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The protein synthesis inhibitor anisomycin reduces sex behavior during a critical period after testosterone treatment in male Syrian hamsters

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ABSTRACT

Testosterone (T) is critical for maintaining male sexual behavior (MSB) in rodents, in part by altering protein synthesis in a well-defined neural circuit. The specific timing of protein synthesis essential for expression of MSB has never been investigated. We administered the protein synthesis inhibitor anisomycin (Ani) to castrated male Syrian hamsters treated sc with 100 μg T in an aqueous vehicle once weekly; this T regimen maintains MSB while elevating circulating T concentrations for only a few hours after each injection. Hamsters were injected sc with the vehicle or 12.5 mg Ani at one of several times relative to T administration; MSB was assessed once per week, 6 days after the previous T injection, for 5 weeks. Anisomycin administered 6–12 h after T injection significantly reduced the expression of sexual behavior, whereas Ani treatment between 3 h before and 3 h after T injection did not impair MSB. This experiment is the first to assess the specific timing of protein synthesis relative to a T pulse that is required for the expression of MSB. The demarcation of a critical interval for T-induced protein synthesis necessary for maintenance of MSB should facilitate specification of the genomic, proteomic, and biochemical cascades that subserve actions of T on male copulation.

1. Introduction

Testosterone (T) controls male sexual behavior (MSB) in many mammals [1–3]. In both male and female rodents copulatory behavior is dependent upon steroid occupancy of nuclear receptors in central nervous system target tissues, and subsequent protein synthesis. MSB always declines and in some mammals is virtually eliminated after castration, but can be fully restored with T replacement. Circulating androgens are, however, unnecessary for the continued expression of MSB, which persists for several weeks or months despite the precipitous decline in circulating T concentrations within hours of orchidectomy [4–6]. Full restoration of MSB in castrated Syrian hamsters typically is first achieved after several weeks of androgen replacement, even when markedly supraphysiological androgen concentrations are maintained by daily injection [7]. The relation of protein synthesis to the maintenance and decline of MSB is poorly understood.

Female sexual receptivity is controlled by estradiol (E_2) and progesterone, which are secreted in a pulsatile fashion; lordosis can be induced in ovariectomized rats by discontinuous exposure of two one-hour pulses of E_2 separated by 4–13 h, administered 24 h prior to progesterone treatment [8,9]. Treatment with the protein synthesis inhibitor (PSI) anisomycin (Ani) at various times relative to E_2 or progesterone treatment, demarcated that protein synthesis within 11 h after the initiation of E_2 treatment is essential for the activation of the lordosis reflex. Ani applied to the VMH coincident with systemic estrogen treatment disrupted lordosis in rats injected with progesterone 4–5 h later [10]. In Syrian hamsters Ani applied to the VMH or ventral mesencephalon of estrogen-primed females 30 min before progesterone treatment reduced lordosis [11]. No comparable information exists concerning the timing of protein synthesis essential for the activation of MSB by T.

The timing of protein synthesis required for acquisition and retention of memories [12], and phase-shifting of circadian rhythms [13], has likewise been established using PSIs. The acute nature of these signals facilitates specification of the time frame during which protein synthesis inhibition disrupts these processes. MSB has not lent itself to this paradigm because prolonged daily hormone treatment of several weeks duration is required to restore male sex behavior. Repeated daily administration of a PSI over such a time span is impractical because of potential cumulative drug toxicity and the complexity of the required experimental design.

Only three studies have explored the role of protein synthesis inhibition in the induction of MSB. Ani applied chronically to the medial preoptic area of rats treated with constant release T-filled Silastic capsules blocked the ejaculatory reflex in 70% of castrated rats in a restoration paradigm [14]. In intact mice, administration of the PSI cycloheximide to the preoptic area decreased the percent of mice that mounted, intromitted or ejaculated 12 h after PSI administration [15]. Application of cycloheximide to the medial preoptic area of rats also decreased MSB [16].

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Because these experiments were conducted in rodents with constantly elevated circulating hormones, we remain ignorant of the time course of androgen-induced protein synthesis necessary to sustain MSB.

Piekarski et al. [17] developed a pulsatile T replacement paradigm that maintains MSB in castrated Syrian hamsters. Injection of low doses of T in aqueous solution that increase blood T concentrations for just a few hours on a single day were sufficient to maintain MSB when administered once per week. Much lower doses were effective than when T is replaced with constant release Silastic capsules or via injection in oil vehicles, both of which elevate T for long intervals. That a pulsatile signal is more effective than a constant signal for maintaining MSB is supported by observations that T secretion in male mammals is basal most of the day and punctuated by pulses that substantially elevate blood concentrations for minutes to several hours. In mice, basal concentrations of 1–3 ng/ml are augmented by surges several times a day that elevate concentrations to 30–40 ng/ml [18]; in Syrian hamsters androgen concentrations increase above baseline values for ~4 h per day [19]. A paradigm that acutely elevates T for just 7 h provides a favorable model system to assess the temporal relation of T availability and protein synthesis critical for MSB.

We administered Ani at different times relative to a once weekly injection of T that maintains MSB in a majority of castrated hamsters. The aim was to identify critical intervals before or after T treatment during which interdiction of protein synthesis interferes with MSB. This approach can help in future investigation of the molecular and cellular mechanisms that underlie MSB.

2. Materials and methods

2.1. Animals

Syrian hamsters (*Mesocricetus auratus*; HsdHan: Aura; 10 weeks old) obtained from Harlan (Indianapolis, IN) were maintained on a 14L:10D photoperiod (14 h light/day, lights off at 1800 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 and conformed to principles enunciated in the NIH guide for the use and care for laboratory animals.

2.2. Experimental procedure

2.2.1. Screening for male sexual behavior

Adult male hamsters were 12 weeks old at the time they were screened in real time for MSB during the late portion of the light phase (~1400–1700 h) with ovariectomized females rendered sexually receptive with standard E_2 plus progesterone treatments. 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 sc on the day of ovariectomy; behavioral estrus was induced by injecting females with 350 μg progesterone sc (Sigma) dissolved in peanut oil (2.5 mg/ml) 4 h prior to the sexual behavior testing sessions. Females were utilized as sexual partners no more frequently than once every 4 days.

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 and kept in the room in which hamsters 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 on two consecutive tests separated by at least a week were considered sexually experienced and retained for the experiment. During the first screening test observations were terminated after the male achieved a single ejaculation. The second screening test that lasted 15 min permitted the emergence of the full suite of sex behaviors, including multiple ejaculations.

We recorded the latencies to the first mount, first intromission, first ejaculation, and the number of ejaculations per test. Males that failed to display any of the behaviors were assigned the maximum latency of 15 min. After the pre-operative tests hamsters were assigned to groups equated with respect to all of the recorded behaviors and body weight.

2.2.2. Surgical procedures

Hamsters were anesthetized with isoflurane vapors (Baxter Healthcare, Deerfield, IL) and castrated through a midline incision in the abdominal cavity. Incisions were closed with sterile sutures and wound clips (Mikron Auto Clip 9 mm, Becton Dickinson, Franklin Lakes, NJ, USA). Hamsters were injected sc with the analgesic 5% buprenorphine (0.2 ml/animal), post-operatively (Hospira Inc., Lake Forest, IL, USA).

2.3. Experimental design

All sexually experienced males were castrated (day 0) and injected sc once every 7 days for 5 weeks with 0.1 ml of 100 μg T dissolved in a 50/50 ethanol–distilled water vehicle. This concentration was selected based on a previous study in which 50 μg T in the same vehicle maintained MSB in a majority of hamsters for 5 weeks [17]. Injections commenced the day after castration. Hamsters were re-tested 1 week after castration. Subsequently, each hamster received either a 12.5 mg injection sc of the protein synthesis inhibitor Anisomycin (Ani; AG Scientific Inc., San Diego, CA) dissolved in 0.3 ml saline or vehicle, once per week for the remaining 4 weeks of the experiment. This dose of Ani is well tolerated by adult Syrian hamsters [20], does not induce observable sickness, crosses the blood-brain barrier, [9,21] and inhibits protein synthesis for 6 h in rats [8], whereas a 50% higher dose inhibits protein synthesis for 9 h in mice [21]. 100 μg T induces supraphysiological circulating concentrations of T (above 2 ng/ml) for 7 h, after which T concentrations decline over the ensuing hours [17]; This closely matches the reported time course of protein synthesis inhibition induced by the present dose of Ani. Because we had no *a priori* knowledge if or when Ani administration would interfere with copulatory behavior, groups of hamsters were administered Ani at −3 (n = 8), 0 (n = 8), 3 (n = 6), 6 (n = 8), 9 (n = 6), or 12 (n = 7) h relative to T injection; choice of time points was guided by research that established the impact of the timing of protein synthesis in response to physiological or environmental cues [9,22]; one group was treated with the saline vehicle instead of Ani, at hour zero (n = 8).

Hamsters were tested for MSB once weekly, 6 days after previous T and Ani treatment, one day before the next scheduled T and Ani injection. This ensured that no circulating T would be present for the 6 days after each T injection [17], and Ani would long since be cleared from the circulation, reducing the possibility of illness during testing. Five post-operative behavior tests were conducted, including 4 after weekly Ani treatments began (weeks 2–5). Tests were 15 min long and conducted in the same manner as in the last pre-operative test.

2.4. Statistical analyses

Pre-operative group equivalence was assessed with one-way omnibus ANOVAs. Mixed ANOVAs tested for changes over the manipulation period of weeks 2–5 post-op, with time-post castration as the within subjects and Ani treatment the between subjects factor. Tukey's HSD test was employed for all post-hoc comparisons after ANOVA. At weeks 4 and 5, after a large proportion of some groups approached the maximum ejaculation and intromission latency, or the minimum number of 0 ejaculations, non-normality of data became a concern. Accordingly, analyses at weeks 4 and 5 for the ejaculation and intromission latencies, and the number of ejaculations, employed the non-parametric Kruskal–Wallis one-way analysis of variance to