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Short duration testosterone infusions maintain male sex behavior in Syrian hamsters

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Abstract

In most mammalian species, reduced androgen availability is associated with marked reductions in male sexuality; conversely, androgen replacement in castrated males restores sex behavior within a few weeks. Testosterone (T) pulse duration, amplitude, frequency, and inter-pulse interval may be as important as total amount of hormone in determining target tissue responsiveness. We remain ignorant of the number and duration of daily T pulses necessary and sufficient to sustain male mating behavior. An in-dwelling infusion system was employed to vary T-pulse frequencies and durations. Daily 4 h infusions of aqueous T (100 μg/0.064 ml) and twice daily 4 h pulses of T (each 50 μg/0.064 ml) were sufficient to maintain ejaculatory behavior of sexually experienced castrated hamsters for 11 weeks post-castration; castrated hamsters infused with vehicle ceased to display the ejaculatory pattern 3 weeks after gonadectomy. Circulating T concentrations of hormone-infused hamsters declined markedly 7 h after the termination of each infusion. These results establish that male sex behavior can be sustained with infusions of relatively low T concentrations for 4 h/day and suggests that the basal concentrations of T sustained by the gonad during inter-pulse intervals may not be necessary for maintenance of sex behavior. 4 h T infusions were sufficient to maintain penile and seminal vesicles weights, but not ventral prostate weights or flank gland dimensions; the threshold for maintaining male sex behavior is lower than that for some androgen-dependent peripheral structures. Development of effective androgen replacement regimens that sustain sex behavior in castrated animals may be useful in the design of androgen replacement therapy for hypogonadal men.

Keywords: Male sexual behavior; Testosterone; Syrian hamster

Introduction

Male sexuality diminishes when androgen availability is reduced; conversely, androgen replacement, usually via continuous release of testosterone (T) from Silastic implants, intracranial implants, or injections of pharmaceutical preparations of T, restores or maintains copulatory behavior and sexual accessory structures in castrated rats and other vertebrates (Meisel and Sachs, 1994). Androgens are not, however, secreted continuously or at a constant rate in any of the well-studied mammalian species. Against a background of low secretory activity, T is released episodically, sometimes with a circadian rhythm superimposed on higher frequency ultradian pulses (e.g., a 3 h T pulse approximately twice a day in rats; Sodersten et al., 1983). In mice, T concentrations in blood are maintained at baseline levels 75% of the time but interrupted 4 times per day by abrupt pulsatile elevations that raise concentrations by more than an order of magnitude (Coquelin and Desjardins, 1982). Tissue sensitivity also reflects the rhythm of pulsatile T release: the period of maximal sensitivity of castrated rats to exogenous T coincides with the time of day that intact males increase androgen secretion (subjective day; Sodersten et al., 1980).

Many studies suggest that hormone target tissues evolved to respond optimally to particular frequencies of hormone secretion. The amount of hormone and the timing of hormone secre-

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tion each encodes information that affects cellular activity in target tissues (reviewed in Chadwick and Goode, 2000). According to this view, pulse duration, amplitude, frequency and inter-pulse interval may be as important as total amount of available hormone in determining target tissue responsiveness. High T concentrations or longer durations of elevated circulating T, achieved either by injection of long-acting preparations or implantation of continuous release capsules, replicate some of the actions produced endogenously by the testes, but not necessarily by the same mechanisms, nor with the same long-term consequences. In typical hormone replacement experiments that investigate the restoration of male sexual behavior, castrated male Syrian hamsters are injected daily with 500–1000 μg/day T or dihydrotestosterone dissolved in an oil vehicle (e.g., Romeo et al., 2001; Arteaga-Silva et al., 2005), which produces plasma T concentrations of 73 ng/ml compared to 2 ng/ml for males with intact gonads (Arteaga-Silva et al., 2005). Higher concentrations of T are required to restore male rat sexual behavior than maintain it (Davidson, 1966). In addition, androgen concentrations necessary to maintain copulatory behavior after castration are an order of magnitude lower than those measured in circulation of intact male rats (Damassa et al., 1977). However, because all of these studies utilized replacement therapies in which daily exposure to circulating androgens were of long duration or constant, they do not address the issue of the minimum durations necessary to maintain or restore male sexual behavior.

We are aware of but two studies concerning the minimum duration of exposure to T necessary to restore male sexual behavior in castrated rodents. Taylor and associates (Taylor et al., 1989, 1990) treated castrated rats with T dissolved in an aqueous solution that elevated blood concentrations for only 4–5 h after injection. Rats that received approximately 50 μg T per day failed to display the behavioral ejaculation pattern, whereas 150 μg T effectively restored ejaculation behavior, although latency to ejaculate was significantly higher when compared with that of intact males. This suggests that brief daily elevation of T is sufficient to restore sex behavior in castrated male rats treated in this fashion for 30 days. Using an arduous approach that involved daily insertion and removal of T-filled Silastic capsules, McGinnis et al. (1989) reported that the ejaculatory pattern was restored in the majority of rats that had been castrated for 3–4 weeks over the course of 10 days of treatment only when the T capsule was *in situ* for more than 18 h (21 and 24 h durations were most effective). Treatments of 18 or fewer hours resulted in a significantly lower proportion of males that displayed copulatory behavior. Cell nuclear androgen receptor occupation declined to castrate levels 6 h after capsule removal.

The Taylor et al. (1989, 1990) claim that 4–5 h/day of elevated T concentrations are sufficient to induce copulatory behavior is in apparent conflict with the findings of McGinnis et al. (1989) that T must be present for >21 h/day to reliably activate the complete sequence of male sex behavior. In the Taylor and McGinnis studies, sex behavior tests began after 37 days and 1 day of hormone treatment, respectively. More stringent T requirements (i.e., longer durations of daily T exposure) may apply in the early phase of restoring sex behavior (days 1–10 of treatment) than later; hormonal stimulation for a few hours either once or twice/day may suffice after the first 2 weeks of treatment. This conjecture is consistent with reports that androgen concentrations necessary to initially activate sex behavior in long-term castrates are substantially higher than those required to maintain the behavior when treatments begin immediately after castration (reviewed in Meisel and Sachs, 1994) and with the finding that a lower dose of a protein synthesis inhibitor is sufficient to disrupt male sex behavior when T is administered in a restoration rather than a maintenance paradigm (McGinnis and Kahn, 1997).

The present study investigated whether a daily pulse of T is sufficient to sustain mating behavior and reproductive structures in Syrian hamsters. In this species, low T concentrations are maintained for most of the day, with a two-fold increase in blood T during the late subjective day that endures for approximately 4 h before declining to basal values (Pieper and Lobocki, 2000). Thus, we infused T in an aqueous solution for 4 h each day to approximate the duration of the natural T pulse. This procedure also permits assessment of the extent to which absence of T availability for most of the day affects tissue responsiveness to peak T concentrations induced by a single 4 h infusion. Because estradiol given in 2 short pulses to ovariectomized rats is much more effective than the same amount given in a single pulse (Parsons et al., 1982; Clark and Roy, 1987), we also determined the effectiveness of two 4 h infusions per day with the total amount of T infused held constant for the single and double infusion groups.

Materials and methods

Animals

Male Syrian hamsters (*Mesocricetus auratus* HsdHan:AURA) purchased from Harlan (Indianapolis, IN) were maintained on a 14L:10D 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 Teklad, 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

50 adult male Syrian hamsters were screened for male sex behavior during the late portion of the light phase. A testing arena was kept in the room in which males were housed; after 5 min in which the male was allowed to acclimate to the arena, behavior was observed after a sexually receptive female was introduced into the arena. Males that ejaculated 2 times within a 10-min test on two consecutive tests separated by a week were considered sexually experienced. All observations were terminated after 10 min. Ovariectomized stimulus females (n=25) were rendered sexually receptive with standard estradiol plus progesterone treatments (e.g., Coolen and Wood, 1999; Wood and Williams, 2001). 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) was implanted s.c. on the day of ovariectomy; to induce behavioral estrus females were treated with 350 μg of progesterone (Sigma, St. Louis, MO) dissolved in peanut oil (2.5 mg/ml) s.c. 4 h prior to the mating test. Most stimulus females were utilized for behavioral testing about 2 times per week.

The information recorded in 10 min tests included: the number of mounts not accompanied by an intromission that preceded an ejaculation,