Disruption of pubertal onset by exogenous testosterone and estrogen in two species of rodents

TONI R. PAK, G. ROBERT LYNCH, D. MATTHEW ZIEGLER, JASON B. LUNDEN, AND PEI-SAN TSAI

Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado 80309

Submitted 9 August 2002; accepted in final form 7 September 2002

Pak, Toni R., G. Robert Lynch, D. Matthew Ziegler, Jason B. Lunden, and Pei-San Tsai. Disruption of pubertal onset by exogenous testosterone and estrogen in two species of rodents. Am J Physiol Endocrinol Metab 284: E206–E212, 2003.—The administration of adult physiological levels of testosterone (T) and 17β-estradiol (E2) to male Siberian hamsters was previously shown to delay the onset of puberty. To examine whether this is a response common to other rodents, we investigated whether exogenous steroids also alter the onset of puberty in Syrian hamsters and mice. Juvenile male Syrian hamsters and mice were implanted with Silastic capsules containing T, E2, or cholesterol control. After 15 days, plasma, pituitaries, and testes were processed for histological analysis or measurements of gonadotropins and circulating steroid hormones. T and E2 implants reduced testis mass and gonadotropin stores in both species and arrested spermatogenesis in Syrian hamsters. In contrast, spermatogenesis in mice was unaffected by T and only modestly affected by E2. Although E2 inhibited circulating follicle-stimulating hormone (FSH) in both species, T inhibited circulating FSH in mice only. Overall, our results demonstrate that the hypothalamic-pituitary-gonadal axis of each rodent species responds uniquely to T and E2 during the pubertal transition. Despite the highly varied effects of T and E2 in these two species, the ability of steroid hormones to alter the timing of puberty appears to be a feature common to many rodents.

testis; steroids; Syrian hamster; mice

Puberty is one of the major physiological events in the life cycle of mammals, and the precise timing of pubertal onset is critical for future reproductive success. We previously showed (16) that, in a highly photoperiodic seasonal breeder, the Siberian hamster (Phodopus sungorus), the onset of puberty can be halted by the administration of adult physiological levels of testosterone (T) and 17β-estradiol (E2) to the juveniles. The ability of steroid hormones to alter the timing of puberty in the Siberian hamster suggests that puberty is a plastic event in these animals and can be easily manipulated by exogenous factors. This hypothesis is supported by a previous report that puberty in the Siberian hamster can also be delayed by short-day photoperiod (9). This plasticity in the timing of puberty may be advantageous for ensuring that their sexual maturation coincides with the breeding season.

It is presently unclear whether the ability of steroid hormones to alter the timing of puberty is a phenomenon common to most rodent species or unique to seasonal breeders such as the Siberian hamster. In most mammals studied, there appears to be a marked decline in the sensitivity of the hypothalamic-pituitary-gonadal (HPG) axis to the negative feedback effects of gonadal steroids, such as T and E2, as the animal undergoes pubertal transition (6, 15, 19). It was thought that this decline in sensitivity might allow the HPG axis to function in adulthood under the influence of higher circulating levels of inhibitory gonadal steroids. That puberty in the Siberian hamster can be inhibited by adult physiological levels of T and E2 (16) suggests that this decline in sensitivity might not occur in the Siberian hamster. In fact, we (17) have previously shown that there is little change in the sensitivity of the HPG axis to the inhibitory effects of T in the male Siberian hamster during the pubertal transition. This raises the possibility that other rodents might be similarly sensitive to the inhibitory effects of gonadal steroids and susceptible to pubertal delay when given exogenous gonadal steroid hormones as juveniles.

In the present study, we examined the ability of T and E2 to delay puberty in rodents other than the Siberian hamster. We chose to study the laboratory mouse (Mus domesticus), a continuous breeder that responds poorly to seasonal cues such as changes in photoperiod, and the Syrian hamster (Mesocricetus auratus), a seasonal breeder highly responsive to photoperiodic cues as adults (20). We examined whether exogenous T and E2 also inhibited puberty and altered downstream reproductive parameters such as circulating follicle-stimulating hormone (FSH), pituitary stores of gonadotropins, and testicular morphology. Results from this study should allow us to determine whether the ability of T and E2 to suppress normal endocrine changes and gamete maturation during pu-
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berty is a phenomenon common to rodents other than the Siberian hamster. Furthermore, results from this study will lay the most fundamental groundwork for any future studies on steroidal modulation of pubertal development in male rodents.

MATERIALS AND METHODS

Animals

Male hybrid mice (C57BL/6J × DBA/2J) were raised from birth in our vivarium under a long-day photoperiod (16:8 light-dark) and weaned at 20 days of age. Adult Syrian hamsters (M. auratus) were purchased from Charles River Laboratories (Wilmington, MA) and bred under a long-day photoperiod. M. auratus hamsters were purchased from Charles River Laboratories (Wilmington, MA) and bred under a long-day photoperiod (16). After 15 days of treatment (35 days of age), animals were weighed and bled by retroorbital puncture under light halothane anesthesia. Plasma samples were stored at −20°C until assayed for FSH, T, or E2 by radioimmunoassays (RIAs). Animals were then killed by cervical dislocation, and their pituitaries and testes were removed for the measurement of either mass and histological analysis (testes) or gonadotropins (pituitaries).

RIAs

Plasma and tissue collection.

Plasma and tissue preparation. Blood samples were collected into heparinized tubes, centrifuged at 3,000 rpm for 8 min, and stored at −20°C. Pituitaries were immediately frozen on dry ice and stored at −70°C. Before the RIA procedure, pituitaries were sonicated in 500 µl of 0.1 M phosphate buffer and processed for the measurements of luteinizing hormone (LH) and FSH by RIA. Pituitary protein content was measured using the Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA).

FSH and LH RIAs. Detection of plasma and pituitary LH and FSH was performed as described previously (16), with the use of rat LH and FSH RIA kits provided by Dr. A. F. Parlow (National Institutes of Health National Pituitary Program). For the FSH RIA, rFSH-110, rFSH-RP2, and rFSH-S11 were used as the iodination stock, RIA standard, and antibody, respectively. For the LH RIA, rLH-110, rLH-RP3, and rLH-S11 were used as the iodination stock, RIA standard, and antibody, respectively. Limits of detection were 0.04 ng/tube for FSH and 0.07 ng/tube for LH. Intra- and interassay coefficients of variation were 6.6 and 8.9%, respectively, for the FSH RIA and 9.3 and 10.1% for the LH RIA.

T and E2 RIAs. T and E2 RIA kits were purchased from Diagnostic Systems Laboratories (Webster, TX). These RIA kits have been previously validated for the measurements of circulating T and E2 in mice (13). For Syrian hamsters, the validity of the T RIA was tested by the serial dilutions of unextracted adult plasma samples, which showed binding displacement completely parallel to the T standard. Because the T RIA kit yielded plasma hormone levels consistent with those reported previously for male Syrian hamsters (see Discussion), no additional validation was done. The E2 RIA was validated in our laboratory. Because of the presence of interfering substances in the plasma during E2 RIA, plasma samples were first extracted with diethyl ether according to the method described by Albers and Prishkolnik (1). Serial dilutions of the extracted plasma samples showed binding displacement completely parallel to that of the E2 standard. The recovery of the E2 standard in test plasma samples after ether extraction was >90%. For the T RIA, the intra- and interassay coefficients of variation were 8.5 and 8.7%, respectively. For the E2 RIA, the intra- and interassay coefficients of variation were 7.5 and 9.4%, respectively. The limits of detection were 80 and 10 pg/ml for T and E2, respectively. Some samples had insufficient volume, so only a single dose was assayed for those.

Histology

Testes from 35-day-old T-treated, E2-treated, and Ch-treated animals were immersion fixed in Bouin’s fixative for 18–24 h and then stored in 70% ethanol. Testes were dehydrated through a series of increasing concentrations of ethanol, defatted in Histoclear. Ten-micrometer sections were cut on a microtome, mounted on poly-l-lysine-coated slides, and stained with hematoxylin and eosin. For morphometric analysis, eight fields per section from a total of 12 sections per animal were scored. A minimum of five animals per treatment group was measured. Sections were all from the center of the testis, and only tubules cut in a cross section that appeared round were measured. General characteristics of cell types and appearance and seminiferous tubule diameters were recorded. Measurements were made using bright-field microscopy with a calibrated ocular micrometer.

Statistics

Differences among groups were analyzed using one-way ANOVA followed by Tukey’s honestly significant difference post hoc test when significant differences were detected. Comparisons between two groups were analyzed using Student’s t-test. The level of significance was defined as P < 0.05. All data are expressed as means ± SE.

RESULTS

Effects of T and E2 on Body Weight and Testis Mass

The effects of T and E2 on body weight of both mice and Syrian hamsters are shown in Table 1. E2 induced a significant reduction in the final and net gain of body weight in mice but not in hamsters. The T implant had no effect on body weight in either species (Table 1).

Chronic administration of T and E2 significantly reduced testis mass in both mice and Syrian hamsters (Fig. 1A). In mice, E2 exerted a greater inhibitory effect on testis mass than T. A similar trend was observed in the Syrian hamster, although this trend was not statistically significant (P = 0.06).

Effects of T and E2 on Testis Morphology

We also examined the effects of T and E2 treatments on testicular morphology. Although both T and E2
treatments significantly decreased testis mass in both species, the effects of these two steroid hormones on testicular morphology differed markedly between Syrian hamsters and mice. In the Syrian hamster, T and E2 treatments reduced interstitial space, resulting in very tightly packed seminiferous tubules (Fig. 2, B and C). In addition, steroid treatments induced the closure of the seminiferous tubule lumen and the pyknosis of a large population of Leydig cells (Fig. 2, B and C). In these animals, the diameters of seminiferous tubules were significantly reduced compared with controls, with E2 exerting a greater inhibitory effect than T (Fig. 1B). Last, T and E2 treatments markedly disrupted spermatogenesis in the Syrian hamster. Within the seminiferous tubules of T- and E2-treated animals, primary spermatocytes, spermatids, and sperm were noticeably absent (Figs. 2, B and C).

In mice, T and E2 also significantly reduced seminiferous tubule diameters, with E2 exerting a greater effect than T (Fig. 1B). However, the effects of T and E2 on testicular morphology were less pronounced in mice. Spermatogenesis did not appear affected in T-treated animals. Primary spermatocytes, spermatids, and mature sperm were still present in T-treated animals (Fig. 2E). On the other hand, E2-treated animals lacked mature sperm in the lumens, although primary spermatocytes and spermatids were present (Fig. 2F). As in the Syrian hamsters, pyknotic nuclei in Leydig cells were observed in mice treated with T and E2 (Figs. 2, E and F).

Effects of T and E2 on Plasma and Pituitary Gonadotropin Levels

T and E2 treatments differentially altered plasma FSH levels in the Syrian hamster and the mouse. In the Syrian hamster, plasma FSH was reduced to undetectable levels by E2 treatment, whereas T had no effect (Fig. 3A). In contrast, T and E2 reduced plasma FSH levels equally in the mouse (Fig. 3A). Both T and E2 treatments significantly reduced pituitary FSH and LH concentrations in both species (Fig. 3, B and C). In both species, there was a trend toward a greater inhibition of pituitary FSH and LH concentrations by E2 compared with T, although this trend was not significant (P = 0.06; Fig. 3, B and C).

Effects of T and E2 on Circulating Gonadal Steroid Hormone Levels

Plasma T and E2 were measured to determine how implants affected circulating levels of gonadal steroid hormones. T implant significantly elevated circulating T in mice but not in Syrian hamsters (Fig. 4A). Because there was insufficient plasma from Ch-implanted mice and Syrian hamsters to measure both T and E2, plasma samples from normal 35-day-old mice and Syrian hamsters (n = 5 for each) were used instead of Ch-implanted controls for the measurement of circulating E2. Compared with age-matched, nonimplanted controls, circulating E2 was significantly elevated in E2-implanted mice but not in Syrian hamsters (Fig. 4B).

DISCUSSION

Although Syrian hamsters and mice displayed some differences in their responsiveness to exogenous T and E2, there was a general inhibition in most reproductive parameters studied in both species. These observations suggest that the ability of exogenous gonadal steroids to alter pubertal onset is not unique to the Siberian hamster. Our results also showed that the final negative impact of T and E2 upon spermatogenesis and testicular maturation was less pronounced in mice.
These observations, along with data showing different steroid-induced changes in circulating FSH between these two species, indicate that the pathways taken by steroids to inhibit puberty might be different between the hamster and mouse. Interestingly, puberty and seasonal reproductive recrudescence were found to share similar endocrine changes (2), such as declining sensitivity to inhibitory steroid hormones (4, 5, 11, 23) and the requirement for high levels of FSH to initiate spermatogenesis (23, 27). Whether or not the species differences observed here can be attributed to the fundamental difference between a seasonal and a nonseasonal breeder remains an interesting avenue to explore.

T treatment severely disrupted spermatogenesis in the Syrian hamster despite normal circulating FSH levels. With T treatment, seminiferous tubule diameter was significantly reduced and spermatogenesis effectively disrupted (Figs. 1B and 2B). This result was unexpected because the progression of spermatogenesis is typically closely correlated with circulating levels of FSH, as has been shown in Siberian hamsters (16). It is possible that T treatment in the Syrian hamster resulted in a reduction of intratesticular T, thought to be important for testicular maintenance in a few species (12, 22, 25, 26, 28), leading to the deterioration of germ cells in the testes. This has been shown previously in adult rats where chronically administered T
and E2 reduced ITT levels and arrested spermatogenesis (28). Overall, our data showed that there is a dissociation between circulating levels of FSH and normal spermatogenesis in the Syrian hamster, suggesting that FSH alone may be insufficient for successful spermatogenesis in this species.

Interestingly, the disruption in spermatogenesis by T and E2 was less pronounced in mice compared with Syrian hamsters. Although T induced prominent abnormalities in testicular morphology, numerous primary spermatocytes and mature sperm remained in the testes of T-treated animals (Fig. 2E), suggesting that spermatogenesis is still ongoing. In E2-treated testes, primary spermatocytes and spermatids were still present, although the presence of mature sperm in the lumens was noticeably lacking (Fig. 2F), indicating that the defect might be at the level of spermiogenesis and not spermatogenesis. Regardless, spermatogenesis took place in T-treated mice with markedly reduced circulating FSH and pituitary gonadotropin stores. This observation suggests that spermatogenesis in mice can also be dissociated from circulating FSH if sufficient T is present. This conclusion is supported by Handelsman et al. (7), who reported that T administration to mutant hypogonadal (hpg) mice initiated and maintained spermatogenesis despite the inherent absence of circulating gonadotropins.

Both T and E2 exerted inhibitory effects on the majority of reproductive parameters measured in this study, with the effect of E2 being significantly more potent than T on testis mass and seminiferous tubule diameter in mice and circulating FSH and seminiferous tubule diameter in Syrian hamsters. The greater potency of E2 in general suggests that the action of T might be mediated, in part, through its aromatization to E2. These results contrast markedly with the results from the Siberian hamster, a species that displays pronounced differences in their responsiveness to T and E2 (16, 17).

Both T and E2 implants significantly elevated circulating T and E2 in mice but not in Syrian hamsters. This discrepancy likely stemmed from implanting the
same size capsule into the larger Syrian hamster. Although implanted Syrian hamsters were exposed to lower levels of steroids from the capsules, the inhibitory effects of T and E2 on testicular morphology were actually more pronounced in Syrian hamsters than in mice. Therefore, the HPG axis of the Syrian hamster might be more sensitive to the inhibitory actions of the gonadal steroids than that of mice. Another explanation for the failure of steroid implants to elevate circulating steroids in the Syrian hamster is that exogenous T and E2 might have a greater inhibitory effect on the production of endogenous T and E2 in these hamsters, resulting in the lack of an overall change in their circulating steroids. This hypothesis also supports the notion that Syrian hamsters are more sensitive to the inhibitory effects of T and E2 than mice. Regardless, the consistent disruption of downstream reproductive parameters in the Syrian hamster by both T and E2 implants attests to the functionality of implants despite the lack of a clear elevation in the circulating steroid hormones.

The levels of circulating steroids in our control mice and Syrian hamsters were consistent with the range previously reported for these two species (10, 13, 21). Our T-implanted mice and Syrian hamsters also had circulating T well within the range reported for intact adult male mice (8, 13) and Syrian hamsters (21). Our T-implant regimen was therefore successful in maintaining circulating T at levels comparable to, but not exceeding, adult physiological levels. E2 implants, however, elevated circulating E2 to levels approximately five times higher than those observed in normal adult male mice (13). This high circulating E2 likely led to a reduction in weight gain in mice (see Table 1), as has been documented previously (3, 18). The E2-induced weight loss was thought to be mediated through both central and peripheral targets (14, 18) and occurred when circulating E2 exceeded a certain threshold level (3). In Syrian hamsters, circulating levels of E2 in both control and E2-implanted males were similar to the values reported for gonadectomized males supplemented with T (1).

Results from the present study and our previous report on Siberian hamsters (16) underscore the presence of large species-related variations in how the HPG axis responds to gonadal steroids. For instance, although T had no effect on circulating FSH in the Syrian hamster, it significantly reduced circulating FSH in the Siberian hamster and mouse (Fig. 3A; Ref. 16). Conversely, E2 treatment significantly suppressed plasma FSH in the Syrian hamster and mouse but had no effect in the Siberian hamster (Fig. 3A; Ref. 16). Furthermore, although both T and E2 treatments significantly reduced pituitary FSH stores in mice and Syrian hamsters, T had no effect in Siberian hamsters (Fig. 3B; Ref. 16). Finally, one of the most pronounced species-related differences in steroidal effects was observed at the level of testes. T potently disrupted spermatogenesis in both Syrian and Siberian hamsters but not in mice (Fig. 2; Ref. 16). E2, on the other hand, potently inhibited spermatogenesis in the Syrian hamster but appeared to primarily alter spermiogenesis in mice and Siberian hamsters (Fig. 2; Ref. 16). Although no clear trends emerged from these data regarding how and why each species responded a certain way to T and E2, we believe these species differences might be partially attributed to the targeting of steroid hormones along different sites of the HPG axis, differential actions of these steroids directly upon the testes, and the different rates at which these species undergo pubertal transition.

To our knowledge, the present study is the first to provide comparative analyses on how E2 alters the male pubertal transition in multiple species of mammals. This type of analysis is particularly important in view of a major concern raised in the past decade regarding the negative impact of environmental estrogens on the reproductive health of vertebrates (reviewed in Ref. 25). Our results clearly showed that, in both rodent species studied, the effects of E2 were more deleterious than the effects of T. This observation attests to the remarkable ability of an estrogen to consistently interfere with reproductive function in multiple mammalian species even well after birth and beyond the neonatal period. This is an alarming phenomenon with potentially far-reaching implications for human reproductive health and warrants further investigations.

At present, the precise mechanisms utilized by T and E2 to disrupt puberty in mice and Syrian hamsters are not clear. Both steroid hormones may have simply acted on the gonad to directly inhibit testicular growth and sperm production. Alternatively, both steroid hormones may have delayed puberty by halting the puberty-associated neuroendocrine shift needed to reduce the sensitivity of the HPG axis to the negative feedback effects of steroid hormones. This is an important question that, unfortunately, cannot be definitively answered in the present study. Our observation that T and E2 could exert inhibitory effects on spermatogenesis independently of their effects on circulating FSH levels further complicates the effort to parse out these two possible mechanisms. In view of the highly varied effects of T and E2 on the HPG axis of each rodent, we hypothesize that the mechanism of pubertal disruption might be, again, highly species and hormone dependent, involving possibly a combination of both mechanisms.

This study provides important comparative information on the differential alteration of puberty by gonadal steroid hormones in two rodent species. Data obtained previously from the Siberian hamster (16, 17), along with results from this study, suggest that the HPG axis of each rodent species responds uniquely to T and E2 during the pubertal transition. Moreover, the ability of T and E2 to delay the progression of puberty is not a phenomenon unique to the Siberian hamster. That said, it is important to note that the extent to which pubertal onset was disrupted varied greatly among the Siberian hamster, Syrian hamster, and mouse. Overall, T and E2 disrupted puberty most potently in the Syrian hamster, with both steroids capable of com-
pletely halting spermatogenesis. The least potent effect was observed in the mice, with spermatogenesis still taking place under both T and E₂ treatments. In sum, we believe that this study has laid the most fundamental groundwork for future studies on how the HPG axis of different rodents responds to sex steroid hormones around the time of pubertal onset. Furthermore, these data clearly speak to the highly varied nature of the HPG axis during the pubertal transition and the danger of extrapolating results from “model” rodent species.

This work was supported in part by National Science Foundation Grant IBN-9996398 to P.-S. Tsai and a research grant from the Council on Research and Creative Work at the University of Colorado to G. R. Lynch.

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