Effects of various steroids on the thymus, spleen, ventral prostate and seminal vesicles in old orchidectomized rats

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ABSTRACT

The effects of several steroids on the regenerating thymus in ageing male rats have been studied. Rats aged from 12 to 15 months were orchidectomized and 7 days later implanted s.c. with silicone elastomer tubing containing 25 mg testosterone, 5α-dihydrotestosterone (DHT), oestradiol, progesterone or corticosterone. One group of rats received an empty implant. Thirty days later the rats were killed and the thymus, spleen, ventral prostate and seminal vesicles weighed and retained for histology. Whole blood was taken for total and differential white cell counts; plasma was prepared for radioimmunoassay of testosterone, oestradiol, progesterone and corticosterone.

After orchidectomy only, a multilobular thymus was present, and histologically the tissue appeared healthy. In testosterone- and oestradiol-treated rats, thymus weight was reduced to about 50% of that in untreated animals. Histologically, much of the thymus taken at autopsy was fat and what remained was poorly organized and contained a much lower density of thymocytes. The total white cell count was significantly reduced in these animals, the effect appearing to be predominantly on lymphocytes. Although treatment with DHT also resulted in a lower mean thymus weight than that of orchidectomized animals, histologically the tissue appeared similar to that of the untreated castrated animals. In rats treated with DHT, the total white cell count was significantly higher than in testosterone-implanted rats. Both progesterone and corticosterone implants resulted in significantly smaller mean thymus weights, although these steroids were not as potent as testosterone or oestradiol. Corticosterone, but not progesterone, appeared to cause a significant reduction in circulating lymphocytes. Dihydrotestosterone possessed only half the potency of testosterone in restoring the weights of the accessory sex organs. Serum concentrations of testosterone in orchidectomized old rats were $0.33 \pm 0.02$ nmol/l and in testosterone-implanted rats $4.8 \pm 0.4$ nmol/l. These results raise the possibility that testosterone and oestradiol may have caused atrophy of the thymus, while DHT may have retarded regeneration of the thymus without any atrophic effect. It remains to be seen whether the different responses between testosterone and DHT, in both the thymus and accessory sex organs, are due to differences in intrinsic action or differences in the metabolism of the steroids.


INTRODUCTION

The thymus is an important organ during development, atrophies after puberty and is generally considered to be unimportant for the immune system during adult life. In a previous study we found that the thymus, which had virtually disappeared in rats aged 15–18 months, was greatly restored 30 days after orchidectomy (Fitzpatrick, Kendall, Wheeler et al. 1985; Greenstein, Fitzpatrick, Kendall & Wheeler, 1987). The regenerated thymus appeared normal histologically, having well-differentiated cortex and medulla, was well vascularized and filled with thymocytes. In these animals the circulating white cell count was double that measured in sham-orchidectomized rats. The regeneration of the thymus in old rats and the enlargement which occurred after orchidectomy of young adults was inhibited by implanting testosterone in quantities which resulted in serum concentrations of the hormone approximating to those found physiologically (Greenstein et al. 1987).

Oestrogens and glucocorticoids cause involution of the thymus in young rodents (see Grossman, 1985). It therefore seemed worthwhile to examine the effects
of various classes of steroid hormones on the regenerating thymus of old rats.

In this series of experiments, testosterone, 5α-dihydrotestosterone (DHT) and oestradiol are shown to inhibit thymus regeneration in old rats. The effect of DHT appeared to differ qualitatively; it did not appear to cause thymic involution, while testosterone and oestradiol were severely atrophic. Some of the results have been presented in abstract form (Fitzpatrick & Greenstein, 1986).

MATERIALS AND METHODS

Male Wistar CSE rats were bred and maintained in the Animal Medical and Dental Schools of Guy's and St Thomas's Hospitals. The animals were kept four or five to a cage under conditions of controlled lighting and heating (lights on from 08.00 to 22.00 h; 21–23 °C), and allowed 100 g daily of Spratt's Laboratory Diet no. 1. These rats weighed from 550 to 650 g and were 12–15 months of age. We have found that if allowed unlimited access to food, the rats became ill, acquired swollen feet, cutaneous tumours and invariably died at about 700 g. On the present restricted diet the rats were healthy and had none of these symptoms.

Rats were orchidectomized bilaterally through the scrotal route under pentobarbitone anaesthesia (Sagatal; May and Baker Ltd, Dagenham, Essex; 40 mg/kg, i.p.). On day 7 after the operation the rats were divided into weight-matched groups and given implants made of Silastic medical-grade tubing (601–335; Dow Corning Corporation Medical Products, Midland, MI, U.S.A.). The implants contained 25 mg testosterone, oestradiol, DHT, progesterone or corticosterone. The rats were anaesthetized with ether and the implants inserted s.c. On day 30 after orchidectomy the animals were anaesthetized with ether and the thorax exposed. Blood was taken in heparinized syringes for total and differential white cell counts, and the thymus, ventral prostate, seminal vesicles and spleen removed, weighed and retained for histology. After routine fixation in formol acetic alcohol and sectioning, tissue sections were stained with haematoxylin and eosin. Total numbers of white cells were estimated in a haematocrit after staining with 1% crystal violet. Differential cell counts were undertaken in May–Grunwald–Giemsa–stained preparations. Radioimmunoassay of testosterone, oestradiol and progesterone was carried out by Dr M. J. Wheeler (Department of Chemical Pathology, St Thomas's Hospital) and radioimmunoassay of corticosterone by Dr M. T. Jones, (Department of Obstetrics and Gynaecology, St. Thomas’s Hospital Medical School).

Results from different treatment groups were compared by one-way analysis of variance and, where appropriate, by unpaired Student’s t-test.

RESULTS

Tissue weights and histology

One month after orchidectomy there was a large well-defined thymus in all the old orchidectomized rats given empty implants. Thymus tissue was also present in all the animals treated with steroids, but in all groups the mean organ weight was markedly lower (Fig. 1). Implants of testosterone, oestradiol and DHT were equally effective in this respect ($P<0.001$) while progesterone and corticosterone were significantly ($P<0.05$) less so. The differences between the effects of the steroids were most apparent when considering the histological appearance of the thymus. In testosterone- and oestradiol-treated animals there was a severe depletion of thymic lymphocytes in the cortical region, which was narrow, and the cortico-medullary junction was ill-defined (results not shown). There were areas of fatty tissue which we have always seen in age-related cases of thymic involution. In contrast, the thymus from DHT-treated animals was normal and indistinguishable from that of orchidectomized sham-treated animals or from normal young rats (results not shown); therefore, although DHT apparently inhibited the regeneration of the thymus, it had no discernible involutional effects on the organ. While corticosterone and progesterone both produced mean thymus weights significantly lower than those in sham-treated orchidectomized rats, the thymus appeared similar in the three groups and there was little visible evidence of lymphocytic involution. The weight of the other lymphoid organ examined, the spleen, was unaffected by testosterone treatment (Fig. 1) and appeared similar histologically to that of orchidectomized rats. All the other steroids suppressed the mean weight of the spleen ($P<0.001$; sham vs steroid). Histologically, however, the tissue appeared similar in all groups (results not shown).

The mean weights of the accessory sex organs were lower after orchidectomy, and of the steroids used, only testosterone and DHT were potent in restoring and maintaining the weights of the glands (Fig. 1) and their secretions. DHT, however, was far less potent than testosterone, especially on the seminal vesicles. Testosterone-treated tissues appeared normal in terms of the appearance of the secretory and germinal epithelium, while in sections from DHT-treated animals there were areas of normal acinar tissue interspersed with other areas of atrophy and stromal thickening (results not shown).
The mean serum concentration of testosterone in orchidectomized rats was $0.33 \pm 0.02$ nmol/l and from testosterone-implanted rats $4.8 \pm 0.4$ nmol/l ($n = 4$). In oestradiol-treated rats, oestradiol concentrations were $312 \pm 21$ pmol/l ($n = 5$) and in corticosterone-treated rats the concentration of corticosterone was $1.36 \pm 0.1$ mmol/l ($n = 5$). In progesterone-treated rats the mean concentration of progesterone was $137.6 \pm 14.9$ nmol/l.

**White Cell Counts**

The pattern of total white cell counts obtained is shown in Fig. 1a. In blood from testosterone-treated rats the total white cell count was significantly ($P < 0.01$) lower than that in blood from sham-treated rats. The total white cell count was also significantly ($P < 0.05$) reduced in oestradiol- and corticosterone-treated rats, but not in rats treated with progesterone or DHT. Table 1 shows the results of the differential white cell count. From this it may be deduced that the reduction was due possibly to changes in the lymphocyte count. In all steroid-treated groups the neutrophil count was markedly increased.

**DISCUSSION**

Thirty days after orchidectomy of old rats there was a large well-defined thymus in all rats, in accord with our initial finding (Fitzpatrick, Kendall, Wheeler et al. 1985). The markedly smaller thymus in animals treated with testosterone, oestradiol and DHT is evidence that the regenerated thymus in old rats resembles that in younger animals in terms of its...
response to these steroids. For example, the involutionary effect of testosterone on the thymus was shown by Selye & Albert (1942a, b); withdrawal of androgens by castration results in hypertrophy of the thymus (Castro, 1974).

Pereira Luz, Marques, Ayub & Riet Correa (1969) treated immature female rats with five daily injections of oestradiol valerate and observed a smaller thymus showing lymphocytic depletion whilst the spleen and lymph nodes appeared smaller. We observed a significant reduction in the weight of the spleen with all hormones tested, except testosterone. Histologically, the tissue appeared normal and there were no signs of lymphocytic depletion. The steroids may therefore have affected non-lymphocytic elements. Glucksman & Cherry (1968) studied the effects of gonadectomy and replacement with testosterone or oestradiol on the cortical epithelium of the thymus in young adult male and female rats. Castration increased the weight of the lymphatic cortex in both male and female rats. Both testosterone and oestradiol reduced the weight of the thymus, but oestradiol stimulated proliferation of the cortical epithelium. We observed a narrower cortical band in both treatment groups. This appeared to be associated with lymphocyte depletion. However, we used a large dose of oestradiol which resulted in a mean plasma concentration of oestradiol of 312 pmol/l, more than double the pro-oestrous concentration of oestradiol in rats (116 pmol/l; Rance, Wise, Selmanoff & Barraclough, 1981). We do not have values for oestradiol in intact old rats. The effects of lower doses should be explored.

Dihydrotestosterone is generally considered to be the potent active metabolite of testosterone, but it appeared to have no involutionary effect on the regenerating thymus, despite the reduction in weight compared with that of untreated animals. Furthermore, DHT did not reduce the total white cell count significantly. A possible explanation for the different effects of testosterone and DHT may be provided by Pearce, Khalid & Funder (1981) who injected DHT chronically into intact and orchidectomized male mice. Dihydrotestosterone had no effect on the thymus of intact mice, but did prevent thymic hypertrophy after orchidectomy. Testosterone markedly involves the thymus in intact males (Selye & Albert, 1942a). It may therefore be surmized that testosterone and DHT may act differently on the thymus of young and old rats. It may be incorrect to assume that the effects of androgens in the thymus can be studied through the use of testosterone or DHT alone.

The total white cell counts measured in old castrated rats agrees with previous data from this laboratory (Fitzpatrick et al. 1985). The present study indicates that raised lymphocyte counts may account for the difference between orchidectomized and untreated groups.

Corticosterone was potent in reducing the white cell count, but relatively weak in suppressing thymus regeneration. Corticosteroids cause species-specific atrophy of the thymus, and the rat is classed as a glucocorticoid-sensitive species in this respect (Shewell & Long, 1956). The presence of the adrenal did not prevent the reappearance of the thymus in old rats after orchidectomy (Fitzpatrick et al. 1985). The adrenal cortex may therefore not play as important a physiological role as do the testes in age-related atrophy of the thymus.

The weights of the accessory sex organs were restored in orchidectomized rats treated with testosterone, and the seminal vesicles were filled with fluid. The effects of DHT, however, were different. DHT is known to be a potent androgenic metabolite of testosterone, yet in these old rats it had only 50% of the efficacy of a similar dose of testosterone. It is possible that the steroid was being rapidly inactivated. Nevertheless, there is evidence that the androgen receptor of the prostate of ageing rats loses affinity for DHT (Greenstein, 1979). After prolonged periods of orchidectomy, the prostate and seminal vesicles are fully restored by testosterone in young but not in old rats (Greenstein, Fitzpatrick, Adcock et al. 1986). It

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**Table 1. Differential white cell count in whole blood from orchidectomized rats implanted s.c. with silicone elastomer capsules containing 25 mg steroid for 30 days. Results are mean percentages ± S.E.M. of three to five estimations**

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Macrophages</th>
<th>Plasma cells</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>80 ± 00</td>
<td>13-94 ± 1-4</td>
<td>1-41 ± 0-2</td>
<td>0-00</td>
<td>3-75 ± 1-10</td>
<td>0-41 ± 0-22</td>
<td>0-50 ± 0-10</td>
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<tr>
<td>Testosterone</td>
<td>42-25 ± 7-3</td>
<td>44-06 ± 5-2</td>
<td>0-98 ± 0-5</td>
<td>0-19 ± 0-1</td>
<td>4-38 ± 0-80</td>
<td>0-78 ± 0-30</td>
<td>0-28 ± 0-08</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>42-15 ± 4-4</td>
<td>48-75 ± 4-4</td>
<td>2-82 ± 0-5</td>
<td>0-00</td>
<td>4-60 ± 0-60</td>
<td>1-05 ± 0-30</td>
<td>0-60 ± 0-20</td>
</tr>
<tr>
<td>DHT</td>
<td>58-23 ± 2-5</td>
<td>32-85 ± 4-1</td>
<td>2-10 ± 0-5</td>
<td>0-03 ± 0-3</td>
<td>4-44 ± 1-0</td>
<td>1-30 ± 0-30</td>
<td>0-65 ± 0-20</td>
</tr>
<tr>
<td>Progesterone</td>
<td>59-43 ± 2-5</td>
<td>31-26 ± 2-0</td>
<td>2-54 ± 0-7</td>
<td>0-15 ± 0-1</td>
<td>4-46 ± 0-60</td>
<td>0-97 ± 0-06</td>
<td>0-91 ± 0-10</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>57-75 ± 1-9</td>
<td>32-25 ± 2-2</td>
<td>3-00 ± 0-4</td>
<td>0-15 ± 0-1</td>
<td>4-80 ± 1-20</td>
<td>1-05 ± 0-30</td>
<td>0-80 ± 0-20</td>
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None, empty implant; DHT, 5α-dihydrotestosterone.
remains to be seen whether these age-related differences in androgen action are due to changes in tissue sensitivity or metabolism of the steroids.

It is apparent that the effects of steroids on the thymus are complex, and it will be necessary to establish the nature of the active compounds. The action of steroids in causing atrophy and preventing hyper trophy needs to be distinguished. The origin of lymphocytes affected by the steroids should be established, perhaps by specific cell marker antibodies, and the consequences for the function of the immune system need to be explored.

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REFERENCES


