) and ERβKO (n = 8) males received a single injection of GnRH-I (Sigma; 1 mg/kg; ip) between 1600 and 1700 h. This dose of GnRH-I was selected because it reliably results in an increase in plasma LH as compared to saline treated controls (Rissman, unpublished data). Forty-five minutes after the injection, the animals were anesthetized and blood was collected and plasma frozen until assay.

Results

Loss of functional ERβ delays behavioral puberty

Wild-type males showed ejaculatory behavior at a significantly younger age as compared to ERβKO males [F(1, 18) = 12.57, P < 0.002; Fig. 1]. However the ages at which WT and ERβKO males show their first mount, mount with thrust, or mount with intromission [F(1, 18) = 0.13, 0.05, and 0.10, respectively] were the same. Sexual behaviors performed on the test when the male first displayed mounting behavior did not differ between WT and ERβKO males. Sexual behaviors performed on the test in which males ejaculated for the first time differed between WT and ERβKO males. ERβKO males took longer to ejaculate [F(1, 18) = 9.04, P < 0.008] and had longer mount-to-ejaculation intervals [F(1, 18) = 12.42, P < 0.003] and longer intromission to ejaculation latencies [F(1, 18) = 17.00, P < 0.0007] as compared with WT males (Table 1). In addition, ERβKO males displayed more mounts [F(1, 18) = 4.82, P < 0.05], more mounts with intromissions [F(1, 18) = 11.54, P < 0.003], and more total mounting behavior [F(1, 18) = 14.64, P < 0.002] as compared to WT males (Fig. 2A). The number of thrusts per mount bouts that include intromissions were not significantly different between the genotypes [F(1, 18) = 0.02]. Because ERβKO males took more time to attain an ejaculation than did WT males, sexual behavior data were normalized for the length of the behavior test. After this adjustment, ERβKO males still displayed a significantly increased number of mounts with intromissions [F(1, 18) = 5.02, P < 0.04] as compared to WT males (Fig. 2B). However, the number of mounts without thrusts or intromissions [F(1, 18) = 3.00] and total mounting behavior [F(1, 18) = 2.50] were no longer significantly different.

Peripubertal: testosterone and seminal vesicle weights

Peripubertal WT and ERβKO males (37–45 days of age) did not have significantly different concentrations of plasma T, but a tendency toward higher levels in ERβKO males was noted [0.335 ± 0.13 ng/ml (WT) vs 0.874 ± 0.4 ng/ml (ERβKO); F(1, 16) = 0.81]. However, there was a significant effect of genotype [F(1, 16) = 7.58, P < 0.01] on seminal vesicle weight [18.7 ± 4.2 mg (WT) vs 37.4 ± 4 mg (KO)].

Adult: genotype affects gonadotropin responses to castration

Castrated ERβKO males had higher levels of plasma LH postcastration than WT males (Fig. 3A). A significant effect of genotype [F(1, 40) = 20.09, P < 0.00007] and hormone

<table>
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<th>Table 1</th>
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<tr>
<td>Latencies (mean ± SEM; in minutes) to display sexual behavior on final sexual behavior test</td>
</tr>
<tr>
<td>WT</td>
</tr>
<tr>
<td>Mount latency</td>
</tr>
<tr>
<td>Thrust latency</td>
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<tr>
<td>Intromission latency</td>
</tr>
<tr>
<td>Ejaculation latency</td>
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<tr>
<td>Mount to ejaculation interval</td>
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<tr>
<td>Intromission to ejaculation interval</td>
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* Significantly different from WT male (P < 0.05).

**Fig. 1.** Mean (± SEM) day of age wild-type (n = 10) and ERβKO (n = 9) males mounted, thrusted, intromitted, and ejaculated. *ERβKO* males were significantly older at the time of ejaculation as compared with WT males (P < 0.05).
treatment \([F(1, 40) = 137.04, P < 0.000001]\) and a significant interaction between genotype and hormone treatment \([F(1, 40) = 14.96, P = 0.0004]\) was noted. Estradiol treatment significantly lowered LH in males of both genotypes \([P < 0.05]\).

Estradiol significantly reduced plasma FSH in males \([F(1, 38) = 24.60, P < 0.00002; \text{Fig. 3B}]\). No effect of genotype \([F(1, 38) = 1.84]\) and no interaction between the two factors \([F(1, 38) = 3.28, P = 0.079]\) was noted. Castrated, untreated males had significantly higher FSH than castrates given \(E_2\) \((P < 0.05)\) regardless of genotype.

No influence of genotype on GnRH-I

ProGnRH-ir neuronal number decreased with \(E_2\) treatment in the POA \([F(1, 29) = 8.11, P < 0.009]; \text{Table 2}\). There was no effect of genotype \([F(1, 29) = 2.90]\) and no interaction between genotype and hormone treatment \([F(1, 29) = 0.18]\). No significant effects were noted for proGnRH-ir cell numbers in any other area quantified.

Castrated WT and ERβKO males had equivalent concentrations of GnRH-I protein in each of the brain area examined (Table 2; \(P > 0.05\)). In addition, GnRH-I treatment resulted in equivalent concentrations of plasma LH in WT and ERβKO males \([4.2 \pm 0.36 \text{ ng/ml (WT)} \text{ vs } 4.23 \pm 0.67 \text{ ng/ml (ERβKO)}; P > 0.05]\).

Discussion

Our data demonstrate that ERβ is important for behavioral pubertal maturation. Male mice lacking functional ERβ had delayed ejaculatory behavior, even though concentrations of T in plasma were not significantly different. In adults, LH concentrations 3 weeks after castration were higher in ERβKO than in WT males. In contrast, no genotypic differences were observed in plasma FSH either before or after \(E_2\) administration. Finally none of the aspects of the GnRH-I system that we quantified differed between ERβKO and WT mice. Thus, lack of functional ERβ affected two different aspects of the HPG axis: onset of sexual behavior and pituitary LH responses to estrogen after castration.

The role of ERβ in male pubertal development

Males lacking functional ERβ showed impaired sexual behavior, requiring more test trials to display complete masculine sexual behavior, which included ejaculation. In
addition, when comparing data from the final behavior test, ER\textsuperscript{KO} males displayed more mounts, more mounts with intromissions, and more total mounting behavior and they took a significantly longer to ejaculate. It is unlikely that these results can be attributed to a deficit in the maturation of the glans penis in ER\textsuperscript{KO} males. Using methods described in rats (Korenbrot et al., 1977), we assessed retraction of the balanopreputial skinfold in ER\textsuperscript{KO} males starting at 38 days of age. Three of four males exhibited normal reflexive responses at this age and the last one was competent 2 days later. The majority of males of both genotypes tested in our study displayed intromission behavior by 42 days of age.

Taken together these results suggest that ER\textsuperscript{KO} males require significantly more stimulation in order to achieve an ejaculation, yet their sexual motivation and arousal are normal. Our data reveal that an earlier report of normal sexual behavior in adult ER\textsuperscript{KO} males may have been premature. In that study the majority (four of six) of WT and (five of nine) ER\textsuperscript{KO} males tested displayed intromissions during one of two 30-min tests, but only one male of each genotype displayed an ejaculation (Ogawa et al., 1997). At present we are examining sexual behavior in adult ER\textsuperscript{KO} and WT males to determine if the behavioral deficit revealed in pubertal males is also apparent in adults.

In our study males received repeated exposure to receptive females. This could have influenced the deficit in pubertal ER\textsuperscript{KO} males. For example, when ER\textsuperscript{KO} and WT males were tested twice between days 38 and 42 days of age, 70% of WT males but only 20% of ER\textsuperscript{KO} males ejaculated (Scordalakes et al., 2002). Yet even more striking is that the other 80% of ER\textsuperscript{KO} males failed to show any sexual behavior by 42 days of age. Thus, when tests began at a later age without repeated exposures to females, ER\textsuperscript{KO} males displayed even greater sexual behavioral deficits. Housing juvenile males with adult females has been shown to advance puberty in mice (Vandenbergh, 1971) and musk shrew (Rissman et al., 1990). In this study, exposure to a female began at 32 days of age. Olfactory cues from the female, which was reintroduced to the male every other day, could account for the equivalent onsets of mounting behavior between ER\textsuperscript{KO} and WT, yet this cues may be insufficient to compensate for the lack of ER\textbeta for normal ejaculatory behavior.

There are several potential mechanisms that could account for the delay of ejaculatory behavior. First, differences in circulating levels of T may account for differences in onset of mating. However, we failed to detect any differences in T concentrations between peripubertal WT and ER\textsuperscript{KO} males. In fact, we did note a trend for the opposite differences with ER\textsuperscript{KO} males having higher T levels than WT males. This transient difference has been reported previously (Nomura et al., 2002a).

A related mechanism that could account for this behavioral difference is variation in numbers of androgen receptor (AR) and/or ER\alpha in nuclei involved in male sexual behavior. These steroid receptors are found in many regions, including the POA, the bed nucleus of the stria terminalis (BNST), and the medial amygdala, and, in the case of ER\alpha and ER\beta, are often found within the same cells (Shughrue et al., 1998). In ER\textalpha male mice treated with equivalent concentrations of testosterone (T) in the BNST are reduced as compared with WT males (Wersinger et al., 1997). In ER\textsuperscript{KO} males, numbers of ER\alpha or ER\beta in several neural sites, including the POA, are not reduced by estradiol treatment as they are in brains of WT littermates (Temple et al., 2001). Thus it is possible that the expression and/or function of ER\alpha and/or AR are modified and downstream pathways that regulate sexual behavior similarly altered in ER\textsuperscript{KO} male brain.

Another potential mechanism is that (the) lack of functional ER\beta effects neuropeptide or neurotransmitter systems involved in male sexual behavior. The ER\beta is highly expressed in the paraventricular nucleus of the hypothalamus (PVN) (Shughrue et al., 1997a, 1997b; Chen et al., 1997; Mitra et al., 2003) Within the PVN a subset of the neurons containing either arginine vasopressin (AVP) or oxytocin (OT) also expressed ER\beta (Alves et al., 1998; Somponpun and Sladek, 2003). Both of these neuropeptides play a role in ejaculatory behavior (Hughes et al., 1987; Witt and Insel, 1994). Oxytocin mRNA in the PVN of both male and female ER\textsuperscript{KO} mice is reduced as compared with WT

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**Table 2**
Numbers (mean ± SEM) of Pro-GnRH-ir cells and GnRH-I content (pg/μg protein) in adult ER\textsuperscript{KO} and WT male mice

<table>
<thead>
<tr>
<th></th>
<th>ProGnRH-ir cell number</th>
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<th>GnRH-I content</th>
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<tbody>
<tr>
<td></td>
<td>OVLT</td>
<td>POA</td>
<td>Other FB areas</td>
<td>OVLT</td>
</tr>
<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrates</td>
<td>39.1 ± 3.6</td>
<td>32.9 ± 5.0*</td>
<td>30.9 ± 7.1</td>
<td>2.74 ± 0.9</td>
</tr>
<tr>
<td>Castrates + E\textsubscript{2}</td>
<td>31.6 ± 4.5</td>
<td>24.1 ± 2.8</td>
<td>30.6 ± 8.3</td>
<td></td>
</tr>
<tr>
<td>ER\textsuperscript{KO}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrates</td>
<td>39.5 ± 5.2</td>
<td>28.3 ± 4.1*</td>
<td>28.6 ± 5.9</td>
<td>1.76 ± 0.9</td>
</tr>
<tr>
<td>Castrates + E\textsubscript{2}</td>
<td>40.8 ± 6.2</td>
<td>16.5 ± 1.8</td>
<td>28.0 ± 4.7</td>
<td></td>
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</tbody>
</table>

* Significantly more Pro-GnRH-ir cells were present in the POA of castrated WT and ER\textsuperscript{KO} males that received no hormone replacement as compared with E\textsubscript{2} treatment (P < 0.05). No genotypic differences were observed in GnRH-I content. OVLT, organum vasculosum of the lamina terminalis; POA, preoptic area; other forebrain (FB) areas, medial septum/diagonal band of Broca and accessory olfactory bulb; MBH, medial basal hypothalamus.
controls (Nomura et al., 2002b; Patisaul et al., 2003). Vasopressin immunoreactivity in the lateral septum, but not in the medial amygdala, is significantly lower in ERαKO as compared with WT males (Scordalakes and Rissman, 2003). Perhaps ERβ regulates vasopression in the medial amygdala and POA.

In addition, serotonin containing neurons coexpress ERβ (Gundlah et al., 2001; Lu et al., 2001; Bethea et al., 2002). In females, ERβKO mice have reduced concentrations of serotonin in the POA (as measured by HPLC) and more 5-HT1A receptors in the amygdala as compared with their WT controls (Krezel et al., 2001; Imwalle et al., under review). At low levels of tetanic stimulation aimed at the basolateral amygdala, ERβKO males were more sensitive than WT males and thus displayed long-term potentiation (Krezel et al., 2001). Interestingly, the sexual phenotype described in adult 5-HT1B receptor knockout male mice is very similar to what we report here in peripubertal ERαKO males (Rodriguez-Manso et al., 2002). There is a large literature in humans showing that treatment with selective serotonin reuptake inhibitors (SSRIs) can have profound sexual side effects, one of the primary complaints in men (Rodriguez-Manso et al., 2002). There is a large literature in humans showing that treatment with selective serotonin reuptake inhibitors (SSRIs) can have profound sexual side effects, one of the primary complaints in men (Rodriguez-Manso et al., 2002). There is a large literature in humans showing that treatment with selective serotonin reuptake inhibitors (SSRIs) can have profound sexual side effects, one of the primary complaints in men (Rodriguez-Manso et al., 2002).

The role of ERβ in adult male HPG axis functioning

Despite recent reports that ERβ is colocalized with a significant proportion of GnRH-I neurons in both rats and mice (Hrabovszky et al., 2001; Herbison et al., 2001; Sharifi et al., 2002), and that ERβ mediates rapid estrogenic responses of GnRH-I neurons (Abraham, 2003), the role of ERβ in male reproduction remains questionable. Here we report that ERβKO males were similar to WT mice in all the HPG axis parameters that we examined except LH levels in response to release from negative feedback. ERβKO castrates had significantly higher levels of LH compared to E2-treated males. This suggests that ERβKO males could be more sensitive to the lack of T than WT males in adulthood. Yet, E2 treatment decreased FSH levels in plasma and resulted in fewer pro-GnRH-ir neurons in the POA for both genotypes. Likewise, lack of ERβ had no effect on GnRH-I content, and equivalent amounts of LH were released in males of both genotypes after a GnRH-I challenge. This suggests that the loss of ERβ has little consequence for adult male HPG axis regulation. More studies are necessary to determine the exact function of ERβ within GnRH-I neurons in adults and also at the time of puberty.

Summary

The data presented here elucidate a previously unreported role for ERβ in the regulation of male sexual behavior. We noted delayed ejaculatory behavior in ERβKO males around the time of puberty. In addition, once sexual activity was initiated, ERβKO males displayed significant differences in the timing of their sexual behavior, taking longer to ejaculate and requiring a greater number of mounts with intromissions before they did so as compared to WT littermates. This finding underscores the fact that even mutant mice reported to be fertile, as are the ERβKO males, may have significant and potentially important reproductive behavior phenotypes. Future studies will investigate the mechanism of ERβ action at the time of puberty and in adulthood to determine whether these effects are direct or whether they occur via interactions with ERα and/or neurotransmitter systems.

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