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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.

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 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.

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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-17β (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 μg 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 a midline 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 μg T (Sigma) (n=5 per group except n=4 for hamsters that received 200 μg T). Concentrations were chosen based on studies of Arteaga-Silva et al. (2005) who reported that 50 μg 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 μg 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 μg T daily (restoration paradigm); the remaining hamsters were distributed evenly among groups and treated with 50 μg 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 μg 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