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Infrequent low dose testosterone treatment maintains male sexual behavior in Syrian hamsters

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Abstract

Testosterone (T) secreted in short pulses several times each day is essential for the maintenance of male sex behavior (MSB) in mammals. Blood T concentrations are relatively low during inter-pulse intervals. Assessment of androgenic influences on MSB of rodents has, with very few exceptions, involved either injections of pure or esterified hormones dissolved in oil or implantation of constant release capsules that generate supraphysiological and/or constantly elevated T concentrations. The minimum daily concentration of T necessary to maintain and restore MSB when T is delivered as a discrete short pulse remains unspecified; nor is it known whether infrequent T pulses in the physiological range sustain MSB. To address these questions, we varied T injection concentrations and frequencies in castrated, sexually-experienced Syrian hamsters. All males injected daily with an aqueous vehicle failed to display the ejaculatory reflex 5 weeks after castration. Once daily 15 µg subcutaneous T injections both maintained and restored MSB, whereas once daily 5 µg T injections resulted in fewer males ejaculating and longer ejaculation latencies. Substantially higher T doses were required to restore MSB in previous studies when T was administered in an oil vehicle. 50 µg T maintained MSB in most hamsters injected once every 4 or 7 days, despite long intervals between injections during which circulating T was undetectable or well below physiological concentrations. Some T regimens that maintained MSB were associated with subnormal seminal vesicle and ventral prostate weights. The demonstration that relatively brief, infrequent elevations of T are sufficient to support MSB provides a useful model to assess the neuroendocrine basis of MSB and raises the possibility that infrequent low dose androgen replacement protocols may restore sex behavior to hypogonadal men without inducing some of the negative side-effects associated with more frequent, higher dose treatments.

Keywords

Testosterone; Copulation; Male Syrian hamsters

Introduction

Male sex behavior (MSB) of mammals depends on gonadal androgen secretion (Meisel and Sachs, 1994; Hull et al., 2002, 2006; Hull and Dominguez, 2007); copulatory behavior declines

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in the absence of testosterone (T) and is restored with T replacement. T replenishment typically has been achieved in rodents by implanting constant release capsules or injecting steroid hormones dissolved in oil vehicles. In rats a single intramuscular injection of 100 µg testosterone propionate elevated T concentrations above the physiological range for 24 h; residual hormone was present in the blood as long as a week after injection (Keating and Tcholakian, 1983). Lingering effects of several days duration have also been reported in rats after a single subcutaneous administration of non-esterified T dissolved in soybean oil (Gerrity et al., 1982). The episodic ultradian rhythms of T secretion of intact rodents are not mimicked by replacement procedures that chronically elevate circulating hormone concentrations (capsules, Damassa et al., 1977) or injections that generate T concentrations well above the physiological range (73 ng/ml in a typical replacement regimen compared to 2 ng/ml in intact male Syrian hamsters; Arteaga-Silva et al., 2005). In mice and rats, T remains at low basal values except for several fleeting surges each day (Coquelin and Desjardins, 1982; Ellis and Desjardins, 1982, respectively). In Syrian hamsters, *Mesocricetus auratus*, T concentrations are elevated for 4 h in the late subjective day (Pieper and Lobocki, 2000). Chronic exposure to hormones in typical replacement paradigms may desensitize target tissues (Wolf et al., 1993) and influence receptor dynamics; low or hormone-free intervals, timing of hormone secretion, and hormone availability in target tissues are important considerations in deconstructing hormone-behavior relations. Duration and frequency of T secretion may be as salient as the amount of available T.

Park et al. (2007) infused Syrian hamsters daily with 100 µg T over 4 h or 50 µg T in each of two 4-hour infusions separated by 8 h. Both regimens maintained MSB despite rapid clearance of circulating T after infusions were terminated. The authors concluded that basal concentrations of T sustained by the gonads during inter-pulse intervals probably are unnecessary for maintenance of MSB. In the present study we employed subcutaneous injections of T dissolved in an aqueous vehicle to elevate blood T concentrations for only a few hours after each injection (e.g., Taylor et al., 1989, 1990). No exogenous treatment can exactly simulate endogenous patterns of T secretion, but this regimen appears to be more representative of the natural condition than those typically employed.

We sought to determine the lowest dose of T necessary to maintain and restore the ejaculatory reflex in castrated Syrian hamsters. Injection frequencies were manipulated to determine if MSB is sustained by infrequent treatments followed by long intervals during which T concentrations are either undetectable or well below physiological values. Answers to these questions may increase understanding of fundamental hormone-behavior relations.

Materials and methods

Animals

Syrian hamsters (*M. auratus*; HsdHan:Aura) obtained from Harlan (Indianapolis, IN) were maintained on a 14 L:10 D photoperiod (14 h light/day, lights off at 1600 h PST). Tap water and Lab Diet Prolab 5P00 were available ad libitum. Hamsters were singly housed at 23±1 °C in polypropylene cages (48×25×21 cm) furnished with Tek-Fresh Lab Animal Bedding (Harlan Teklab, Madison, WI). All procedures were approved by the Animal Care and Use Committee of the University of California at Berkeley.

Experimental procedure

Pre-screening for male sexual behavior—Adult male hamsters were screened for MSB during the late portion of the light phase (~1400–1600 h). The testing arena consisted of a clear Plexiglass box (41×21×21 cm) set above a slanted mirror to facilitate observation of intromissions and ejaculations. This apparatus was kept in the room in which males were

housed. After 10 min during which the male was acclimated to the apparatus, a sexually receptive female was introduced and MSB recorded. Males that ejaculated within 15 min on two consecutive tests separated by a week were considered sexually experienced and retained for the experiments. During pre-screening tests only the occurrence of the ejaculatory reflex and its latency were recorded. All observations were terminated after 15 min or after the male had ejaculated and displayed one subsequent intromission, whichever occurred first. Ovariectomized females were rendered sexually receptive with standard estradiol plus progesterone treatments (Park et al., 2007). A Silastic capsule (Dow Corning, Midland, MI, USA; 4 mm in length; ID 1.98 mm, OD 3.18 mm) filled with estradiol-17 β (Sigma, St. Louis, MO) and sealed with silicone adhesive, was implanted s.c. on the day of ovariectomy; behavioral estrus was induced by injecting females s.c. with 350 μ g progesterone (Sigma) dissolved in peanut oil (2.5 mg/ml) 4 h prior to the mating test. Most females were utilized for behavioral testing 1–2 times per week.

We recorded the number of mounts not accompanied by an intromission that preceded the first ejaculation, the number of intromissions that preceded ejaculation, latencies to the first mount, first intromission, and first ejaculation, and the duration of the post-ejaculatory interval (minutes between the ejaculation and the next intromission). Males that failed to ejaculate were assigned the maximum ejaculation latency of 15 min.

Surgical procedures

Sexually experienced males were anesthetized with isoflurane vapors (Baxter Healthcare, Deerfield, IL) and castrated through amidline incision in the abdominal cavity. Stimulus females were ovariectomized via a midline incision. Incisions were closed with sterile suture and wound clips (Mikron Auto Clip 9 mm, Becton Dickinson, Franklin Lakes, NJ). Hamsters were injected s.c. with the analgesic 5% buprenorphine (0.1 ml/animal), postoperatively (Hospira Inc., Lake Forest, IL).

Experiment 1

Phase 1—To determine the lowest dose necessary to maintain MSB, sexually experienced males were injected daily beginning on the day after castration with 0.2 ml of a 50% ethanol-distilled water vehicle that contained 0, 50, 100, 200, or 400 μ g T (Sigma) ($n=5$ per group except $n=4$ for hamsters that received 200 μ g T). Concentrations were chosen based on studies of Arteaga-Silva et al. (2005) who reported that 50 μ g T in oil was ineffective in restoring MSB in Syrian hamsters. Animals were tested for sexual behavior 2, 5 and 11 weeks post-castration approximately 5 h after that day's injection (see Fig. 1 for time line).

Phase 2—We observed that daily treatment with 50 μ g T maintained MSB in phase 1. We then assessed the effects on MSB of less frequent injections. Beginning on week 12 post-castration, all hamsters that had been injected with vehicle during phase 1 received 50 μ g T daily (restoration paradigm); the remaining hamsters were distributed evenly among groups and treated with 50 μ g T every 2, 4 or 7 days (maintenance paradigm). Hamsters were tested for sexual behavior 17 weeks post-castration, i.e., 5 weeks after phase 2 treatment began (Fig. 1). Sex tests occurred the day prior to their next scheduled injection.

Experiment 2

Phase 1—Because all T concentrations in experiment 1 sustained MSB we again sought to establish the threshold T dose sufficient to maintain MSB. A new group of sexually-experienced adult male hamsters, tested and castrated as in experiment 1, received either vehicle or 15 μ g T daily ($n=7$ and 11, respectively) for 5 weeks beginning the day after castration (maintenance paradigm). MSB was assessed after 2 and 5 weeks of treatment (Fig. 1).

Phase 2—At the end of 5 weeks of treatment, hamsters previously injected with the vehicle solution were treated with 15 µg T daily (restoration paradigm); those previously injected with 15 µg T daily transitioned to 5 µg T injections daily (maintenance paradigm). Hamsters were re-tested 2 and 5 weeks after the beginning of phase 2 treatment (weeks 7 and 10 in Fig. 1).

Blood and tissue analysis

All hamsters were bled one day after the week 11 and week 17 tests of Experiment 1, and at weeks 5 and 10 of Experiment 2. Blood was obtained from the retro-orbital sinus from hamsters lightly anesthetized with isoflurane vapors 17 h prior to the next scheduled injection (~1730 h) under dim red illumination. Samples were centrifuged at 4 °C for 20 min at 3000 rpm, and the serum collected and frozen at –80 °C until assayed.

Approximately 48 h after the final behavior tests in each experiment the seminal vesicles and ventral prostate of deeply anesthetized hamsters were removed and dry weights were recorded (± 0.01 mg).

Testosterone radioimmunoassay

T was measured in a separate assay for each experiment using a solid-phase ^{125}I radioimmunoassay kit (DSL-4000; Diagnostic Systems Laboratories, Webster, TX). Serum samples were aliquoted in 50-µl amounts into duplicate assay tubes and incubated with tracer for 1 h at 37 °C. The lower limit of detectability of the assay reported by the manufacturer was 0.08 ng/ml T. The intra-assay and inter-assay coefficients of variation were 3.7% and 4.1%, respectively. We validated this kit (Park et al., 2003) and have used it previously to measure T concentrations in Syrian hamsters (Park et al., 2007).

Statistical analyses

The Statview program (Statview 5; SAS Institute, Cary NC, USA) was used in all analyses. Differences between pre-operative and post-operative values were assessed with paired *t*-tests. Differences between groups at specific time points were assessed with unpaired *t*-tests. Separate repeated measures ANOVAs were used to analyze changes in the number of mounts, intromissions, mount, intromission and ejaculation latencies and the post-ejaculatory interval between groups over time. Serum samples with undetectable T concentrations were assigned the minimal detectable concentration of the assay (0.08 ng/ml). Post-hoc comparisons were conducted where appropriate using Fisher's Protected Least Significant Difference test. Observed differences were considered significant if $p < 0.05$ and this value is reported for all significant comparisons regardless of the actual significance level.

Results

Effects of T concentrations on male sex behavior of castrated hamsters

Experiment 1

Phase 1: 5 weeks after castration no vehicle-injected hamster (0 µg T) displayed the ejaculatory reflex (ER: ejaculation latency of 15 min, Fig. 2A). Ejaculation latencies did not differ after 11 weeks of treatment among groups receiving different doses of T (Fig. 2A; $F_{3,15}=1.06$, $p > 0.05$); hamsters injected with the lowest dose (50 µg) generated more mounts at weeks 5 and 11 and more intromissions at week 11 prior to ejaculation (Figs. 2B, C; $F_{1,17}=22.55$, 17.63, and 28.2 respectively, $p < 0.05$) than did groups treated with higher T doses. At week 5 the vehicle-injected hamsters did not intromit and had significantly longer mount latencies than did all other groups ($F_{1,21}=39.53$, $p < 0.05$; not illustrated). By week 11 no vehicle injected hamster mounted or intromitted. Additionally, at week 11 the 400 µg group was slower to intromit than either the 200 or 100 µg group ($t_7=3.0$ and 6.23 respectively, $p < 0.05$; not

illustrated). There were no differences between groups in the post-ejaculatory intervals. No other differences between groups were observed.

Phase 2: 50 µg of daily T treatment restored ejaculatory behavior in 4 of 5 hamsters castrated 11 weeks earlier and previously treated with the vehicle. All hamsters administered 50 µg T every 2 days ($n=7$) continued to ejaculate, as did 4 of 6 hamsters injected every 4 days and 4 of 6 hamsters injected every 7 days. Hamsters that ejaculated in the group treated every fourth day had been injected with 100 µg ($n=1$), 200 µg ($n=2$) and 400 µg ($n=1$) during phase 1; the two hamsters that failed to ejaculate had previously received 50 or 100 µg T. Corresponding Phase 1 treatments for the group injected once per week were 50 µg ($n=1$), 100 µg ($n=1$) and 400 µg ($n=2$) for those that ejaculated; the two hamsters that failed to ejaculate were previously injected with 50 or 200 µg T. Ejaculation latencies increased significantly in hamsters injected every 4th or 7th day compared to values of hamsters treated every 2 days (Fig. 3A; $t_{11}=-2.69$ and -4.04 respectively, $p<0.05$), but did not differ significantly from values of hamsters injected daily ($t_8=-1.51$, $p=0.17$ and $t_8=-2.27$, $p=0.053$, respectively). A greater number of mounts (Fig. 3B) preceded ejaculation in hamsters injected once every 4 or 7 days compared to values for groups injected daily or every other day (daily vs. 4 days, $t_8=-2.94$, daily vs. 7 days, $t_8=-2.40$, 2 days vs. 4 days, $t_{11}=-2.67$ and 2 days vs. 7 days, $t_{11}=-2.22$, $p<0.05$). When the analysis was restricted to hamsters that ejaculated, males injected every 1 or 2 days had significantly shorter ejaculation latencies and fewer mounts than those injected once every 7 days ($F_{1,12}=4.93$ and 9.97 respectively, $p<0.05$); hamsters injected every 4 days did not differ significantly from any of the other groups (not illustrated). The number of intromissions preceding ejaculation was equivalent for groups treated daily or every 2 days and significantly lower than values for hamsters injected every 4 or 7 days (Fig. 3C; $t_9=0.76$ and $F_{1,16}=20.75$, respectively). Hamsters injected every 2 days had significantly shorter post-ejaculatory intervals than hamsters injected either every 4 or 7 days (not illustrated; $t_{11}=-2.89$ and -2.73 respectively, $p<0.05$).

Experiment 2

Phase 1 (Fig. 4): Two weeks after castration, vehicle-treated hamsters (0 µg) displayed more mounts (Fig. 4B), longer ejaculation latencies (Fig. 4A) and longer post-ejaculatory intervals (not illustrated) than hamsters injected with 15 µg T ($t_{16}=-5.21$, -3.38 and -3.57 , respectively, $p<0.05$), but there were no significant differences in number of intromissions (Fig. 4C) or in mount or intromission latencies (not illustrated) between the groups ($t_{16}=-1.02$, 0.84 , and 1.87 , $p>0.05$). By the end of phase 1 (5 weeks), no vehicle-injected hamster displayed an ejaculatory reflex, whereas 15 µg T maintained this behavior in all hamsters with latencies that did not differ from pre-castration values (Fig. 4A; week 5 vs. pre-op values, $t_{10}=-0.05$, $p>0.05$).

Phase 2 (Fig. 5): 5 weeks of 15 µg T treatment (weeks 5–10) restored copulation in all hamsters that had previously ceased to ejaculate in phase 1 during treatment with the vehicle, but with ejaculation latencies longer than their pre-castration values (Fig. 5A; week 10 vs. pre-op values, $t_{10}=-4.77$, $p<0.05$). In contrast, a reduction of the T dose from 15 to 5 µg from week 5 to week 10 eliminated ejaculation in 3/11 hamsters, significantly lengthened ejaculation latencies (Fig. 5A, week 5 vs. week 10, $t_{10}=7.67$, $p<0.05$), and increased the number of mounts and intromissions at week 10 (Fig. 5B, C; $t_{10}=-4.05$, $t_{10}=-5.6$, respectively, $p<0.05$). Mount and intromission latencies did not differ in hamsters treated with 15 µg T in the maintenance and restoration regimens (week 5, maintenance vs. week 10 restoration, $t_{16}=-1.35$ and -0.90 respectively, $p>0.05$, not illustrated). Hamsters in the restoration regimen had significantly more intromissions preceding ejaculation during treatment with 15 µg T than those in the maintenance regimen (~ 18 vs. ~ 35 ; Fig. 5C; week 5 maintenance vs. week 10 restoration $t_{16}=-3.2$, $p<0.05$).

T concentrations induced by injection regimens

Experiment 1—T concentrations of each group differed significantly from all others when blood was sampled 7 h after injection after 11 weeks of T or vehicle treatment (Phase 1; Fig. 6A; multiple *t* tests, $p < 0.05$). Hamsters injected with the vehicle had T concentrations below the lower limit of detectability. T concentrations of hamsters treated with 50 μ g T ranged between 0.9 and 1.3 ng/ml, which is below the values for intact males (Arteaga-Silva et al., 2005; Park et al., 2007, both reported values of ~ 2.0 ng/ml).

Hamsters were bled after the week 17 behavior tests 7, 31, 79, or 151 h after the most recent 50 μ g T injection (Phase 2). T concentrations at 7 h ranged from 0.47 to 0.9 ng/ml and were significantly higher than values at all subsequent time points (multiple *t* tests, $p < 0.05$; Fig. 6B), which did not differ from each other; hamsters treated every 2 days tended to have higher T concentrations than those injected every 4 and 7 days ($t_{11} = 2.15$ and 2.12 , respectively, $p = 0.055$ and 0.057 respectively). Nine of 19 hamsters sampled between 31 and 151 h post-injection had undetectable T concentrations; T concentrations in the remaining 10 hamsters ranged from 0.09 to 0.21 ng/ml; an equal number of hamsters had non-detectable concentrations in groups injected every 4 and 7 days.

Experiment 2

Phase 1: Hamsters treated with 15 μ g T in the maintenance paradigm had significantly higher T concentrations (< 0.4 ng/ml) than those injected with the vehicle (non-detectable values) after 5 weeks of post-castration treatment (Fig. 6C; $t_{16} = 3.74$, $p < 0.05$), but well below the ~ 2 ng/ml values of intact males (Park et al., 2007). T concentrations were equivalent after 5 weeks of treatment in the maintenance and restoration paradigms (Fig. 6C; $t_{16} = -0.67$, $p > 0.05$). The 5 μ g treatment produced T concentrations intermediate between and significantly different from those of hamsters injected with the vehicle or 15 μ g T (Fig. 6C; $t_{16} = 2.19$ and $t_{16} = -3.91$ respectively, $p < 0.05$); T was undetectable in 3 hamsters and ranged between 0.11 and 0.32 ng/ml in the 8 remaining hamsters in this group.

Effects on androgen-responsive peripheral tissues

Experiment 1

Phase 1: 50 μ g T injections every day or every 2 days maintained higher ventral prostate weights than did the less frequent treatments (Fig. 7A; daily vs. every 4 and 7 days; $t_9 = 4.14$ and 3.03 . Every 2 days vs. every 4 and 7 days; $t_{11} = 2.86$ and 2.36 , $p < 0.05$). Injections every 1 or 2 days also maintained heavier seminal vesicles than did T treatment every 7 days (Fig. 7B; $t_9 = 6.35$ and $t_{11} = 7.8$ respectively, $p < 0.05$). Treatments during the first phase of the study were not significantly correlated with terminal seminal vesicle or prostate weights during phase 2 ($p > 0.05$, not illustrated).

Experiment 2

Phase 2: Prostate and seminal vesicle weights of hamsters treated with 15 μ g T daily (Fig. 7C) were lower (~ 130 mg and 35 mg, respectively) than those of hamsters treated daily with 50 μ g T in phase 2 of experiment 1 (~ 250 mg and 70 mg, respectively; compare Fig. 7C to Figs. 7A, B; $t_{11} = -5.91$ and -7.39 , $p < 0.05$), but did not differ between the 15 and 5 μ g treatment groups ($t_{16} = -0.26$ and 1.3 respectively, $p > 0.05$).

Discussion

Male sex behavior (MSB) of Syrian hamsters was sustained by daily subcutaneous injection of 15 μ g T in both maintenance and restoration paradigms. Transition to 5 μ g T was accompanied by a decrease in copulatory efficiency as evidenced by the substantial increase

in number of mounts and intromissions preceding ejaculation and an increase in the number of males that failed to ejaculate; the threshold dose probably falls between these values but a definitive conclusion awaits tests in which 5 µg T treatment is extended for more than 5 weeks. The present work indicates that MSB is maintained by T concentrations substantially lower than required when T is dissolved in oil; 50 µg T injected daily in an oil vehicle was ineffective in restoring MSB in Syrian hamsters castrated 5 weeks earlier (Arteaga-Silva et al. 2005). The use of an aqueous vehicle in the present study produced relatively brief elevations of blood T, which more closely simulates endogenous episodes of T secretion (Pieper and Lobocki, 2000). Higher and/or more rapidly achieved peak T tissue concentrations may result when the hormone is administered in an aqueous vehicle; the rate of change in hormone concentration, in addition to the absolute concentration of T, may influence MSB.

T concentrations were undetectable in 20% of hamsters as early as 7 h after injection; T values were well below peak physiological concentrations or undetectable in most hamsters 31 h post-injection of 50 µg T. It is notable that 8 of 12 hamsters treated with this dose sustained MSB during treatments spaced 4 or 7 days apart. Some of these hamsters likely had undetectable blood T concentrations for 6 days between injections. Future work utilizing the present injection regimen will be necessary to establish the lowest frequency of T injections capable of maintaining MSB. Persistence of the ejaculatory reflex after infrequent T injections was manifested by hamsters that had been injected with a range of doses during phase 1 of Experiment 1. Because the ejaculatory reflex is absent 3 weeks after castration (e.g., Park et al, 2007), treatment effects from phase 1 are unlikely to have affected the outcome of phase 2 testing 5 weeks later. Whether the once weekly T injections would support MSB for many months is unknown. The experiment was terminated after 17 weeks to reduce stress associated with more prolonged treatments.

It remains to be established whether the minimalist replacement regimen described herein, and even more extended spacing between T injections, will affect the amount of sexual behavior males display before achieving sexual satiation (Arteaga et al., 2000) or the generation of long intromissions (Huck and Lisk, 1985). Our tests were terminated after a single intromission following the first ejaculatory series. Daily treatment with the lowest dose in phase 1 of Experiment 1 (50 µg T) was sufficient to maintain ejaculation with latencies that did not differ from pre-operative values or from those of hamsters treated post-operatively with higher T doses; the number of mounts and intromissions that preceded ejaculation were each elevated in the group receiving 50 µg T compared to those receiving higher T doses, which is indicative of reduced copulatory efficiency in the former group.

Each T pulse appears to initiate neural processes that bridge long gaps during which circulating T is below physiological concentrations. T affects MSB by binding to androgen receptors (ARs) in the cytosol of target tissues of a mating network (Wood, 1997). Subsequent migration of the bound hormone to the cell nucleus affects transcription factors that direct protein synthesis and support MSB (e.g., McGinnis and Kahn, 1997). A given T pulse may continue to activate protein synthesis after the hormone is absent from target tissues. Krey and McGinnis (1990) measured occupied AR complexes in target tissues of castrated rats just before, 0.5 h, and as late as 24 h after insertion of T capsules that produced blood concentrations in the 2–5 ng/ml range. Large increases in brain nuclear ARs were noted through 8 h, with no further increase from 8–24 h; nuclear receptor number decreased by 60% 2 h after capsule removal and values after 8 h were no different from those of untreated males. In Syrian hamsters migration of ARs from the nucleus to the cytosol, assessed immunocytochemically, is not entirely complete two weeks post-castration (Wood and Newman, 1993). Residual nuclear ARs may continue to direct lower levels of protein synthesis for a number of days after the withdrawal of androgens, thereby slowing the decline in post-castration MSB. The gradual decline of MSB after gonadectomy may depend on lingering effects of pre-synthesized proteins in specific neurons.

The protein synthesis inhibitors anisomycin and cycloheximide applied to the preoptic area of rats and mice, respectively, inhibit the display of MSB (McGinnis and Kahn 1997; Quadagno et al. 1976), supporting the canonical view that classical genomic actions of steroids and *de novo* protein synthesis are essential mediators of MSB. Time-limited ligand-independent activation of steroid receptors also may slow the decline in MSB after castration (Blaustein, 2004).

The maintenance of nuclear ARs is not sufficient to support MSB. For example, treatment of prepubertal Syrian hamsters with DHT is at least as effective in increasing brain ARs in juvenile as in adult males, but only the latter show components of sexual behavior (Meek et al., 1997; Romeo et al., 2001).

The means by which T maintains MSB is unclear. T may act via aromatization to estrogens in neural tissue and 5- α reduction to DHT in peripheral structures which are implicated in maintenance of MSB in Syrian hamsters. The doses previously used to address these issues induced markedly supraphysiological blood hormone concentrations. Castrated males treated daily with 1000 μ g DHT showed no interest in estrous females, nor any copulatory behavior, whereas males treated with 50 μ g estradiol (E2) were sexually motivated but did not display the ejaculation reflex (Arteaga-Silva et al., 2005). Combined treatment with DHT and E2 restored all components of MSB; the physiological significance of the latter finding is uncertain; DHT and E2 concentrations of intact males were 0.2 and 0.07 ng/ml, respectively, 2 orders of magnitude lower than 45.3 and 8.0 ng/ml, respectively, in the hamsters treated with the DHT plus E2 cocktail that restored MSB in castrates (Arteaga-Silva et al., 2005). It is unknown whether the very low concentrations of T herein shown to maintain and restore MSB are mediated in whole or part by conversion to estrogens. The quantities of T in the present study likely generate much lower concentrations of estradiol than those effective in restoring MSB in previous experiments.

In summary, the present study establishes that relatively brief elevations in blood T concentrations at infrequent intervals sustain male sex behavior. This protocol provides a complementary way to explore the relation of nuclear androgen receptor occupancy and protein synthesis on the one hand, and sex behavior on the other.

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Experiment 1

Experiment 2

Fig. 1.

Schematic of the experimental design. Hamsters were castrated (♂) at week 0 and tested postoperatively for sex behavior at weeks 2, 5, 11 and 17 in Experiment 1 and weeks 2, 5, 7 and 10 in Experiment 2. "B" indicates blood withdrawal one day after the mating test. Arrows indicate when reproductive tissues were removed and weighed. The frequency and/or dose of T injection, varied between phases 1 and 2.

Fig. 2. Mean±S.E.M. (A) ejaculation latencies, (B) number of mounts, and (C) number of intromissions of hamsters tested pre-operatively and at 2, 5 and 11 weeks post-castration in Experiment 1, phase 1. Hamsters that failed to ejaculate were assigned a latency of 15 min. Pre-operative data were available only for ejaculation latency. *Significantly different from all other groups ($p<0.05$). Only hamsters injected with the vehicle manifested increased ejaculation latencies over the course of 11 weeks. On the week 11 test, hamsters injected with 50 µg T generated more mounts and intromissions prior to ejaculation—a sign of reduced copulatory proficiency. However, their ejaculation latency did not increase.

Fig. 3. Mean $\pm$ S.E.M. (A) ejaculation latencies, (B) number of mounts, and (C) number of intromissions in Experiment 1, phase 2. Hamsters that failed to ejaculate were assigned the maximum test latency of 15 min. Bars designated with different letters differ significantly from each other ($p < 0.05$). An increase in mounts and intromissions prior to ejaculation suggests a decrease in copulatory proficiency.

Fig. 4.

Mean±S.E.M. (A) ejaculation latency, (B) number of mounts, and (C) number of intromissions by hamsters treated with 15 µg T (●) or hormone vehicle (■) each day for the first 5 weeks post-castration (phase 1, Experiment 2). Hamsters that failed to ejaculate were assigned the maximum ejaculation latency of 15 min. Pre-operative data were available only for ejaculation latency. *Differs significantly from value of group treated with 15 µg T. Daily treatment with 15 µg T maintains male copulatory behavior.

Fig. 5.

Mean±S.E.M. (A) ejaculation latency, (B) number of mounds, and (C) number of intromissions of hamsters in which daily treatment increased from 0 to 15 µg T/day (■) or decreased from 15 µg T to 5 µg T/day (●) during weeks 6–10 post-castration. *Significantly different from pre-operative values. **Significantly different from baseline value at week 5. 15 µg T restores male sex behavior in long-term castrates previously treated with hormone vehicle. A decrease in T concentration from 15 to 5 µg T/day results in less efficient male sex behavior, indicated by a significant increase in ejaculatory latency and a significant increase in the number of mounds and intromissions preceding ejaculation.

Fig. 6. Mean±S.E.M. (A) T concentrations after 11 weeks of daily treatment with different doses of T, (B) T concentrations after 5 weeks of injections of 50 µg T at different frequencies denoted on the *x*-axis and underscored by the number of hours from the last injection to the blood draw, and (C) T concentrations after 5 and 10 weeks of daily treatment with vehicle (0 µg), 5 µg or 15 µg T in the maintenance and restoration paradigms. N.D. non-detectable. Bars designated with different letters represent values that differ significantly from each other ($p<0.05$).

Fig. 7. Mean±S.E.M. tissue weights 17 weeks post-castration of (A) ventral prostate, (B) seminal vesicles for hamsters with different injection frequencies (Experiment 1, phase 2) and (C) comparable measures in Experiment 2, phase 2. Arrows in panel C indicate that treatments changed from 15 μ g to 5 μ g or 0 μ g to 15 μ g T, 5 weeks prior to collection of tissues. Other conventions as in Fig. 6.