Ornithine decarboxylase activity as a marker of androgen and antiandrogen action in the rat epididymis

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Summary. After castration, there was a marked decrease in serum androgen concentration at 6 h, and a dramatic inhibition of ornithine decarboxylase (ODC) at 12 h. Administration of testosterone propionate to castrated rats at a dose of 0.05 mg/animal restored ODC activity to the normal value. However, no change was observed when intact rats were treated with testosterone even at a 40-fold higher dose, indicating that endogenous androgens present in intact rats are far in excess for maintenance of maximal levels of activity. Administration of the antiandrogen flutamide to intact rats caused a moderate decrease in epididymal weight, whereas this effect was more pronounced in castrated, androgen-treated rats. In the latter, the effect of flutamide was significant at the lowest dose used (0.5 mg/day). ODC activity was significantly decreased by flutamide treatment of intact rats, but even at the highest dose used (10 mg/day) only a 39% inhibition was observed. In flutamide-treated rats, LH concentrations were markedly increased, as were serum and epididymal androgens. In androgen-treated castrated rats, flutamide caused epididymal ODC to fall to undetectable values. These results show that: (1) androgens are essential for the maintenance of ODC activity in the epididymis; (2) epididymal ODC activity is maximally stimulated by endogenous androgens, at least in the pubertal rat; (3) the apparent potency of flutamide is substantially lowered by an increase in epididymal androgens. We suggest that ODC is a sensitive marker of the action of androgens and antiandrogens in the epididymis.

Keywords: androgen; antiandrogen; epididymis; ornithine decarboxylase; rat

Introduction

It is now well known that androgens are the principal regulators of epididymal function (Hamilton, 1975; Brooks, 1981). In several species, androgens are essential for the normal growth and development of the epididymis, as well for the maintenance of its differentiated function, i.e. the acquisition of fertilizing ability by spermatozoa (Cameo & Blaquier, 1976; Orgebin-Crist et al., 1981). It is therefore likely that antiandrogens, acting through binding to the androgen receptor, interfere with the androgen-dependent processes at the epididymal level. Among these compounds, 4'-nitro-3'-trifluoroethyl-isobutyranilide (flutamide), a non-steroidal antiandrogen, is believed to be a 'pure' antiandrogen (Poyet & Labrie, 1985) since it is devoid of any hormonal activity (Neri et al., 1972).

Several authors have attempted to show the antiandrogenic activity of steroidal and non-steroidal antiandrogens, such as cyproterone acetate and flutamide, on epididymal function by means of biochemical measures usually regarded as markers of androgen action on this organ (Brooks et al., 1974; Dhar & Setty, 1976; Rajalakshmi et al., 1976; Rastogi et al., 1979; Dhar et al., 1982). However, the results do not fully support the androgen-dependence of the epididymal secretory activity and the response to antiandrogens. De las Heras & Calandra (1987) have shown that epididymal ornithine decarboxylase (EC 4.1.1.17, ODC), the rate-limiting enzyme in polyamine
biosynthesis, which is considered to be a critical step in cell growth and differentiation (Tabor & Tabor, 1984; Russell, 1985), is androgen-dependent. In the present study, we further examined the effect of androgens on epididymal ODC. In addition, we evaluated the effect of flutamide on this enzyme in intact and castrated rats.

Materials and Methods

Chemicals. L-[1-14C]Ornithine (sp. act. 49–56 mCi/mmol), [1,2,6,7-3H]testosterone (sp. act. 98.8 Ci/mmol), [1,2-3H]5α-androstan-3α,17β-diol (sp. act. 40.9 Ci/mmol) and Omnifluor were purchased from New England Nuclear, Boston, MA, U.S.A. The purity of labelled steroids was regularly checked by thin-layer chromatography, and [14C]ornithine was purified as described by Jänne & Williams-Ashman (1971). Testosterone propionate was a gift from Laboratorios Gador (Buenos Aires, Argentina). Flutamide was kindly supplied by Schering Canada Ltd (Québec, Canada) through the courtesy of Dr F. Labrie. Solvents were from Merck, Darmstadt, F.R.G. All other reagents were purchased from Sigma Chemical Co, St Louis, MO, U.S.A.

Animals and treatments. In all experiments, male Sprague–Dawley rats (45 days old) were used. The animals were maintained in a temperature-controlled room, with lights on between 07:00 and 19:00 h, and had free access to laboratory chow and tap water.

Bilateral castration was performed by the scrotal route under light ether anaesthesia. For studies on androgen action, intact or 24 h-castrated rats were injected subcutaneously (s.c.) with testosterone propionate (0.02-2 mg in corn oil) every 2 days for 7 days. Flutamide (0.5–10 mg/day) was injected s.c. as a suspension in 0.9% NaCl solution containing 1% gelatin, twice a day for 7 days. Control animals received vehicle only. For studies on flutamide action in rats castrated 24 h earlier and treated with androgen, flutamide was injected under the same schedule as described above, together with 0.05 mg testosterone propionate/day. In all cases, the animals were killed by decapitation 16 h after the last injection.

Hormone measurements. Trunk blood was collected and centrifuged, and the serum was frozen until assayed. Serum prolactin and LH were measured by double-antibody RIA according to the methods previously described (Moguilevsky et al., 1981, 1985). Results were expressed as ng/ml on the basis of the standards (rat PRL RP-1 and LH RP-1) supplied by the NIAMDD Rat Pituitary Distribution Program. The within-assay and interassay coefficients of variation were less than 7 and 11%, respectively for both assays. The sensitivities were 1.7 ng prolactin/ml serum and 2 ng LH/ml serum.

Serum testosterone and 5α-dihydrotestosterone (DHT) were measured as androgen by RIA as previously described (Barañao et al., 1981). The lower detection limit was 12.5 pg, and the within-assay and interassay coefficients of variation were <12%.

Epididymal androgen content was measured by the method validated in this laboratory (Suescun et al., 1985), with minor modifications. Briefly, tissues were homogenized in acetone, the resulting homogenates centrifuged, and supernatants were evaporated to dryness. After resuspension in water, the samples were submitted to a sequence of solvent partitions: water–diethyl ether (1:10, v/v), 70% methanol–hexane (1:1, v/v) and methanol–dichloromethane (1:3, v/v). Samples were evaporated, and the residues suspended in buffer. DHT and 5α-androstan-3α,17β-diol (androstanediol) were measured by RIA using highly specific antibodies (DHT-1-CMO–BSA and androstanediol-15-CMO–HSA) from Immunotech Diagnostic, Montreal, Canada (Belanger et al., 1980). The within-assay and interassay coefficients of variation were <12 and <14%, respectively. The sensitivities of the DHT and androstanediol tissue assays were each 12.5 pg/tube. Recovery of internal standards was 70.7 ± 2.4 (mean ± s.e.). The results were expressed in terms of ng/g tissue.

ODC assay. ODC activity was measured in 20,000 g supernatants from whole epididymides as previously described (de las Heras & Calandra, 1987). The enzymic activity was expressed in terms of pmol 14CO2 released/h/mg protein or pmol/h/organ.

Other methods. Protein concentration was measured by the method of Lowry et al. (1951) using BSA as standard. Statistical analysis was made by Student’s t test or Duncan’s test for multiple comparisons (Li, 1964). P > 0.05 was considered not significant.

Results

Effect of castration and testosterone on epididymal ODC

As seen in Fig. 1, a marked reduction (83%) in circulating androgens was observed 6 h after castration. At this time, ODC activity was unaffected, but decreased by 71% after 12 h. At 72 h, ODC was barely detectable, and after 7 days it was not detected.
Fig. 1. Short-term effect of castration on serum androgen (□) and epididymal ODC activity (■). Values represent mean ± s.e.m. for 5 animals. *P < 0.01 compared with value at 0 h.

On the basis of these data, epididymal ODC was measured in intact or 24 h-castrated rats treated with testosterone propionate. Even high doses of the androgen (up to 2 mg) failed to show any significant effect on ODC in epididymides from intact rats, whereas a low dose (0.05 mg) restored ODC to normal values in castrated animals (Fig. 2). Similar patterns were obtained when results were expressed per organ or gram of tissue (not shown).

**Effect of flutamide on epididymal ODC**

Administration of flutamide to intact rats resulted in a moderate but significant decrease in epididymal weight. No significant differences were detected at the doses of 0.5 and 1 mg but at 10 mg, the highest dose used, epididymal weight fell by 34% (Table 1).

The effect of flutamide on epididymal ODC in intact rats is shown in Table 1. No significant differences were evident at the doses of 0.5 and 1 mg, and only a 39% inhibition was observed at
Table 1. Effect of flutamide on epididymal weight and ODC activity in intact rats

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Epididymal wt (mg/100 g body wt)</th>
<th>Epididymal ODC activity pmol/h/mg protein</th>
<th>pmol/h/organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (controls)</td>
<td>69.9 ± 4.9</td>
<td>618 ± 28.0</td>
<td>2185 ± 258</td>
</tr>
<tr>
<td>0.5</td>
<td>64.4 ± 3.0</td>
<td>648 ± 55.4</td>
<td>2126 ± 138</td>
</tr>
<tr>
<td>1</td>
<td>59.2 ± 3.8</td>
<td>526 ± 60.6</td>
<td>1984 ± 248</td>
</tr>
<tr>
<td>5</td>
<td>53.9 ± 3.9*</td>
<td>460 ± 18.9*</td>
<td>1438 ± 52*</td>
</tr>
<tr>
<td>10</td>
<td>46.1 ± 4.8**</td>
<td>400 ± 48.2**</td>
<td>1012 ± 151**</td>
</tr>
</tbody>
</table>

Values represent mean ± s.e.m. for 5 animals.
*P < 0.05; **P < 0.01 compared with vehicle-treated controls.

Table 2. Serum LH, prolactin and androgen (testosterone + DHT) concentrations and epididymal androgen (DHT and androstanediol) concentration in intact flutamide-treated rats

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Serum LH (ng/ml)</th>
<th>Serum androgen (ng/ml)</th>
<th>Serum prolactin (ng/ml)</th>
<th>Epididymal DHT (ng/g)</th>
<th>Epididymal androstanediol (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (controls)</td>
<td>122 ± 30</td>
<td>2.30 ± 0.66</td>
<td>36.0 ± 2.6</td>
<td>6.01 ± 1.25</td>
<td>82.4 ± 11.0</td>
</tr>
<tr>
<td>0.5</td>
<td>225 ± 22</td>
<td>2.02 ± 0.50</td>
<td>28.3 ± 4.5</td>
<td>9.41 ± 0.94</td>
<td>85.2 ± 5.3</td>
</tr>
<tr>
<td>1</td>
<td>238 ± 73</td>
<td>2.99 ± 0.65</td>
<td>29.8 ± 2.6</td>
<td>10.6 ± 0.70</td>
<td>101.9 ± 9.2</td>
</tr>
<tr>
<td>5</td>
<td>375 ± 73**</td>
<td>4.47 ± 1.20</td>
<td>30.6 ± 5.4</td>
<td>13.7 ± 1.75*</td>
<td>129.6 ± 16.8</td>
</tr>
<tr>
<td>10</td>
<td>462 ± 63**</td>
<td>7.72 ± 1.16**</td>
<td>32.2 ± 5.4</td>
<td>23.1 ± 3.94**</td>
<td>159.6 ± 28.9**</td>
</tr>
</tbody>
</table>

Values represent mean ± s.e.m. for 5 animals.
*P < 0.05; **P < 0.01 compared with vehicle-treated controls.

To evaluate possible changes in the hormonal environment after flutamide treatment, serum LH, prolactin and androgen concentrations were measured. Serum LH concentrations increased in flutamide-treated rats, resulting in high values of serum androgens. No significant differences in prolactin concentrations were observed at any of the doses tested (Table 2).

Concentrations of DHT and androstanediol were effectively increased in the epididymis after treatment with flutamide in parallel to serum androgen changes (Table 2). Therefore, the ratio

\[ F' = F \times \frac{(DHT)_o}{(DHT)} \]

where \( F \) = flutamide dose, \((DHT) =\) epididymal DHT concentration in experimental animals, \((DHT)_o\) = epididymal DHT concentration in control animals, is a more reliable index of the effective dose of the antiandrogen. As seen in Fig. 3, a linear correlation exists between epididymal ODC activity and log \( F' \).

To evaluate the effect of flutamide in the presence of constant androgen concentrations, 24 h-castrated rats were injected with testosterone propionate with increasing doses of flutamide (Table 3). In contrast with results in intact rats, a sharp decrease was observed in epididymal weight at the dose of 0.5 mg, and reached 58% at 10 mg. Table 3 also shows that the androgen dose administered was effective in maintaining normal values of activity. In addition, flutamide provoked a pronounced decay of activity even at low doses. At the dose of 1 mg, epididymal ODC was no longer detected.
Fig. 3. Correlation between the effective dose of flutamide $F'$ and epididymal ODC activity. Intact rats were treated with flutamide (0.5–10 mg/day). $F'$ values were calculated from epididymal DHT concentrations attained at each dose of the antiandrogen (see text for details). Correlation coefficient, $r = 0.997$.

Table 3. Effect of flutamide on epididymal weight and ODC activity in androgen-treated castrated rats

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Epididymal wt (mg/100 g body wt)</th>
<th>Epididymal ODC activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pmol/h/mg protein</td>
</tr>
<tr>
<td>0 (controls)</td>
<td>65.1 ± 6.5</td>
<td>839 ± 89.4</td>
</tr>
<tr>
<td>0.5</td>
<td>42.5 ± 4.6*</td>
<td>331 ± 27.4*</td>
</tr>
<tr>
<td>1</td>
<td>34.6 ± 4.2*</td>
<td>138 ± 10.9*</td>
</tr>
<tr>
<td>5</td>
<td>32.2 ± 1.7*</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>27.3 ± 1.6*</td>
<td>ND</td>
</tr>
</tbody>
</table>

Castrated rats were treated with 0.05 mg testosterone propionate/day together with flutamide at the doses indicated. Values represent mean ± s.e.m. for 5 animals. ND = not detected.

$*P < 0.01$ compared with vehicle-treated controls.

Discussion

The present results show that ODC is under strict androgenic control in the rat epididymis: ODC activity is decreased by more than 70% as early as 12 h after orchidectomy. The observed delay in ODC response to castration is in excellent agreement with a report by Aafjes & Vreeburg (1972) who showed that, after castration, DHT disappeared from the epididymis with a half-life of 4–5 h, and so epididymal DHT content would fall about 80% within 12 h. Aafjes & Vreeburg (1972) also reported that DHT content is reduced to about 30% by 6 h after orchidectomy. In the present study, ODC was unaffected at that time, indicating that endogenous DHT content is much greater than necessary to cause maximal stimulation of ODC, at least in the pubertal rat. The lack of effect of exogenous testosterone on ODC in intact rats (Fig. 2) strongly supports this concept. The high sensitivity of epididymal ODC to androgens is revealed by the fact that 0.05 mg testosterone propionate, administered every 2 days, restored ODC to normal values in 24-h castrated animals. However, a difference exists between the androgen response of rats that had been castrated 24 h and 7 days before treatment. A 2-fold stimulation of ODC, compared to intact controls, in the
latter case at the dose of 0-2 mg testosterone propionate, has been previously reported (de las Heras & Calandra, 1987), whereas in the present study the response was less marked. One possible explanation for this difference in sensitivity is that long-term castration may result in the appearance of an androgen receptor population showing a higher affinity constant value, and immediate androgen replacement would prevent this effect. This assumption is in accordance with a report (Tezón & Blaquier, 1983) showing that castration for 6 days leads to an increase in $K_a$ of epididymal cytosolic androgen receptors, whereas this effect was not observed at 2 days after castration.

At present, flutamide is the only antiandrogen acting at the receptor level without any other hormonal activity (Raynaud et al., 1979; Schmidt & Katzenellenbogen, 1979; Simard et al., 1986). In mouse kidney, it has been shown that flutamide inhibits the androgen induction of ODC and ODC-mRNA (Kontula et al., 1985). In our study, flutamide was effective in reducing the weight of the epididymis as well as ODC activity in this organ. However, high doses were necessary to achieve this effect. Two factors could account for this low response to the antiandrogen. First, epididymal androgen concentrations are higher than in other androgen-responsive tissues (Pujol et al., 1976). Androgen is supplied to the epididymis by the intraluminal fluid and via the systemic circulation (Pujol et al., 1976). As shown in Fig. 2, low androgen levels reaching the epididymis via systemic blood are sufficient to maintain ODC activity. Therefore, the epididymal lumen might act as a reservoir of androgens, thus preventing flutamide competition for androgen receptors. Second, epididymal androgen content is further increased by flutamide, presumably as a result of the rise in androgen production, which results, in turn, from a reflex increase in LH by the action of the antiandrogen at the pituitary level (Södersten et al., 1975; Poyet & Labrie, 1985). This is strongly supported by the severe decline in ODC activity when flutamide is administered to castrated, androgen-treated rats.

Since prolactin has been shown to increase the nuclear concentration of DHT in the epididymis of hypophysectomized rats (Baker et al., 1979) and specific prolactin receptors have been described in this organ (Orgebin-Crist & Djiane, 1979), an increase in prolactin concentrations could result in an enhanced sensitivity to androgens. This possibility was, however, ruled out since serum prolactin values remained unaltered by flutamide treatment.

Several enzymic activities have been used in an attempt to monitor the metabolic activity of the epididymis under different hormonal backgrounds (Rastogi et al., 1979; Brooks, 1981). However, most of them are not a clear reflection of the androgenic status of the organ, as they are unaffected or even increased by long-term castration (Rastogi et al., 1979). Moreover, the activity of glycolytic enzymes is reduced after castration (Brooks, 1976), but the time required to reach a steady state is rather long, probably due to their low turnover rate. ODC has an unusually short half-life (Tabor & Tabor, 1984; Russell, 1985), and so its activity is rapidly modified when synthesis and/or degradation are altered. Therefore, a rapid ODC response to androgen withdrawal was expected to occur, as shown herein.

In summary, we have shown that ODC is a sensitive marker for the study of androgen action on the epididymis. Further studies on the relationship between polyamine metabolism and other metabolic pathways will contribute to elucidation of the mechanism of androgen action in this organ, as well as the involvement of polyamines in sperm maturation and storage.

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References


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