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Testosterone Affects Food Intake and Body Weight of Weanling Male Rats

ANTONIO A. NUNEZ AND MARJORIE GRUNDMAN

Department of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824

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NUNEZ, A. A. AND M. GRUNDMAN. *Testosterone affects food intake and body weight of weanling male rats.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 933–936, 1982.—The effects of testosterone propionate (TP) on food intake and body weight were investigated using castrated prepubertal male rats. Regardless of dose (1 mg, 0.2 mg or 0.1 mg), daily injections of TP increased body weight gain and food intake during the prepubertal period (from 22 to 40 days to age). Considering previous results, the present observations suggest that in the male rat sensitivity to the effects of gonadal hormones on feeding develops earlier than in females.

Development Food intake Body weight Testosterone Sex differences Gonadal hormones Androgens Testosterone metabolism

THE androgenic modulation of feeding and body weight in adult male rats has been extensively investigated [4, 5, 6, 11, 14, 19]. Several experiments have shown that castration produces a permanent hypophagia [4], and a reduction in body weight gain [4, 6, 19]. Exogenous testosterone reverses the effects of castration when administered in low doses [4, 5, 11]. However, long-term treatment with doses of testosterone propionate (TP) higher than 0.2 mg/day reduces food intake, body weight and carcass lipid content [4, 5, 11]. The anorexic effects of TP are also evident after direct hormonal stimulation of the ventromedial hypothalamus [7].

In contrast to the available data on adult rats, little is known about the effects of testicular androgens on food intake and body weight during the prepubertal period. In 21-day old male rats, castration reduces body weight and body length [13]; but, the effects of castration do not become evident until well after the sham-operated controls reach puberty. Therefore, these measures may not be sensitive to androgens during the prepubertal period. In weanling female rats, estradiol decreases body weight [10,15]. However, weanling female rats are unresponsive to this hormone with respect to food intake [10, 15, 18]. Estradiol decreases both food intake and body weight in adult females [16,17].

The purpose of the present experiment was to determine the effects of TP treatment on the food intake and body weight of castrated weanling males. In the first part of the study, we administered two doses of TP (0.2 mg or 1 mg/day) previously used in experiments with adult animals, and observed the rats over 18 days of treatment. Since the effects of TP on feeding and body weight depend upon dose and length of treatment [4,11], in the second part of the experiment we treated the animals with doses of 0.2 mg or 0.1 mg of TP/day and extended the treatment period to 27 days.

METHOD

Animals and Housing

Male Sprague-Dawley rats were weaned at Day 21 of age and castrated as described below. Immediately after surgery, the rats were individually housed in hanging cages with free access to food pellets (Wayne Laboratory) and tap water. A 12:12 light-dark cycle (lights on at 0800 hr) was in effect for the duration of the experiment.

Procedure

Part one. Thirty rats were castrated via a single scrotal incision under methoxyflurane (Metofane; Pittman-Moore) anesthesia. At 22 days of age, the animals were divided into three groups (n=10 each), matched for mean postsurgical body weight. The animals received daily subcutaneous injections (0.05 ml) of either the oil vehicle, 1 mg of TP (Steraloids Inc) or 0.2 mg of TP for 18 days. Base line mean (±SEM) body weights (g) were: oil group=51.3±0.9, 1 mg TP=50.7±1.0, 0.2 mg TP=51.1±0.8. Food intake (nearest 0.1 g) and body weight (nearest g) were measured daily between 1000 and 1100 hr. At the end of the experiment, the rats received an overdose of sodium pentobarbital (Nembutal), and nasoanal lengths were measured. Seminal vesicles were dissected and weighted wet (nearest mg).

Part two. Following the procedures described above, 24 additional animals were castrated and divided into three groups, (n=8 each) matched for body weight. Daily injections of oil, 0.2 mg of TP or 0.1 mg of TP began the day after surgery (22 days of age) and continued for 27 days. Baseline mean (±SEM) body weights (g) were: oil group=61.8±1.7, 0.2 mg TP=62.0±1.7, 0.1 mg TP=61.6±1.6. Body weight and food intake were measured every three days. Nasoanal lengths and seminal vesicle weights were obtained at the end of the experiment.

Data Analysis

For both parts of the study, the data on food intake and body weight gain were divided into blocks of 9 days each and subjected to individual nonparametric analyses of variance (Friedman two-way analysis of variance for matched sam-

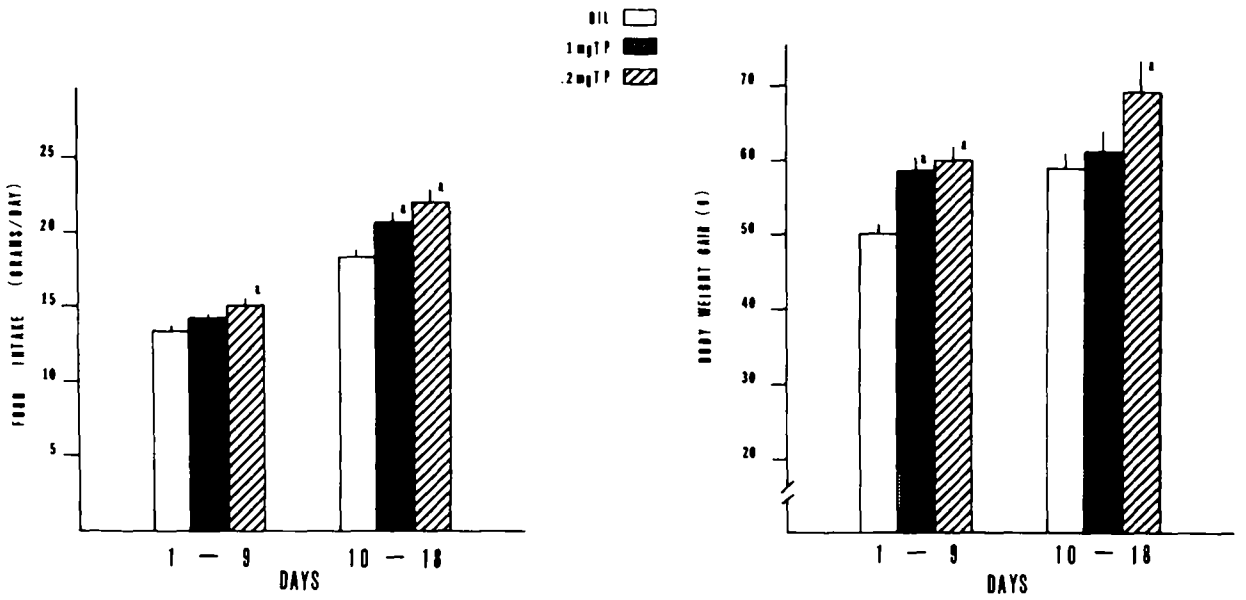

FIG. 1. Effects of treatment with testosterone propionate (TP; 1 mg, 0.2 or oil) on food intake (left panel) and body weight gain (right panel). For both measures, the data are grouped in 9-day blocks and presented as means (+SEM). [a]Significantly different from the oil group.

ples [12], p. 166). Planned comparisons between pairs of treatment groups used the Wilcoxon matched-pairs signed-ranks test ([12], p. 75). The data on anonasal length and relative seminal vesicle weight were analyzed with the same statistical tests used for the feeding and body weight data. Differences between groups were considered statistically significant when $p<0.05$ (two-tails, in the case of comparisons using the Wilcoxon test).

RESULTS

Part One

The treatment groups differed significantly in their food intakes (Fig. 1) over the first, $\chi_r^2=7.9$, $p<0.02$, and the second, $\chi_r^2=12.6$, $p<0.01$, 9-day blocks. For both 9-day blocks, the 0.2 mg TP group had significantly higher food intakes than the oil group, T=3 and 0 respectively, $p<0.01$ for both cases. The 1 mg TP group ate significantly more than the control group only during the second block of treatment days, T=2, $p<0.01$. No significant differences in food intake were found between the two TP groups.

Significant group differences in body weight gain were observed during the two treatment blocks, χ_r^2s=12.1 and 7.4, $df=2$, $p<0.01$ and $p<0.05$, respectively. Both TP groups gained significantly more weight than the oil group during the first 9 days of treatment, T=0, $p<0.01$ for both comparisons. During the second 9-day block, only the 0.2 mg TP group gained significantly more than the oil group, T=3, $p=0.01$. The differences in weight gain between the two TP groups failed to reach statistical significance.

Significant differences in nasoanal length, $\chi_r^2=9.8$, $p<0.01$, and relative seminal vesicle weight, $\chi_r^2=20.0$, $p<0.001$, were found across groups (see Table 1). The rats in the 0.2 mg TP group were significantly longer than those in the oil group, T=0, $p<0.01$, or the 1 mg TP group, T=2, $p<0.01$. The high dose of TP was more effective than the low dose in increasing relative seminal vesicle weight, T=0, $p<0.01$.

TABLE 1

MEAN (+SEM) NASOANAL LENGTH AND RELATIVE SEMINAL VESICLE WEIGHT

Group	Nasoanal Length (cm)	Seminal Vesicle Weight (wet wt/b. wt mg/g)
Oil	18.0 ± 0.10	0.32 ± 0.04
1 mg TP	18.1 ± 0.11	7.42 ± 0.57*
0.2 mg TP	18.5 ± 0.14†	3.70 ± 0.39‡

*Significantly different from the oil group and the 0.2 mg TP group.

†Significantly different from the oil group and the 1 mg TP group.

Part Two

Both doses of TP stimulated food intake during the first, $\chi_r^2=13.0$, $p<0.001$, and second, $\chi_r^2=12.3$, $p<0.001$, blocks of treatment days (see Fig. 2). Over these two blocks, no significant differences in food intake were found between the two TP groups. For the last block, the overall differences between groups were also significant, $\chi_r^2=7.8$, $p<0.02$; however, only the 0.1 mg TP group ate significantly more than the oil group, T=2, $p=0.02$. The 0.2 mg TP group ate significantly less than the 0.1 mg TP group during the last 9 days of treatment, T=3, $p<0.05$.

Differences in body weight gain were significant across

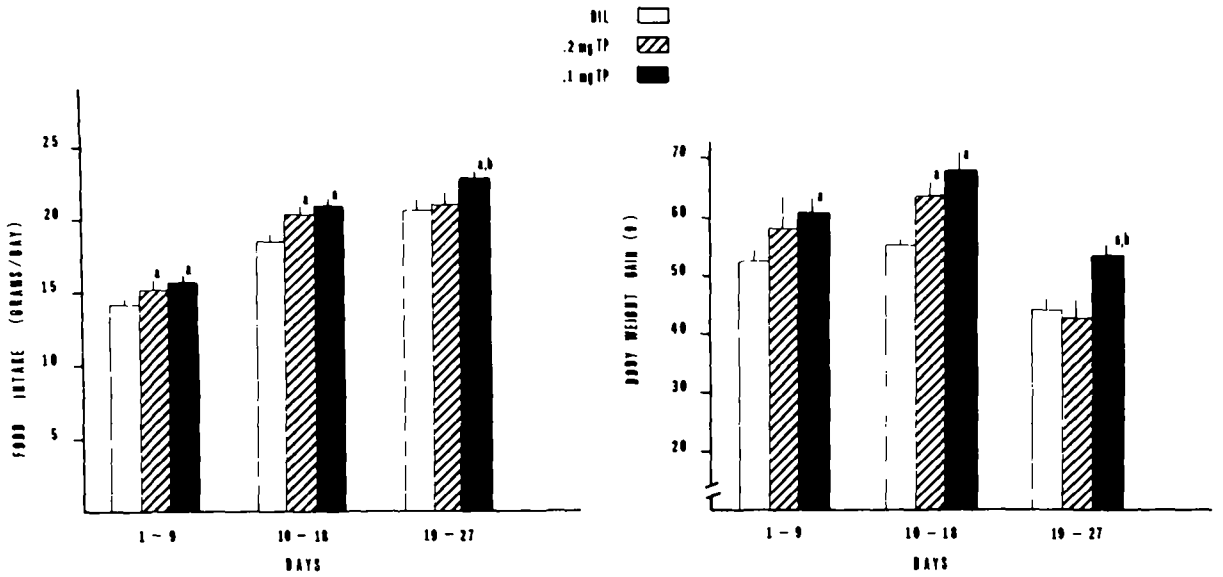

FIG. 2. Effects of treatment with testosterone propionate (TP; 0.2 mg, 0.1 mg or oil) on food intake (left panel) and body weight (right panel). For both measures, the data are grouped in 9-day blocks and presented as means (+SEM). [a]Significantly different from the oil group. [b]Significantly different from the group injected with 0.2 mg of TP.

groups over the first, $\chi^2_r = 10.6$, $p < 0.005$, and second, $\chi^2_r = 12.0$, $p < 0.002$, 9-day blocks (Fig. 2). During the first 9 days of treatment, the 0.1 mg TP group, but not the 0.2 mg TP group, gained significantly more weight than the oil group, $T = 0$, $p = 0.01$. Over the second 9-day block, both TP groups gained more than the oil group, $T = 0$, $p = 0.01$ for both comparisons. For the last 9 days of treatment, the overall group differences were not significant, $\chi^2_r = 4.75$, $p = 0.12$, and body weight gains for the oil group and the 0.2 mg TP group were nearly identical (see Fig. 2). However, individual comparisons showed that the 0.1 mg TP group had gained significantly more weight than the other two groups, $T = 3$, $p < 0.05$ for both comparisons.

No differences in nasoanal length were detected across groups, $\chi^2_r = 1.75$, $p = 0.531$. Treatment with TP increased seminal vesicle weight, $\chi^2_r = 12.0$, $p < 0.002$; however, no differences between the effects of the two doses of TP were found on this measure (Table 2).

TABLE 2

MEAN (±SEM) NASOANAL LENGTH AND RELATIVE SEMINAL VESICLE WEIGHT

Group	Nasoanal Length (cm)	Seminal Vesicle Weight $\left(\frac{\text{wet wt/b. wt}}{\text{mg/g}}\right)$
Oil	19.6 ± 0.22	0.46 ± 0.03
0.2 mg TP	19.9 ± 0.88	5.13 ± 0.42*
0.1 mg TP	20.1 ± 0.47	4.89 ± 0.29*

*Significantly different from the oil group.

DISCUSSION

The present results show that weanling male rats are sensitive to the effects of TP on food intake and rate of body weight gain. Regardless of dose, TP produced increments in these two measures during the prepubertal period (i.e., between 22 and 40 days of age). In contrast, previous work with ovariectomized females showed that daily injections of TP (2 mg/day) from 30 to 50 days of age failed to affect body weight [1]. Sex differences in sensitivity to TP, with respect to body weight and feeding, have been observed in adult animals and depend, in part, upon the action of androgens during the first five days of postnatal life [2,3].

Relatively low doses of TP were more effective than the high dose in stimulating body weight gain and food intake. In adult males, estrogenic metabolites of TP have been shown to mediate the reductions in food intake and body weight produced by high doses of TP [4, 5, 7, 11]. A similar mechanism may be responsible for the dose-dependent effects reported here. It is suggested that when a large dose of TP is administered, aromatized metabolites of the hormone act to mask the effects of testosterone (or its non-estrogenic metabolites) on food intake and body weight. That an aromatase inhibitor prevents the weight-reducing effects of high doses of TP [5] lends credence to this hypothesis.

After 18 days of treatment (*Part One*), the animals receiving a high dose of TP (1 mg) were signficantly shorter than those injected with 0.2 mg of TP. Here again, as in the case of body weight gain and feeding, estrogenic metabolites of TP may be responsible for the observed difference. In weanling females, injections of estradiol reduce nasoanal length, and concurrent treatment with progesterone reverses this effect [10]. Treatment with a low dose of TP, between 22 and 40 days of age, facilitated linear growth (*Part one*); but this effect was not seen after 27 days of treatment, when the animals were 49 days of age (*Part two*).

Finally, the positive effects of TP seen in this study seem to contradict the data from castration experiments [13]. Animals castrated at age 21 do not differ from sham-operated controls during the prepubertal period [13]. These observations suggest that the prepubertal male rat is insensitive to testicular secretions. Two lines of evidence may serve to reconcile the present findings and those of castration studies. First, recent experiments [8,9] have established that during the prepubertal period (from 10 to 40 days of age) testosterone is not the major secretory product of the Leydig cells [8,9]. Before puberty, there is a period of enhanced testicular 5α-reductase activity, and the major secretory products are 5α-reduced metabolites of testosterone [9]. Second, 5α-reduced products of testosterone (i.e., 5α-dihydrotestosterone) have been found to be significantly less effective than testosterone in stimulating food intake and body weight gain [4,11]. In adult castrates, replacement treatment with TP, but not 5α-dihydrotestosterone propionate, increases carcass protein content [11]. Thus, before 40 days of age the testicles primarily produce androgens that have only weak effects on food intake and body weight. Therefore, withdrawal of testicular androgens by prepubertal castration fails to have dramatic effects on these measures. On the other hand, the present results indicate that sensitivity to testosterone may be present early in development. However, the amounts of hormone injected in this experiment almost certainly exceeded physiological levels of testosterone.

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