EFFECTS OF TESTOSTERONE PROPIONATE TREATMENT OF NEONATALLY OVARIECTOMIZED RATS ON GROWTH AND SUBSEQUENT RESPONSIVENESS TO OESTROGEN

By

M. F. Tarttelin, J. E. Shryne and R. A. Gorski

ABSTRACT

There was no significant difference in body weight between neonatally ovariectomized (OvX) rats whether given oil treatment or 90 μg testosterone propionate (TP) on day 3, when examined up to 23 weeks of age. When these two animals were injected with oestradiol benzoate (3 μg/day for 2 weeks), the neonatally OvX TP treated rats showed a significantly smaller depression in body weight than did the control neonatally OvX rats. Measurement of food intake also showed that TP treated rats responded significantly less to the depressant effects of oestrogen than did the controls. These data are consistent with the hypothesis that the ovary does restrain body weight in TP rats but that androgen treatment in the neonatal period may not have a specific effect on growth but may alter the sensitivity of growth regulating processes to the inhibitory effects of oestrogen.

Neonatal treatment of the rat with testosterone propionate (TP) is known to alter hypothalamic regulation of gonadal function and sex behaviour towards the male type (see Gorski 1971, 1974). Furthermore, TP treatment in female rats

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has also been reported to induce significant increases in body weight (Harris 1964; Barraclough 1966; Beatty et al. 1970; Bell & Zucker 1971). We have previously shown that doses of TP of 30–270 µg were effective in inducing increased growth as measured by body weight when given on days 2 or 3 of life (Tarttelin et al. 1975). However, even the higher dose of 270 µg TP on day 4 or 5 did not increase growth compared to that of oil injected controls, although this treatment still produced the typical impairment of sex behaviour and gonadal function. Thus, these data suggest that there may be a “critical period” during which androgen can alter body weight regulating processes, apparently independent of its effect on gonadal function or sexual behaviour.

In the same study we found that after ovariectomy neonatally oil treated rats reached the same body weight as intact TP treated rats. Moreover, although ovariectomy of the TP treated rats still increased body weight, this increase was significantly less than in the control ovariectomized rats. These data suggest the possibility that TP treated rats grow more than intact controls because they are less sensitive to a growth inhibiting effect of oestrogen than are the controls. To test this further, in the present study we compare neonatally ovariectomized rats treated with TP or oil. If the growth increases normally seen in TP treated rats are due to a direct effect of TP, then one would expect a difference between oil and TP treatment even in neonatally ovariectomized rats. On the other hand, if the difference in body weight between TP and oil treated rats is mediated principally by an action of oestrogen, then there should be no difference between TP and oil treatment following neonatal ovariectomy, as in both cases the endogenous source of oestrogen has been removed.

MATERIALS AND METHODS

Fourteen pregnant Simonsen Sprague-Dawley rats of known fertilization date were housed in the rat colony with controlled lighting (14 h light, 10 h darkness; lights off at 7 p.m.) and temperature for 7 days prior to parturition.

Part 1

Ninety female neonatal pups were divided at random among 4 groups; two groups were ovariectomized (OvX) on day 2 and two groups sham operated (Table 1). The day of birth was defined as day 1. One group of neonatally OvX rats and one group of sham operated animals were given 90 µg TP (in 0.05 ml sesame oil) on day 3 as a single injection under the skin of the back; the other groups were given oil only. Data from a previous study (Tarttelin et al. 1975) indicated that in this strain 90 µg TP given on day 3 will produce significant growth changes, sterility, and impaired sex behaviour. Treatments were coded by removal of the distal phalanx of specific toes from individual animals. The pups were distributed so as to include all treatments in the same litter, and the litters were adjusted to 8 animals by adding males if necessary. Two of the mothers had 9 and 11 pups, all of which were retained. Any pups showing evidence of leakage from the injection site were discarded.
Table 1.
Summary of experimental and control procedures.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Neonatal treatment</th>
<th>Adult treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surgery Day 2</td>
<td>Injection Day 3</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>OvX&lt;sup&gt;a&lt;/sup&gt;</td>
<td>TP&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>OvX</td>
<td>oil</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>sham</td>
<td>TP</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>sham</td>
<td>oil</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ovariectomized.
<sup>b</sup> Testosterone propionate.
<sup>c</sup> Oestradiol benzoate.

Fifty-three pups were weaned at three weeks, individually identified by ear numbers, and weighed (correct to 1 g) weekly until the end of the experiment. Rats were housed by litters with pelleted Purina rat chow and tap water available <i>ad libitum</i>. From the twenty-first day the intact rats were checked daily for vaginal opening. In order to assess the effect of TP treatment on gonadal function, vaginal opening dates were recorded and daily vaginal smears obtained from this time. In addition, at day 45 these rats were laparotomized and the right ovaries examined for corpora lutea.

Part II

At 25 weeks of age, the 22 neonatally OvX rats were transferred to individual cages in an isolated rat room kept at constant temperature with regulated light (14 h light, 10 h darkness; lights off at noon). Rats were fed ground Purina rat chow in non-spill containers and provided with tap water. Food containers and water bottles were weighed twice weekly, but all intake data are expressed as weekly means. After a two week adjustment period, food and water intake were recorded for a four week period, then the rats were given daily injections of sesame oil for one week followed by 3 μg oestradiol benzoate (OeB) in 0.05 ml sesame oil daily for two additional weeks.

At the conclusion of the experiment, three weeks after the cessation of hormone treatment, the rats of groups 1 and 2 were anaesthetized and their nose-anal lengths recorded. The body weight data from Part I were subjected to computer analysis of variance comparing the four treatment groups each week from the time of weaning. When the “F” test was significant, a <i>t</i>-test matrix was examined in order to identify the groups which contributed to the significant differences. Because food and water intakes (Part II) were quite variable, these parameters were analyzed after transforming the absolute weekly intake into a percentage change with respect to the mean intake for the first two weeks of the control period. This transformation makes possible accurate comparisons of differences in magnitude and rate of change between groups although individual animals may vary considerably. In the case of body weight, the data were analyzed both as raw data and after similar transformation.
RESULTS

Part I

Body weight data from the 6th–14th week of age are presented in Fig. 1. There were no differences between the body weights of the neonatally OvX rats treated with TP or oil. Neonatally OvX TP or oil treated rats were significantly heavier than the intact oil treated rats from the ninth week onwards ($P < 0.01$). Intact TP treated rats were significantly heavier than the oil treated rats from the 10th week onwards ($P < 0.05$). However, there were no significant differences between the neonatally OvX oil or TP treated rats and the intact TP treated rats.

Body weight data obtained from the fourteenth to the twenty-third week are not illustrated, but there remained no statistically significant difference between the neonatally OvX oil or TP treated rats throughout this period. The neonatally OvX TP treated rats became significantly heavier than the intact TP rats at the seventeenth week ($P < 0.05$; ranging to $P < 0.01$ at the twenty-third week), and neonatally OvX oil treated rats only reached significantly heavier weights than the intact TP rats at the twenty-second week ($P < 0.01$).
Body weight data from rats in Groups 1 and 2 are illustrated in Fig. 2. In neonatally OvX TP treated rats there was a significant ($P < 0.005$) reduction in the depression in FI and BWt seen following treatment with OeB as compared to the oil treated rats. Food and water intake data for these animals are summarized in Table 2. No differences in food and water intake were detected at the beginning of this portion of the experiment, but following treatment with OeB, the food intake of the oil treated rats fell significantly more than the TP treated rats ($P < 0.01$). Water intake was very variable and no statistically significant differences were observed ($P > 0.05$); however, water intake showed a tendency to increase following OeB treatment as previously reported (Tarttelin & Gorski 1973).

![Graph showing body weight data](image)

**Fig. 2.**

The effect of the daily injection of 3 µg oestradiol benzoate (indicated by the thick horizontal line) on the body weight of neonatally ovariectomized (OvX) rats given either 90 µg testosterone propionate (TP) or oil on the third day of life. See key for group identification. The abscissa indicates time in weeks from the beginning of Part II of this study. The upper graph presents mean raw data, while the lower graph illustrates the same data transformed to a percentage change using the mean of the first two weeks of measurement as the basis for the transformation.
Table 2.
Food and water intake in adult rats neonatally ovariectomized and injected with testosterone propionate (TP) or oil. During the "treatment period" rats were given daily injections of 3 µg oestradiol benzoate.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Percentage deviation from two week control period</th>
<th>Intake parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-treatment period&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1 (neo-OvX TP)</td>
<td>10</td>
<td>-4 ± 1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-2 ± 1.0</td>
</tr>
<tr>
<td>2 (neo-OvX oil)</td>
<td>12</td>
<td>-7 ± 2.1</td>
<td>-2 ± 1.4</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1 ± 3.0</td>
<td>-3 ± 2.1</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>-2.9 ± 2.6</td>
<td>-1.5 ± 3.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> In weeks.
<sup>b</sup> Animals given oil vehicle only during this week.
<sup>c</sup> Mean ± Standard Error.
<sup>*</sup> Significantly different from each other (P < 0.01).
Examination of the ovaries of the intact rats (Groups 3 and 4) at day 45 showed that all the TP treated rats were anovulatory as judged by the absence of corpora lutea. In agreement with these observations, all their vaginal smears proved to be fully cornified. At sacrifice there was no difference between the nose-anal lengths of the neonatally OvX rats treated with TP (22.73 ± 0.4 cm) or oil (22.63 ± 0.1 cm).

**DISCUSSION**

The most striking finding of the present study is the lack of difference between the body weights of the neonatally OvX rats, whether given TP or oil, even when studied up to twenty-three weeks of age. This is in marked contrast to the growth promoting effect of TP in the intact animal. One interpretation of this lack of effect of TP on the body weight of neonatally OvX rats is that TP does not act directly on growth promoting processes. Note that the present data indicate that the ovaries of TP treated rats do restrain growth processes since the neonatally OvX TP treated rats eventually (week 17) become significantly heavier than the intact TP treated animals. However, this restraint is less than that seen in normal animals since oil treated neonatally OvX females are significantly heavier than their intact counterparts after only 9 weeks. This temporal difference clearly suggests that the body weight suppressing effects of oestrogen are less and/or delayed in TP treated rats compared to oil controls.

The present data also demonstrate that neonatal treatment with TP reduces the responsiveness of OvX rats to exogenous oestrogen. Neonatally OvX oil treated rats exhibit a significantly greater depression in body weight and food intake than neonatally OvX TP treated animals to the same dose of OeB. This observation of the reduced effectiveness of exogenous OeB, plus the fact that there is no effect of neonatally administered TP in the OvX rat, suggest that TP could have its effect on growth or food intake indirectly by altering responsiveness to oestrogen.

A similar conclusion was reached by Bell & Zucker (1971), who reported that early androgen treatment reduces the responsiveness of the female rat to the weight and food intake suppressing effects of a regime of oestrogen and progestosterone. However, the results obtained in the present study conflict with this experiment in an important detail, as neonatal OvX abolished the body weight stimulating effect of TP compared to neonatally OvX controls. Bell & Zucker (1971) reported that neonatally OvX TP treated rats were significantly heavier compared to similarly OvX but oil treated controls. However, this was apparently true only during the period between 15–17 weeks of age. The present identical body weight response of TP and oil treated neonatally OvX rats argues against the direct action of TP on the organization of growth regulating
processes and for the intermediate involvement of oestrogen. We have also
taken this study further by treating neonatally OvX rats with OeB and demon-
strating its reduced effectiveness in those rats also treated with TP.

In this regard, there are numerous studies which suggest that neonatal treat-
ment with TP reduces oestrogen responsiveness of the brain. For example, the
androgenized female rat is reported to be behaviourally less sensitive to oestro-
gen (Gerall & Kenney 1970; Whalen et al. 1971; Hendricks 1972), although the
physiological significance of this observation is unclear (see Gorski 1974). Simi-
larly, several studies suggest that the uptake and/or binding of labelled oesstra-
diol by the brain of the TP treated rat is less than in the normal (Flerkó &
Mess 1968; Anderson & Greenwald 1969; Vértes & King 1971), although this
has not been a uniform finding (Green et al. 1969; Maurer & Woolley 1975).
Finally, the androgenized rat does not exhibit oestrogen positive feedback; that
is, treatment with OeB in a sequence which results in a surge of LH in the normal
female is ineffective in the androgenized female (Mennin & Gorski 1975). Thus,
these data appear to add support to the concept that neonatal androgen treat-
ment also renders body weight control processes less responsive to the weight
inhibiting action of oestrogen.

Although this interpretation adequately explains the present data, the concept
that TP modifies growth processes only indirectly through the changes in oestro-
gen sensitivity needs further elucidation. Note, for example, that much of the
experimental evidence which suggests that androgenization induces a decrease
in oestrogen sensitivity is based on treatment which induces “masculinization”
of the regulation of gonadotrophin secretion and of sexual behaviour; in fact,
this decrease in oestrogen (or hormonal) sensitivity is reported to be critical
for the syndrome as normally defined. In marked contrast, we have reported
that rats that are fully masculinized in these terms still have normal body weight
(Tarttelin et al. 1975). We have suggested that there is a critical period for the
organization of body weight regulatory mechanisms independent of that for
gonadotrophin or behavioural regulation. The concept of a limited period of
time during which androgen exposure can alter body weight and composition
has been recently confirmed (Tarttelin & Clark 1975). If a loss in oestrogen
responsiveness is the main explanation of the effects of androgen exposure, then
we must postulate that the action of TP in the neonatal rat is specific to certain
oestrogen responsive (or potentially so) cells or systems and not others, specific
either because of their sensitivity to androgen or differences in their temporal
pattern of development. It is also possible that the current emphasis on the
neural effects of oestrogens is too restrictive. Clark & Tarttelin (1975), for
example, have suggested that oestrogen may inhibit growth at a peripheral level.
ACKNOWLEDGMENTS

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