Report

Testosterone in a Cyclodextrin-Containing Formulation: Behavioral and Physiological Effects of Episode-like Pulses in Rats

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Testosterone, administered in the form of an inclusion complex with 2-hydroxypropyl-β-cyclodextrin by subcutaneous injection, enters the circulation in a manner markedly similar to the natural episodic release by the testes. The effects of a regimen of once-a-day administration of complexed testosterone to adult (castrated or intact) rats and to senescent (intact) rats were investigated. Although this procedure left the castrated animals with concentrations of circulatory hormone far below physiological levels for much of the day, a significant improvement in androgen-sensitive behavior and physiology was obtained. Furthermore, the testosterone effects were more pronounced when high doses were used periodically rather than when the same total amount of testosterone was equally divided among doses. The same supplementation to intact rats intensified androgen-sensitive behavior and physiology over normal levels. In senescent rats uniform pulses of the testosterone complex also improved behavior and physiology. Specifically, spermatogenesis was stimulated and, notably, the treatment increased muscle weight without substantial enlargement of the prostate. Since the testosterone-cyclodextrin complex also can be effectively administered as a sublingual tablet, the data suggest that similar regimens may be recommended for elderly men suffering from decreases in muscle mass.

KEY WORDS: testosterone, buccal administration; testosterone, administration by bolus; cyclodextrin-testosterone; hydroxypropyl-β-cyclodextrin; androgen-substitutional therapy.

INTRODUCTION

Hormones not only convey crucial messages between tissues but also modulate transmission of messages and sensitivity of responses by target tissues. These modulations arise from the effects hormones exert on the synthesis of their circulating carrier proteins and on the number of functional hormonal receptors in target tissues. The pulsatile release of hormones that has been demonstrated for several glands may be a way to encode these modulations and messages into a system in which a single compound is used for all the signaling (1–3). The androgenic system illustrates these features well. The pituitary gland releases luteinizing hormone intermittently (3). The blood level of testosterone, a hormone which is downstream in the cascade from luteinizing hormone, also varies over time; there is a basal level above which the concentration rises a few times a day in episodes lasting approximately an hour (2,4). These episodes may be triggered by conditioning (5). Testosterone maintains normal activity in various aspects of physiology and behavior but also may induce hyperplasia and support neoplastic growth (6,7). Thus, it is of both theoretical and therapeutic interest to find out which of the androgenic effects require a steady level of circulatory hormone and which effects are cued by episodic increases. The effects of steady levels of testosterone can be evaluated since depot preparations with constant testosterone release have been available for some time (8). The effects of episodic release are difficult to address (9,10) since previous pharmaceutical forms of testosterone generate depots in tissues from which hormone is released gradually into the circulation (11). Only recently has a pharmaceutical form of testosterone been developed which allows convenient administration of physiologically meaningful amounts of hormone in a manner which imitates the natural rapid rise and fall of hormonal levels occurring in episodes (12). This pharmaceutical form is based on an inclusion complex of testosterone with 2-hydroxypropyl-β-cyclodextrin. In this complex a molecule of hormone is included in the cavity of the host molecule; the formation or dissociation of such a complex in solution is very rapid. Consequently, the hormone can quickly transfer from the circulating solution into a tissue, while the carrier 2-hydroxypropyl-β-cyclodextrin stays in the solution. Aqueous solutions of the inclusion complex are stable and do not form precipitates upon dilution. Importantly also,
2-hydroxypropyl-β-cyclodextrin lacks toxicity or irritancy toward tissues; used in another context a dose of 0.5 g/kg/24 hr intravenously was tolerated in a human male (13). In the present work this pharmaceutical form of testosterone was evaluated on rats which had subnormal levels of testosterone, due either to castration (14,15) or to senescence (16-20).

MATERIALS AND METHODS

Animals

Long-Evans rats were individually housed in a facility with constant temperature (20-22°C), humidity (50%), and 12-hr light/12-hr dark cycle; food and water were freely available. The males were sexually experienced. Assignment of animals to groups was random. In the experiments with young adult male rats (150-200 days old), all animals were anesthetized with ether and either castrated (N = 64) or sham operated (N = 12). In the experiment with senescent rats, 24-month-old gondally intact males (N = 14) of the same strain were used. Adult female rats used were ovariec-tomized under pentobarbital sodium anesthesia and allowed a week’s recovery time. Females were induced to estrus by subcutaneous injection of estradiol benzoate (200 µg) followed 48 hr later by injection of progesterone (800 µg); animals were then used within several hours. For details see Refs. 9, 10, and 21.

Testosterone Preparation and Administration

Testosterone (2.5 g) and 2-hydroxypropyl-β-cyclodextrin (25 g; degree of substitution, 6.2) were stirred in distilled water (100 ml) at room temperature for 3 days. The suspension was then filtered through a Millipore filter (0.45 µm). The clear filtrate was diluted to 200 ml with distilled water and freeze-dried. The resulting white nonhygroscopic powder (26.3 g) contained 7.7 ± 0.04% (w/w) (N = 3) of testosterone as determined by spectrophotometry (ε = 16,600 at 250 nm). This powder was dissolved in isotonic saline solution and injected subcutaneously. Both the powder and the solution were stored at room temperature.

Behavior Tests

In aggression tests each experimental male was paired with an intact male of the same age and strain selected at random from the general animal colony (N = 30). Interactions of the pair were observed and aggressive behavior was recorded during a 20-min session (22).

To test sexual performance an estrous female was introduced into a cage in which the male had been placed 15 min earlier. Latencies of the first intromission (seconds) and first ejaculation (minutes) were recorded. The session lasted for 30 min.

In tests of sexual motivation, an inaccessible estrous and nonestrus female were separately contained in small mesh-wire cages placed at each end of a large cage (23). Then the male was placed in the large cage for 20 min. The time spent in that half of the large cage which contained the estrous female was recorded (seconds). Further, urinary markings on fields (altogether 600) in the vicinity of the cages of each female were counted.

Physiological Examination

After the behavioral tests, each male was sacrificed with an overdose of pentobarbital sodium. Androgen-sensitive structures of interest (Tables I and II) were excised using ligation to prevent less fluid where applicable, and wet weights were obtained. Note that in Table II weights relative to body weights were given to enable easier comparison with senescent rats of other strains.

Testosterone in Serum

Sixteen of the males were castrated and tested 2 days later for the half-life of the testosterone in serum. Blood samples from rats were obtained by cardiac puncture and testosterone in serum measured by radioimmunoassay using rabbit antiserum supplied by the Department of Pharmacology, University of Heidelberg, and [3H]testosterone from Amersham-Buchler, Braunschweig. The intrassay coefficient of variation was 4.6%.

Data Analyses

All groups contained six or more animals. Results from the experiment on behavioral/physiological features of the young adult rats were analyzed with a series of 2 × 2 analyses of variance with the schedules (uniform or rhythmic) and testosterone dosage as main factors. In post hoc group comparisons Tukey’s HSD (P < 0.05) tests were used. Findings from experiments on senescent rats were examined with t tests (P < 0.05). Within-group changes in behavior before and after supplementation with testosterone were analyzed, as well as differences between treated and untreated groups. One senescent animal died during the experiment and his data were not included.

RESULTS

The concentration of testosterone in the circulation of intact rats varies with time, rising above baseline levels in irregularly spaced episodes which occur a few times each day. A typical episode (2) is shown in the left panel in Fig. 1. Circulating testosterone levels in castrated rats were an order of magnitude below baseline levels of intact rats (Fig. 1, middle panel). When castrated rats were given a single subcutaneous injection of complexed testosterone (400 µg/kg body weight), the concentration in serum rose dramatically and began a decrease immediately (Fig. 1, middle panel) at a rate that appears to mimic an episode of an intact rat. Multiple administration of testosterone complex did not lead to any buildup of hormone in the animals. When castrated rats (N = 3) were given 30 daily injections of complexed testosterone at a much higher dose (1600 µg/kg body weight) than used in the behavior-physiology study, the circulatory testosterone on the day following the last administration was at typical castrate levels (Fig. 1, middle panel).

The principal experiment with young adult rats involved once-a-day supplementation of complexed testosterone by subcutaneous injection for 39 days. During days 37-39 behavior tests were performed, on the 40th day animals were killed, and physiology-related parameters were measured. Four groups of animals were supplemented: the dose-response study on castrates used daily means of 100, 300, or