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Photoperiodic Regulation of Compensatory Testicular Hypertrophy in Hamsters[1]

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ABSTRACT

In mammals, removal of one testis results in compensatory testicular hypertrophy (CTH) of the remaining gonad. Although CTH is ubiquitous among juveniles of many species, laboratory rats, laboratory mice, and humans unilaterally castrated in adulthood fail to display CTH. We documented CTH in pre- and postpubertally hemi-castrated Syrian and Siberian hamsters and tested whether day length affects CTH in juvenile and adult Siberian hamsters. Robust CTH was evident in long-day hemi-castrates of both species and was preceded by increased serum FSH concentrations in juvenile Siberian hamsters. In sharp contrast, CTH was undetectable in short-day hemi-castrated Siberian hamsters for several months and only made its appearance with the development of neuroendocrine refractoriness to short day lengths; serum FSH concentrations of juveniles also did not increase above sham-castrate values until the onset of refractoriness. Long-day hemi-castrated Siberian hamsters with hypertrophied testes underwent complete gonadal regression after transfer to short days, albeit at a reduced rate for the first 3 weeks of treatment. Blood testosterone concentrations of adult hamsters did not differ between long-day hemicastrates and sham-castrates 9–12 weeks after surgery. We conclude that CTH is suppressed by short day lengths in Siberian hamsters at all ages and stages of reproductive development; in short day lengths, but not long day lengths, the remaining testis produces sufficient negative feedback inhibition to restrain FSH hypersecretion and prevent CTH.

compensatory testicular hypertrophy, follicle-stimulating hormone, inhibin, photoperiodism, reproductive axis, seasonal reproduction, testis, testosterone, unilateral castration

INTRODUCTION

Removal of a single testis results in significant hypertrophy of the remaining gonad in several mammalian species (rats: 1, bulls: 2, rams: 3, boars: 4, stallions: 5, dogs: 6, bonnet monkeys: 7, rhesus monkeys: 8, rabbits, badgers, and field voles: cited in 9, guinea pigs: 10, rice rats: 11). This compensatory testicular hypertrophy (CTH) is the result of increased size and number of germ cells as well as increased size of Sertoli cells [4, 7–9, 12–14].

Decreased inhibin negative feedback on FSH secretion after hemi-castration appears to mediate CTH. Hemi-castration results in increased FSH, but not LH secretion [3, 15, 16]. Administration of inhibin-rich porcine follicular fluid to prepubertal hemi-castrated rats prevents FSH hypersecretion and CTH [17]. Active immunization of sheep against inhibin resulted in increased scrotal circumference [18]. Finally, reductions in blood inhibin concentrations after unilateral castration persist for many days, whereas the decrease in testosterone secretion persists for no more than 6–8 hours in unilaterally castrated rats and Syrian hamsters [19, 20].

Short day lengths (SD) induce marked increases in negative feedback sensitivity of the hypothalamic-pituitary axis of hamsters to gonadal steroids; much lower concentrations of testosterone, dihydrotestosterone, or estradiol are sufficient to suppress gonadotropin secretion after bilateral castration in SD Syrian hamsters than are needed in LD hamsters [21–24].

Plasma inhibin concentrations of Syrian hamsters transferred from long to short day lengths decrease in parallel with testicular involution and reach nadir concentrations in 6–10 weeks [25, 26]. Injections of inhibin antiserum are associated with elevated plasma FSH concentrations in LD hamsters [19], but this response is attenuated in SDs [reviewed in 25]. The role of inhibin in restraining FSH secretion in the latter stages of short-day induced testicular regression was discounted by Kirby and colleagues [25]. Thus in SDs, Syrian hamsters seem to rely solely on the increased steroid negative feedback sensitivity to restrain FSH secretion.

Few studies have assessed CTH in seasonal mammals. Using indirect surgical procedures, one study raised the possibility that the neuroendocrine axis of short-day Syrian hamsters might be incompatible with CTH [27]. In the marsh rice rat, CTH was prevented in animals housed in short but not long days [11], but the role of reproductive hormones was not assessed. These studies raise the possibility that SD-induced changes in steroid and inhibin feedback render the hormonal output of a single SD testis sufficient to restrain FSH secretion and thus prevent CTH. In rams, however, non-breeding photoperiods do not prevent CTH [9].

In the present study, we characterized the CTH phenomenon in juvenile and adult Siberian and Syrian hamsters and assessed the impact of day length on CTH and reproductive hormone secretion (FSH and testosterone) in unilaterally castrated Siberian hamsters.

MATERIALS AND METHODS

Animals and Housing Conditions

Siberian hamsters (*Phodopus sungorus*) from our breeding colony were derived from animals originally provided by Bruce Goldman (University of Connecticut) and later outbred to hamsters received from Katherine Wynne-Edwards (Queen's University). The breeding colony was maintained on an LD photoperiod (14L; lights off at 1800 h PST). For experiments on juvenile

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Siberian hamsters, adult females and males were paired and either maintained in the same 14L photocycle or immediately transferred to a SD photocycle (10L; lights on at 0800 h PST). Offspring were weaned at 20 (± 1) days of age; only the males were used in the current study. Syrian hamsters (*Mesocricetus auratus HsdHan:AURA*) purchased from Harlan (Indianapolis, Indiana) were maintained on an LD photoperiod (14L, lights off at 1600 h PST). These hamsters were bred in our laboratory, and their progeny used as subjects. Tap water and Purina Rodent Chow 5015 and Lab Diet Prolab 5P00 were available ad libitum for all Siberian and Syrian hamsters, respectively. Hamsters were housed at 23 ± 1°C in polypropylene cages (Siberian hamsters: 25 × 14 × 12 cm, 1–3 per cage, Syrian hamsters: 48 × 25 × 21 cm, 1–2 per cage) furnished with Tek-Fresh Lab Animal Bedding (Harlan Teklad, Madison, WI). Hamsters were fitted with ear tags for individual identification. Siberian and Syrian hamsters were considered to be juveniles until 60 days of age, by which age testicular development had been completed by long-day animals of both species [28, 29].

All procedures were approved by the Animal Care and Use Committee of the University of California at Berkeley and were in accordance with the National Research Council publication *Guide for Care and Use of Laboratory Animals*.

Surgeries

Surgeries were performed under deep anesthesia induced with isoflurane vapors. The left testis was externalized through an abdominal incision left of the midline. In unilateral castrates the testicular vein was ligated, and the testis and epididymis were removed. The remaining tissue was replaced, and the incision closed with sterile surgical sutures. The wound was treated with antibiotic ointment, and animals were given Buprenorphine (0.05, 0.1, 0.1, and 0.2 ml of 0.015 mg/ml s.c. for juvenile Siberian, adult Siberian, juvenile Syrian, and adult Syrian hamsters, respectively) as a postoperative analgesic. Sham-castrates underwent identical procedures, except the testicular vein was not ligated and no tissue was removed. Un-operated animals were anesthetized with isoflurane vapors but not subjected to surgical manipulation.

Testis Measures

Right testis weight. At killing, hamsters were anesthetized with isoflurane vapors, and the right testis was exposed, removed, cleared of extraneous tissue, and weighed on an analytical balance.

Estimated testis volume. Hamsters were anesthetized with isoflurane vapors, and the length and width of the right testis were measured externally (± 0.1 mm) with calipers by an experimenter unaware of the experimental treatment. The product $(\text{testis width})^2$ x (length) is highly correlated with paired testis weight [30] and was used as a measure of estimated testis volume (ETV). This procedure permitted repeated non-invasive measurement of testis size, and thus the full profile of testicular development was documented in individual hamsters.

Blood Sampling

Hamsters were anesthetized with isoflurane vapors, and approximately 0.6 ml of blood was withdrawn from the retro-orbital plexus into non-heparinized Natelson collection tubes. Blood was transferred to 1.5 ml centrifuge tubes and placed on ice for ~ 3 h. Samples were then centrifuged at 2600 rpm for ~ 20 min. The supernatant was transferred to a clean centrifuge tube and stored at −80°C until assayed.

Radioimmunoassay

Serum FSH concentrations were determined in duplicate in a single assay with rFSH RP-3 as standard, and anti-rat FSH 11 antibody (NIDDK, Rockville, MD), as previously validated [31, 32]. The lower limit of detection was 1.0 ng/ml and the intra-assay coefficient of variation was 10.7%.

Serum testosterone concentrations were determined in a single assay using a solid-phase ^{125}I radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX), previously validated in our laboratory [33]. Samples were divided into duplicate 50 µl aliquots and incubated with tracer for 1 h at 37°C. Cross-reaction of the antibody with 5α-dihydrotestosterone and the lower limit of hormone detection for this kit were 5.8% and 0.08 ng/ml, respectively. The intra-assay coefficient of variation was 7.4%.

Experimental Procedures

Experiment 1a: Ontogeny of CTH in Siberian hamsters. One hundred twenty-two males housed in a 14L photoperiod underwent unilateral castration or sham surgery at 20, 30, 60, 90, or 180 days of age. An additional un-operated group was anesthetized at 20 days of age. Six weeks later, right testis weight (RTW) and body mass (BM) were recorded.

Experiment 1b: Ontogeny of CTH in Syrian hamsters. Hamsters born in our laboratory to Harlan stock were housed in 14L. At 25 days of age, 12 hamsters were unilaterally castrated (left testis removed), and the remainder underwent the sham-castration procedure. At 21 weeks of age, previously sham-castrated hamsters were either hemi-castrated (n = 13) or sham-operated (n = 10). The 3 groups thus formed were: Sham (sham surgery at wk 3 and wk 21), Hemi-Juvenile (hemi-castration at week 3), and Hemi-Adult (sham-castrated at wk 3 and hemi-castrated at wk 21). ETV and BM measures were recorded two days before the initial surgery and at 2-week intervals thereafter.

Experiment 2a: Juvenile Siberian hamsters – ETV. Sixty-seven hamsters gestated and maintained in either SDs (10L; n = 32) or LDs (14L; n = 35) were unilaterally castrated (hemi; left testis removed) or sham-operated (sham) at 20 (± 1) days of age. ETV and BM were recorded every 2 weeks for 26 weeks beginning at the time of surgery. LD hamsters that failed to develop the LD testicular phenotype (i.e., ETV < 400) by 6 weeks after surgery were removed from all analyses (n = 10; 6 sham and 4 hemi-castrate animals). SD hamsters that did not respond to the SD photoperiod (ETV > 200 at or before wk 8) were also excluded from all analyses (n = 3). Blood was withdrawn from the retro-orbital plexus between 3–4 h after light onset on days 0, 2, 14, 70, 98, 140, and 182 after surgery. To reduce the amount of blood taken from each hamster, individuals were bled a maximum of 4 times. The blood was processed and analyzed by radioimmunoassay (RIA) for FSH. By week 26, after all groups had undergone testicular development, the right testis was removed and weighed.

Experiment 2b: SD-Juvenile Siberian hamsters – RTW. ETV measures may not be sufficiently sensitive to detect small but significant increases in testicular size of SD-housed juvenile Siberian hamsters. Thus, we repeated the SD manipulations in a separate set of hamsters, but recorded testis weight, a more direct measure of testis size. Forty hamsters, gestated and kept in SDs (10L) thereafter, were unilaterally castrated (hemi; left testis removed; n = 14), sham operated (sham; n = 13), or left un-operated (anesthetized only; n = 13) at 20 (± 1) days of age. Six weeks later, RTW and BM were recorded.

Experiment 3a: CTH in photo-regressed adult Siberian hamsters. Forty adult hamsters (3–4 months of age) were transferred to a SD photocycle (10L; lights off at 1600 h PST). Eleven age-matched control hamsters remained in 14L. Nine weeks later most SD hamsters had fully regressed testes (ETV < 100; n = 25), whereas others with ETV > 400 were photo non-responders (n = 12). These distinct SD groups were designated SD regressed (SDreg) and SD non-responders (SDnr). Nonresponsiveness to SD photoperiods is common in a subset of Siberian hamster populations [e.g., 34]. Three hamsters with intermediate-sized testes (100 < ETV < 400) were removed from the study. The following day, hamsters underwent hemi-castration (hemi) or sham surgery. The resulting sample sizes were: LD-sham, n = 4; LD-hemi, n = 7; SDnr-sham, n = 5; SDnr-hemi, n = 7; SDreg-sham, n = 12; SDreg-hemi, n = 13. Five weeks later, RTW and BM were recorded.

Experiment 3b: Testicular regression in adult Siberian hamsters with CTH. Adult LD hamsters (2–4 months of age) with fully developed gonads (ETV > 400) were unilaterally castrated (n = 34) or sham operated (n = 34). Seven weeks later a second ETV measure was determined for all animals to verify CTH in the unilateral castrates. Twenty-two hamsters in each surgical group were then transferred to SDs; the remaining hamsters were maintained in the LD photoperiod. Data from four hamsters that died during the experiment and from one LD hamster with a highly aberrant testicular pattern (testicular involution) were removed from the analyses. Non-responder hamsters (ETV > 400 nine weeks after transfer to SDs) constituted a separate SD group. The resulting sample sizes were: LD-hemi, n = 12; LD-sham, n = 9; SD-hemi, n = 15; SD-sham, n = 13; SDnr-hemi, n = 6; SDnr-sham, n = 7. ETV and BM were recorded at 3-week intervals until gonadal growth that culminated in complete testicular recrudescence was documented 39 weeks after transfer to SDs. Blood samples were drawn from the retro-orbital plexus 2–3 hours before the onset of darkness one day after ETV measures were recorded. One group of hamsters was first bled at wk 0, the second group at wk 3. Each group was then bled at alternating time-points. Testosterone concentrations were determined for the samples obtained 9 and 12 weeks after photoperiod transfer. At both time-points, most hemi- and sham-castrated hamsters housed in SDs had fully regressed testes, whereas CTH was evident in the LD hemi-castrates (see results).

Statistical Analyses

Differences in RTW and BM of Siberian hamsters in experiments 1a and 3a, were assessed using two-way ANOVA (Statview 5.0; Abacus Concepts, Berkeley, CA). A one-way repeated measures ANOVA was used to determine

TABLE 1. RTW (mg; mean ± SEM) of LD-housed Siberian hamsters 6 wk after hemicastration, sham surgery, or anesthetization only (Unop) at various ages.

Age at surgery (days)	Hemi (n)	Sham (n)	Unop (n)
20	423 ± 35** (12)	320 ± 12 (12)	326 ± 23 (12)
30	447 ± 28* (12)	288 ± 23 (10)	NA
60	430 ± 25* (12)	291 ± 26 (12)	NA
90	434 ± 24* (13)	315 ± 25 (12)	NA
180	445 ± 19* (12)	381 ± 20 (11)	NA

* Significantly greater than Sham controls ($P < 0.05$).
** Significantly greater than Sham and Unop controls ($P < 0.05$).

overall differences in ETV and BM for Syrian hamsters in experiment 1b. Testis and BM measures were analyzed using two-way repeated measures ANOVA in experiment 2a and a one-way ANOVA in experiment 2b. Because differences in BM were present prior to surgical manipulation in experiment 2b, percent change in BM was used to assess this trait. To assess differences in gonadal development between hemi- and sham-castrates in experiment 2a, rate of change of ETV per 2 weeks was calculated for time-points occurring around the time of substantial gonadal growth (wk 0–8 and wk 10–24 postsurgery for LD and SD juvenile groups, respectively), and data from hemi- and sham-castrates in each photoperiod were analyzed with *t*-tests. Presurgical FSH concentrations were analyzed using a one-way ANOVA. All other FSH data were assessed with two-way ANOVA. In experiment 3b, ETV and BM measures were split into three separate one-way repeated measures ANOVAs (LD, SDreg, and SDnr). The regression profile of the SDreg hamsters was analyzed by comparing the percent change in ETV using *t*-tests between hemi-castrates and sham-castrates at wk 3, 6, and 9. Rate of gonadal recrudescence in SDreg hamsters was assessed using rate of change of ETV per 3 weeks for wk 21–39, and data from hemi- and sham-castrates were analyzed with *t*-tests. Testosterone comparisons were made between hemi- and sham-castrates for each condition using *t*-tests. Because testosterone concentrations for each group did not differ at weeks 9 and 12, values from these time-points were combined to increase sample size. Analysis of the wk 9 and 12 time-points separately, however, did not change the outcome of the statistical tests. Post-hoc group comparisons were made using Fisher's PLSD where appropriate. For all analyses, significance was assumed if $P < 0.05$ and is reported as such even when actual values are lower.

RESULTS

Ontogeny of CTH in Siberian and Syrian Hamsters

In contrast to the majority of species tested [5, 7–10, 35, 36], humans, several strains of laboratory rats, and laboratory mice do not display CTH after postpubertal hemi-castration [1, 12, 37–39]. The adult reproductive system of the latter three species is minimally affected by seasonal changes in day length [40], and it has been suggested that persistence of the CTH response into adulthood is restricted to seasonal breeders [9]. To test the hypothesis that CTH is retained in seasonally breeding adult rodents, two hamster species whose reproductive cycles are regulated by day length were subjected to hemi-castration at different stages of reproductive development.

Experiment 1a: Ontogeny of CTH in Siberian Hamsters

Right testis weight. CTH was evident in all unilaterally castrated groups housed in long days regardless of age at surgery ($P < 0.05$; Table 1). Mean RTW ranged from 423 to 447 mg for hemi-castrates and from 288 to 381 mg for control hamsters. Sham surgery did not affect RTW, which did not differ between un-operated and sham-operated controls ($P > 0.05$).

Experiment 1b: Ontogeny of CTH in Syrian Hamsters

Estimated testis volume. ETVs were equivalent in all groups from wk 3 to 7 ($P > 0.05$; Fig. 1). CTH developed 6 weeks after unilateral castration in both the hemi-juvenile and hemi-adult hamsters (wk 9 and 27, respectively; $P < 0.05$) compared with sham-castrated hamsters. At week 27, ETVs were equivalent in males hemi-castrated as juveniles or adults ($P > 0.05$) and significantly greater than those of sham-operated controls ($P < 0.05$).

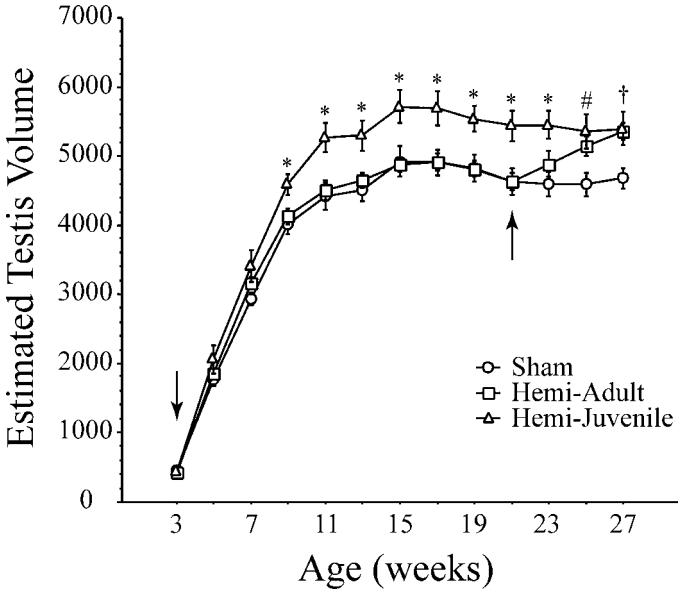

FIG. 1. Mean (± SEM) ETV of Syrian hamsters hemi-castrated at 3 wk of age (hemi-juvenile; downward arrow), hemi-castrated at 21 wk of age (hemi-adult; upward arrow), or sham-castrated at 3 and 21 wk of age (sham). Arrows indicate time of surgeries. Hemi-juvenile values (*) were significantly different from those of other groups. Hemi-juvenile and sham values (♯) differ significantly; hemi-adults did not differ from hemi-juveniles, but differed marginally from sham operates ($P = 0.05$). Sham group values (†) were significantly lower than those of hemi-adult and hemi-juvenile groups.

Effects of Photoperiod on CTH in Juvenile Siberian Hamsters

In the following experiments, we tested the hypothesis that day length modifies the CTH and FSH responses to unilateral castration. Initial experiments were conducted on juvenile Siberian hamsters. Gonadal growth trajectories differ markedly between Siberian hamsters housed in long versus short day lengths; puberty is delayed by ~ 2–3 months in the SD cohort [41]. Delayed development in SDs is likely achieved by delaying the decrease in steroid negative feedback sensitivity requisite for pubertal development in hamsters [29].

Experiment 2a: Juvenile Siberian Hamsters

Testis measures of long-day hamsters. CTH was evident in hemi-castrates 4 weeks after surgery (Fig. 2; day 28). The rate of testicular development was greater in hemi-castrated than in sham-operated hamsters between weeks 2 and 4 and weeks 4 and 6 after surgery ($P < 0.05$). Increased testicular volume persisted through the last time-point recorded. Twenty-six weeks after surgery (day 182), mean RTW of hemi-castrates was 146% of the value for sham-castrates ($P < 0.05$).

Testis measures of short-day hamsters. In contrast, the ETVs of SD hemi-castrates did not differ from those of SD-sham controls in the first several months after surgery (Fig. 2). At the onset of testicular development (weeks 12–14), the rate

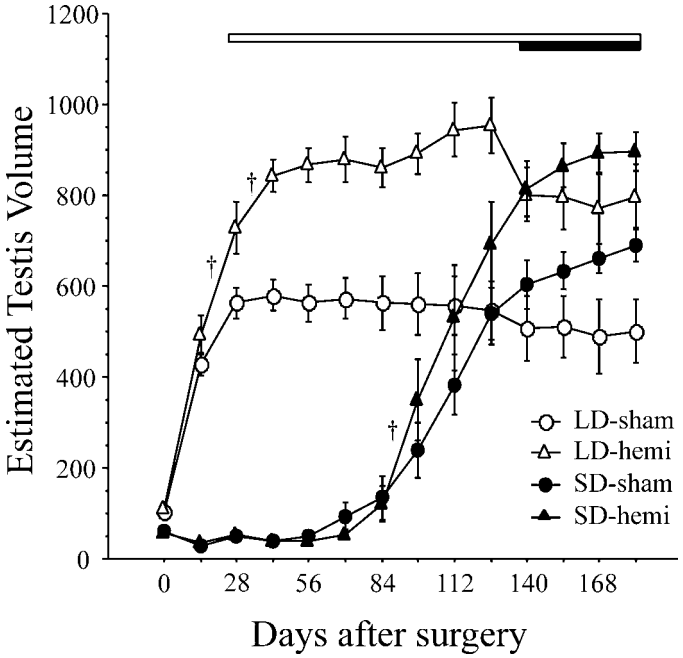

FIG. 2. Mean (± SEM) ETV of hemi-castrated (triangles) and sham-castrated (circles) hamsters gestated and reared in 14L (open symbols) or 10L (closed symbols). Surgery occurred at 20 (± 1) days of age and is designated day 0. Open and closed bars at the top of the graph indicate significant difference between hemi-castrated and sham-castrated groups for 14L and 10L treatments, respectively ($P < 0.05$). Significantly increased rate of testicular development (†) in hemi-castrates compared with sham-castrate controls ($P < 0.05$).

of testicular growth was greater in the hemi-castrated than sham-castrated males. This initial rapid increase, contributed to a significant difference in testis dimensions that became apparent at week 20 and persisted for the remainder of the experiment (mean hemi ETV > sham value during wk 20–26, $P < 0.05$). At autopsy in week 26, the RTW of hemi-castrates was 133% of the sham-control value ($P < 0.05$).

FSH concentrations: Overview. Serum FSH concentrations were higher in LD than SD hamsters preoperatively (day −1, $P < 0.05$; Fig. 3). FSH concentrations of LD-sham

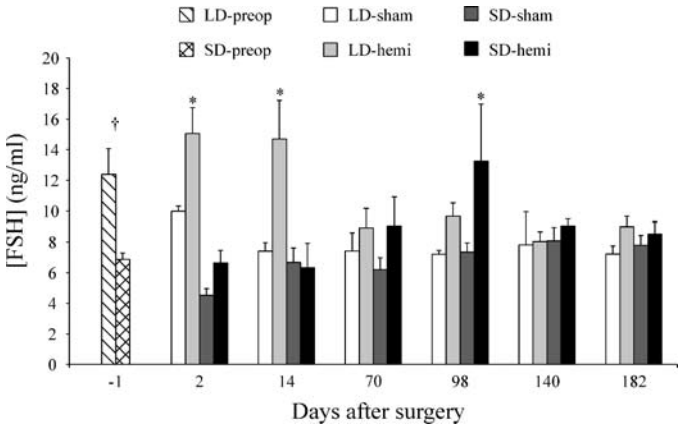

FIG. 3. Mean (+ SEM) FSH concentrations before and after hemi-castration (hemi) or sham surgery (sham) in hamsters gestated and reared in 14L or 10L (surgery at 20 ± 1 days of age = time 0). Significant difference (*) between hemi-castrates and surgical controls in the same photoperiod ($P < 0.05$). Significant difference (†) between LD and SD preoperative groups ($P < 0.05$).

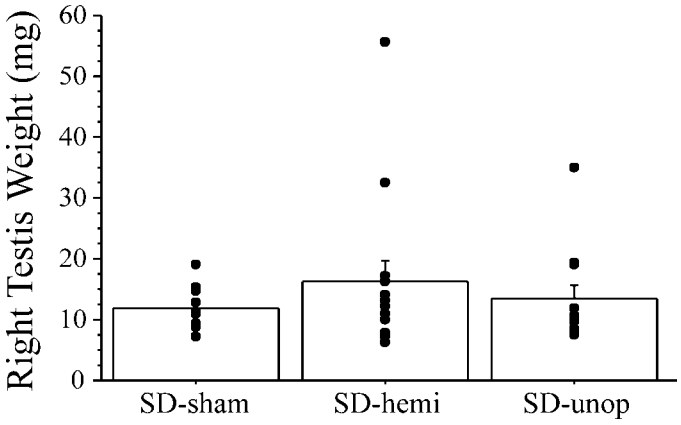

FIG. 4. Mean (+ SEM) RTW of hemi-castrated, sham-castrated, and unoperated hamsters gestated and reared in 10L. Surgery was at 20 (± 1) days of age and RTW was recorded 6 wk later. Points indicate values for individual hamsters. There were no differences between groups ($P > 0.05$).

hamsters declined over the next two weeks (days 2 and 14), as previously reported for peri-pubertal males [28].

FSH concentrations of long-day hamsters. Two days after surgery, FSH concentrations were significantly higher in hemi- than sham-castrated hamsters ($P < 0.05$; Fig. 3). This difference was maintained 14 days after castration, but was not statistically significant at any subsequent time-point (70, 98, 140, and 182 days after surgery).

FSH concentrations of short-day hamsters. In contrast to LD hamsters, serum FSH concentrations were not affected by hemi-castration in the first 2 weeks after surgery (days 2 and 14; Fig. 3). A nonsignificant trend toward increased FSH in the hemi-castrates on the second postoperative day was no longer evident by day 14. FSH concentrations of hemi-castrates were significantly elevated above control values only on day 98, which coincided with the early phase of testicular development (Fig. 2).

Experiment 2b: SD-Juvenile Siberian Hamsters

Testis measures in short-day housed hamsters. RTW, determined 6 weeks after surgery, did not differ among the groups ($P > 0.05$; Fig. 4) confirming the absence of CTH in SD juvenile males based on serial ETV measurements in experiment 2a.

Effects of Photoperiod on CTH in Adult Siberian Hamsters

Few studies have assessed the impact of photoperiod and seasonality on CTH of adult mammals. Some evidence suggests that SDs suppress CTH in adult Syrian hamsters and prairie dogs [27, 42], but this effect is not observed in adult rams [9]. Because of these contrasting results, and the fact that in some species the expression of CTH itself depends upon the age at hemicastration [1], we assessed the effects of photoperiod on CTH in postpubertal hamsters transferred to SD conditions 9 weeks before (experiment 3a) and 7 weeks after (experiment 3b) surgery. The goal was to determine whether the suppressive effects found in juveniles are also found in adults. In addition, to further characterize the reproductive hormonal response to unilateral castration and its regulation by photoperiod, blood testosterone concentrations were measured 16–19 weeks after hemi-castration in experiment 3b.

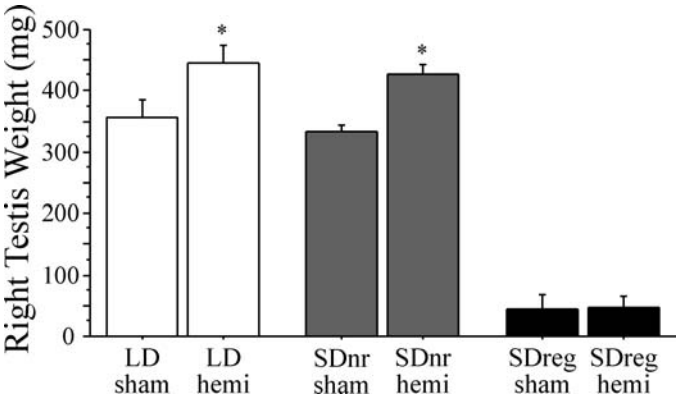

FIG. 5. Mean (+ SEM) RTW of hemi-castrated and sham-castrated adult Siberian hamsters maintained in 14L (long days, open bars) or transferred to 10L (short days, SD; shaded bars) for 14 wk. Surgery occurred after 9 wk of SD exposure, and right testis weight was recorded 5 wk later. SD hamsters were categorized into groups that did (SDreg, black bars) and did not (SDnr, gray bars) undergo testicular regression after 9 wk of SD exposure. *Indicates significant difference between hemi-castrates and corresponding sham-castrate controls.

Experiment 3a: CTH in Photo-Regressed Adult Siberian Hamsters

Right testis weight. At week 14, 5 weeks after surgery, the overall analysis documented a significant effect of surgery and condition (LD, SDreg, SDnr; $P < 0.05$). The interaction between these variables fell short of significance ($P = 0.10$) in the overall analysis, but post-hoc comparisons revealed a significant interaction between these variables. RTWs of LD and SDnr hemi-castrates exceeded those of their respective sham controls ($P < 0.05$; Fig. 5). In contrast, RTWs did not differ between SDreg hemi-castrates and the SDreg sham controls ($P > 0.05$; Fig. 5). Large variation in RTW values among all groups and the small sample sizes of the LD and SDnr sham groups may have accounted for the non-significant interaction effect in the overall ANOVA.

Experiment 3b: Testicular Regression in Adult Siberian Hamsters with CTH

Estimated testis volume. Hemi-castrated hamsters displayed CTH 7 weeks after surgery, before transfer to SDs (Fig. 6A-C, wk 0; $P < 0.05$ for all hemi vs. sham comparisons at wk 0). LD hemi-castrates displayed robust CTH at all postsurgical time-points (Fig. 6A).

The majority of hamsters transferred to SDs underwent testicular regression during the first 6 weeks after photoperiod transfer (SDreg; Fig. 6B). A subset, however, did not undergo gonadal involution in SDs (ETV > 400 at wk 9); data from these SD-non-responders (SDnr, Fig. 6C) were analyzed separately. The number of non-responders in the hemi-castrate and sham SD groups did not differ (n = 6 and 7, respectively).

After transfer to SDs, ETVs of the SDreg-hemi group were higher than those of the SDreg-sham hamsters at wk 3 ($P < 0.05$), but did not differ significantly from each other during wk 6–24 ($P > 0.05$), while the testes were regressed (Fig. 6B). Differences re-emerged with the onset of gonadal recrudescence (SDreg-hemi > SDreg-sham, $P < 0.05$ for wk 27–39; Fig. 6B). The rate of gonadal growth in the SDreg-hemi hamsters was significantly greater than that of the SDreg-sham animals from wk 24–27. ETVs of SDnr-hemi hamsters were significantly higher than those of their sham-controls at most postsurgical time-points (Fig. 6C). A trend toward elevated

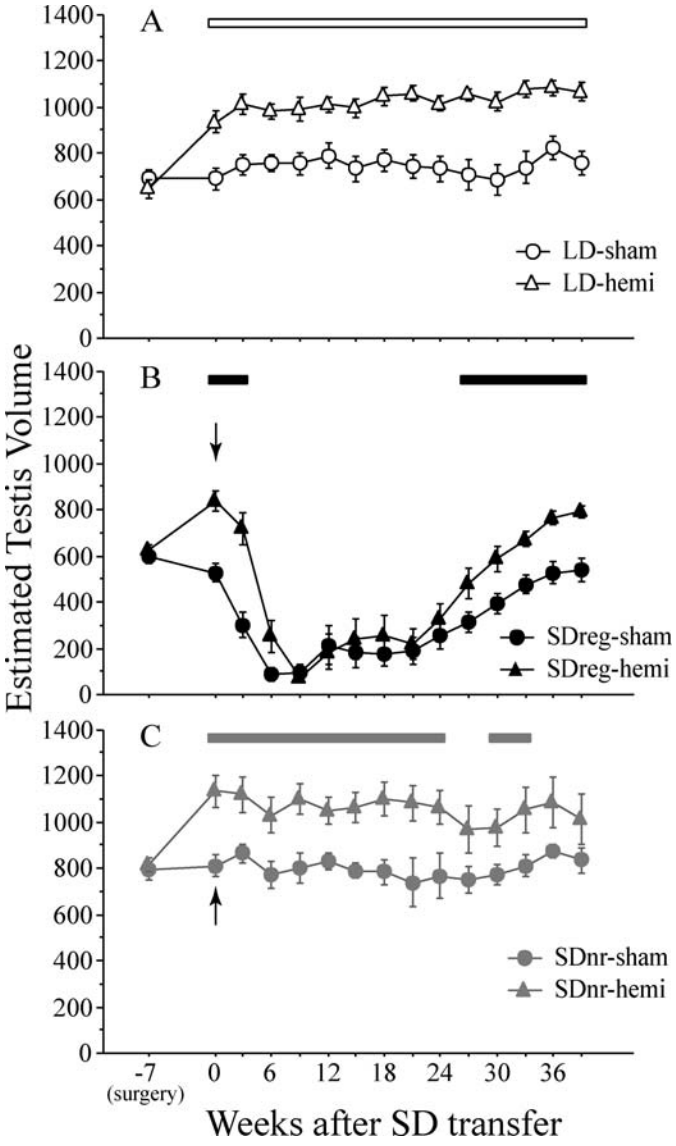

FIG. 6. Mean (± SEM) ETV of adult Siberian hamsters hemi-castrated or sham-castrated in 14L. Hamsters were either maintained in 14L (**A**) or transferred to 10L (SD; **B** and **C**) at week 0 (arrow indicates time of transfer to SDs). SDreg hamsters (black symbols) underwent testicular regression during 9 wk of SD treatment (**B**) whereas SDnr (gray symbols) did not (**C**). Open, black, and gray bars at the top of each panel indicate significant differences between hemi-castrated hamsters and their respective sham-operated controls.

ETVs in the SDnr-hemi hamsters was present at wk 27 and 36 ($0.09 > P > 0.05$).

The rate of testicular regression during the first 3 wk of SD treatment was greater in SDreg-sham than SDreg-hemi hamsters ($P < 05$; Fig. 6B). Statistical analysis of regression rate was abandoned for the later time-points because SDreg-sham hamsters were fully regressed by week 6; the absence of differences would likely be attributable to a floor effect. SDreg-sham animals reached nadir ETV values earlier than SDreg-hemi hamsters (wk 6 vs. wk 9), probably due to lower initial ETVs and more extensive testicular regression in the first 3 weeks of SD exposure ($P < 0.05$). Final nadir testicular dimensions did not differ between these groups; mean nadir ETVs (± SEM) for SDreg-hemi (wk 9) and SDreg-sham (wk 6) were 87.5 (± 26.6) and 69.3 (± 12.2), respectively.

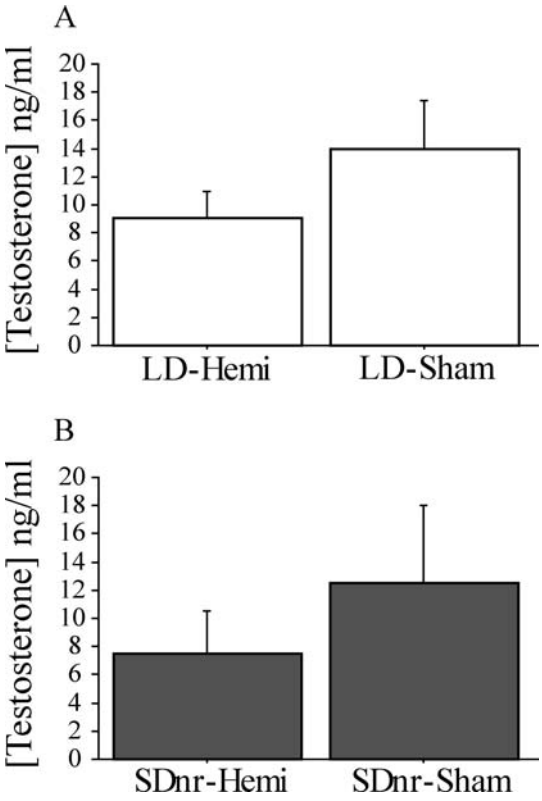

FIG. 7. Mean (+ SEM) serum testosterone concentrations of hemi- and sham-castrated LD (**A**) and SDnr hamsters (**B**). Blood samples were obtained 9 or 12 wk after photoperiod transfer. Sample means did not differ for wk 9 and 12 measures and were combined into a single analysis to increase sample size; collapsing across these time-points did not alter the outcome of statistical analyses. Final sample sizes were: LD-hemi, n = 12; LD-sham, n = 9; SDnr-hemi, n = 6; SDnr-sham, n = 7. Testosterone concentrations did not differ significantly between hemi and sham groups in either the LD ($P = 0.2$) or SDnr ($P = 0.47$) conditions.

Testosterone concentrations. Five SDreg hamsters that initiated gonadal recrudescence (ETV $>$ 200 followed by progressive increases the following weeks) before blood sampling were excluded from the analysis. Testosterone concentrations were below the limit of detection for 20 of the remaining 23 hamsters in the SDreg groups, which precluded evaluation of inter-group differences. For both LD and SDnr groups, testosterone concentrations of hemi- and sham-castrates did not differ significantly ($P > 0.05$ for each comparison; Fig. 7), although in each instance the values for the hemi-castrates were lower.

Body Mass

In each of the six experiments, unilateral castration had no effect on body mass.

DISCUSSION

Ontogeny of CTH in Siberian and Syrian Hamsters

Siberian and Syrian hamsters maintained in long day lengths display CTH after both pre- and postpubertal unilateral castration. A literature survey suggests that CTH is universal among prepubertal hemi-castrates [1–4, 6, 11]. CTH following unilateral castration in adulthood, however, is not manifested by all species: whereas most species tested do develop CTH after postpubertal hemi-castration [stallion: 5; bonnet monkey: 7; rhesus monkey: 8; rabbit, badger, and field vole: cited in 9; guinea pig: 10; ram: 35; bull: 36; Siberian and Syrian hamster: present investigation], humans, laboratory rats, and laboratory mice do not [1, 12, 37, 39]. The mechanism(s) that controls the adult reproductive axes of mice, rats, and humans evidently differs from that operative in many other mammals.

The occurrence of CTH in adult rams [35], guinea pigs [10], bulls [36], and stallions [5] indicates that the loss of CTH in adulthood is not an inevitable consequence of domestication. Documentation of CTH in adulthood for two seasonally breeding hamster species in the present study suggests that this phenomenon is characteristic of seasonal mammals [9], but confirmation of this conjecture awaits further comparative investigations. CTH may be an ancestral trait that is undetectable in adults of some mammalian species. For example, CTH is expressed in postpubertally gonadectomized rhesus and bonnet monkeys, but not humans [7, 8, 39], and in some, but not other, postpubertal rodents [1, 37, experiments 1a and 1b].

Effects of Photoperiod on CTH in Siberian Hamsters

CTH is absent in both juvenile and adult Siberian hamsters that exhibit the SD photoregressed phenotype. Similar findings have been reported in juvenile marsh rice rats [11] and may be characteristic of all photoperiodic rodents. Here we demonstrate that counteraction of CTH in SD Siberian hamsters is neither restricted to the prepubertal stage nor contingent upon unique restraint of FSH secretion prior to gonadal maturation. The prepubertal testis and the seasonally regressed gonad of adult male Siberian hamsters are morphologically similar [43] and under similar endocrine control: GnRH, LH, FSH, and testosterone concentrations are comparably low in both states. The absence of CTH in both juvenile and adult SD Siberian hamsters further indicates a similar neuroendocrine state with respect to the mechanism(s) governing CTH. We cannot rule out, however, that photoperiod acts through different mechanisms to regulate CTH in pre- and postpubertal hamsters.

Although the hemi-castrate hamsters that expressed the SD phenotype did not undergo CTH, testicular morphology may have been affected. Changes in Leydig cell size and testicular vasculature have been reported in adult hemi-castrated rats that do not exhibit CTH [44, 45]. Whether the SD testis of hemi-castrates that fails to undergo CTH is structurally equivalent to that of sham-castrates awaits future histological analysis.

The gonads of Siberian hamsters develop with the onset of refractoriness to SDs; both juvenile and adult SD hemi-castrates manifest accelerated rates of testicular growth compared with sham-operated controls. Testicular hypertrophy ensues several weeks later. In juveniles, markedly elevated FSH concentrations precede the development of CTH.

The duration of nocturnal pineal melatonin secretion provides day length information to the neuroendocrine axis of mammals [46]. Injections of melatonin 2 h before the onset of darkness suppress CTH in juvenile marsh rice rats, a photoperiodic, seasonally breeding rodent [11]. These data suggest that SD suppression of CTH, in common with other photoperiodic traits, is dependent on long duration nocturnal melatonin signals [46]. Siberian hamsters that failed to undergo testicular regression in SDs, unlike their photoperiodic counterparts, exhibited robust CTH and in this respect resembled LD hamsters. The absence of gonadal regression in SD nonresponsive Siberian hamsters is due to a circadian anomaly that truncates nocturnal melatonin secretion [47]; injections of melatonin induce the SD phenotype in such nonresponsive Siberian hamsters [48]. Thus, the expression of

CTH in SD nonresponsive hamsters is consistent with the suggestion that photoperiodic regulation of CTH is mediated via the duration of nocturnal melatonin secretion. We cannot rule out, however, postpineal contributions.

Hormonal Responses to Unilateral Castration in Siberian Hamsters

As in other species, the development of CTH in Siberian hamsters is preceded by significant and sustained increases in FSH secretion that appear to mediate CTH.

As in other mammals [6, 7, 16, 19, 20, 36], testosterone concentrations in LD and SD photo-nonresponsive, unilaterally castrated Siberian hamsters return to values typical of intact hamsters. The remaining testis increases testosterone secretion and in this respect compensates for the loss of the other gonad. This is consistent with the hypothesis that decreases in circulating inhibin, rather than testosterone concentrations, mediate increases in FSH and thus CTH after hemi-castration [15]. In Syrian hamsters and rats, the compensatory testosterone response occurs no later than 6–8 hours after hemi-castration [19, 20]. Testosterone concentrations for most SD hamsters with regressed testes were below the limit of detection; we therefore were unable to determine whether compensatory testosterone secretion occurs in these hemi-castrates, or whether SDs suppress this response.

A Possible Mechanism for Photoperiodic Regulation of CTH

In short but not long day lengths, secretions from the remaining testis prevent CTH. In Siberian hamsters, the SD testis remains responsive to FSH; exogenous FSH induces testis growth, re-establishes mature seminiferous tubules, and restores spermatogenesis as well as Sertoli cell nuclear structure and size in SD photoregressed Siberian hamsters [49–51]. These findings, in conjunction with the present demonstration that FSH concentrations are not elevated in SD hemi-castrates for the first few months after surgery, suggest that the absence of CTH in SD hamsters reflects the failure of hemi-castration to elicit FSH hypersecretion, rather than loss of testis responsiveness to FSH. Whether SD decreases in testis sensitivity to FSH contribute to the photoperiodic suppression of CTH is presently unknown.

Increased non-testicular restraint of FSH, decreased gonadotropin availability, or changes in hypothalamic GnRH in short day lengths could diminish dependence on testicular feedback and contribute to the absence of CTH. Although gonad-independent increases in FSH restraint [52], decreased pituitary FSH hormone and mRNA content [52, 53], and changes in the number of GnRH-ir cells [54] are characteristic of SD Siberian hamsters, FSH secretion in SD bilateral castrates is elevated to values above those of intact LD hamsters [52]. The neuroendocrine system of SD Siberian hamsters evidently contains sufficient hormone to stimulate gonadal growth. Some testicular factor(s) is required for the maintenance of typical low SD FSH concentrations in Siberian hamsters. The present studies indicate that a single SD testis produces this factor(s) in sufficient quantities to restrain FSH. Thus, the lack of CTH in SD hamsters likely reflects increased hypothalamic and/or pituitary sensitivity to testicular hormonal feedback.

We consider two possible hormonal mechanisms through which SDs suppress CTH. Persistent decreases in inhibin after hemi-castration are thought to trigger CTH [15]. Consequently, SD suppression of CTH may depend on 1) increased sensitivity of the SD pituitary to inhibin negative feedback or 2) decreased dependence on inhibin feedback in SDs. The former is congruent with increased sensitivity of SD pituitaries to Sertoli cell products in vitro [55], whereas the latter is compatible with the notion that increases in steroid negative feedback sensitivity [21, 22, 24] render inhibin feedback unnecessary in SDs [25]. Thus, the absence of CTH in SDs is likely due to either parallel increases in the sensitivity to steroid and inhibin negative feedback, or increased sensitivity to steroid negative feedback that renders inhibin superfluous for restraint of FSH. Future studies are required to determine the exact nature of this photoperiodic change.

Alternatively, the remaining testis of SD hemi-castrates may increase its secretion of inhibin to compensate for the loss of the other gonad. Because a reliable assay for blood inhibin in Siberian hamsters is not yet available [56], we were unable to evaluate this conjecture, but consider this a remote possibility; Sertoli cells, the primary cellular sites of inhibin synthesis in males [57], show morphological signs of inactivity upon gonadal regression [43].

Hemi-castrated Siberian hamsters with hypertrophied gonads underwent complete testicular regression upon transfer to SDs, but at a reduced rate during the first 3 weeks. Inhibin has been implicated in suppression of FSH during the initial, but not latter, stages of testicular involution of Syrian hamsters [25]. Thus, decreased inhibin in hemi-castrates would be expected to disrupt early, but not late, gonadal regression.

Neural projections play a major role in compensatory hypertrophy of the adrenal gland [58] and ovary [59] and the immediate compensatory testosterone secretion in hemi-castrates [60], raising the possibility of a similar neural component to CTH. Reports of neural and testicular asymmetry after unilateral orchidectomy are consistent with this conjecture [59, 61–63]. At present, however, neural contributions to CTH remain to be established.

In conclusion, seasonal changes in the hypothalamic-pituitary-gonadal axis affect expression of CTH, which is manifested in hamsters housed in long but not short day lengths. Increases in feedback sensitivity to gonadal factors are so pronounced in short day lengths that output from a single testis is sufficient to restrain FSH secretion and prevent CTH.

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