ACTION OF TESTOSTERONE AND TESTOLOLACTONE ON ISOCITRIC DEHYDROGENASE, ASPARTATE TRANSCARBAMYLASE AND GULONATE NADP OXIDOREDUCTASE IN THE RAT IN VIVO

By

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ABSTRACT

The action of testosterone and testololactone, alone or in combination, was tested in castrated male rats in vivo. No effect was found on isocitric dehydrogenase activity in the ventral prostates and seminal vesicles of the rats or on gulonate NADP oxidoreductase in the liver or aspartate transcarbamylase in the kidney. It was shown that the effect of aspartate transcarbamylase in the liver is testosterone-dependent and that testololactone which has no androgenic properties inhibits the effect of testosterone on the aspartate transbarbamylase in the liver.

The action of androgens, administered in vivo, on the enzyme contents of several organs is well known (Mann & Lutwak-Mann 1951). Testololactone is a steroid the structure of which is similar to that of testosterone but which has no androgenic properties. However, it has about the same action as testosterone on metastatic female breast cancer (Segaloff et al. 1960; Groupe Européen du Cancer du Sein 1962). It thus seemed of interest to compare the action of these steroids on several enzymes in order to see whether these two compounds exert their action through a common metabolic pathway.

METHODS

Male rats, C. D. Charles River, weighing from 300 to 400 g were used. They were castrated and injected according to a randomized plan. Normal non-castrated rats were used as controls.
The rats were castrated on the first day of the experiment. From the third to the eighth day they were injected daily with 0.2 ml of a solution of physiological saline containing in suspension either 1 mg testosterone, 10 mg testololactone or 1 mg testosterone + 10 mg testololactone.

The rats were killed on the eighth day by a blow on the neck. The sexual organs (ventral prostates and seminal vesicles) of 9 rats from each group were pooled in order to obtain an adequate weight of these organs. The livers and kidneys of 3 rats were pooled when the enzyme contents of these tissues were determined.

The determination of isocitric dehydrogenase (ICD) was according to the method of Wolfson & Williams-Ashman (1957). The activity is expressed in \( \mu \text{M} \text{NADPH}_2 \) transformed per min per g wet weight. The determination of gulonate NADP oxidoreductase was according to the method of Salomon & Stubbs (1961). (Units are expressed in \( \mu \text{M} \text{NADPH}_2 \) oxidized per min per g wet weight).

The determination of aspartate transcarbamylase follows the method of Bresnick & Mosse (1966). (Units were expressed in \( \text{nM} \) aspartate transformed per 15 min per g wet weight.

The determination of DNA follows the method of Dische (1955).

RESULTS

Action on isocitric dehydrogenase activity

Table 1 gives results which are representative of those generally obtained. We performed 4 experiments each with 5 determinations (intact rats, castrated rats, rats injected with testosterone, rats injected with testololactone, rats injected with both steroids).

We did not observe any statistically significant effect of castration, testosterone or testololactone on the activity of isocitric dehydrogenase in the ventral prostates and seminal vesicles of the rats.

Action on aspartate transcarbamylase activity in the kidney

We performed 12 experiments. Table 1 gives the results which are representative of those generally obtained. Whether expressed as per g organ or \( \mu \text{g} \) DNA, it was found that castration, administration of testosterone, testololactone or both substances together had no effect on the enzyme activity.

Action on aspartate transcarbamylase in the liver

12 experiments were performed. Table 1 gives representative results. Castration diminished the activity \( (P = 0.02) \). Administration of testosterone to castrated rats increased the activity again \( (P = 0.001) \). Administration of testololactone alone had no effect. Administration of testololactone together with testosterone had an inhibitory effect on testosterone \( (P = 0.001) \). If the activities are expressed as \( \text{nM} \) aspartate transformed per 15 min per \( \mu \text{g} \) DNA, one finds a decrease of activity with castration \( (P = 0.001) \), an increase with
Table 1.
Typical results of the determination of the enzymes.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>TC</th>
<th>T</th>
<th>TL</th>
<th>T + TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICD in the ventral prostates</td>
<td>6.80</td>
<td>8.43</td>
<td>6.55</td>
<td>3.80</td>
<td>4.65 (\mu\text{m} \text{NADPH}_2) transformed per min per g wet weight</td>
</tr>
<tr>
<td>ICD in the seminal vesicles</td>
<td>4.38</td>
<td>6.50</td>
<td>2.88</td>
<td>8.50</td>
<td>2.95 nm aspartate transformed per 15 min per g wet weight</td>
</tr>
<tr>
<td>Aspartate transcarbamylase in the liver</td>
<td>630</td>
<td>600</td>
<td>720</td>
<td>1070</td>
<td>390</td>
</tr>
<tr>
<td>Aspartate transcarbamylase in the kidney</td>
<td>2470</td>
<td>1610</td>
<td>2490</td>
<td>2260</td>
<td>2200</td>
</tr>
<tr>
<td>Gulonate NADP oxidoreductase in the liver</td>
<td>96</td>
<td>92</td>
<td>92</td>
<td>99</td>
<td>80 (\mu\text{m} \text{NADPH}_2) oxidized per min per g wet weight</td>
</tr>
</tbody>
</table>

NC : normal non-castrated rats
TC : castrated rats
T : castrated rats + testosterone
TL : castrated rats + testolactone
T + TL: castrated rats + testosterone + testolactone.
the administration of testosterone \((P = 0.001)\) and an inhibition with the administration of both steroids \((P = 0.01)\).

Action on gulonate NADP oxidase of the liver

12 experiments were performed. No statistical significant effect of castration or of hormone administration were found.

**Discussion**

It is well known that testosterone increases the activity of citric acid in the male accessory organs. No effect was, however, found on isocitric dehydrogenase. Our results show that neither testosterone or testololactone had any effect on its activity. Gulonate NADP oxidoreductase plays a role in the reduction of glucuronate into gulonate and as such represents a pathway in the synthesis of ascorbic acid. It was shown that this acid was diminished in the castrated rat as well as in the hypophysectomized rat (Stubbs et al. 1967). Our results showed no effect by the steroids on gulonate oxidase activity.

Aspartate transcarbamylase is the enzyme which catalyses the irreversible transcarbamylation of aspartic acid with carbamyl phosphate. This enzyme is increased in several animal as well as in human tumours (Calva et al. 1958; Van Rymenant et al. 1968). Our results show that this enzyme activity is testosterone-dependent in the liver of the rat. It is interesting to see that testololactone has an inhibitory effect on this stimulation due to testosterone. This could be due to some competition for the binding sites of the hormones. Further work is necessary in order to clarify this finding.

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**References**

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