EFFECT OF MALE SEX HORMONES ON ASCORBIC ACID METABOLISM IN RATS

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Castrated male rats were injected intramuscularly with testosterone (5 mg/day) and dehydroepiandrosterone (5 mg/day) continuously for two weeks. Animals were sacrificed and the various metabolites of ascorbic acid in liver and urine were determined. Total ascorbic acid and the activities of hepatic ascorbic acid synthesizing enzymes were also determined. The activities of both liver and kidney ascorbic acid degrading enzymes were studied.

In contrast to testosterone, dehydroepiandrosterone failed to modify the ascorbic acid metabolism in castrated rats. Testosterone administration to castrated rats was found to restore the hepatic contents of ascorbic acid metabolites to the sham control levels. It was suggested that the functional OH group at C-17 in androgens might have some role in ascorbic acid metabolism.

Castration was found to cause decrease in the activities of two out of the three biosynthetic enzymes of ascorbic acid, namely D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase in rat liver. The activity of L-gulono-γ-lactone hydrolase was found to be unchanged in castration. This result which is in slight disagreement with the earlier work of STUBBS et al. has been discussed. Testosterone was observed to restore the decrease in the activities of both D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase in castrated rats. Testosterone was also found to restore the increased activity of both renal and hepatic dehydroascorbatase observed in castration to the level of sham control rats.

However, exogeneous administration of testosterone intramuscularly (5 mg/day) for two weeks to normal rats did not show any significant alteration in the ascorbic acid metabolism.

Sex hormones have been known to be associated with ascorbic acid metabolism in various animals. BISWAS (1) had reported an increase in the renal biosynthesis of ascorbic acid both in normal and castrated toads following testosterone admini-
istration. The content of liver and kidney ascorbic acid was also found to be elevated in capons treated with testosterone (2). Storage of adrenal ascorbic acid was observed by Jakowicki (3) to be increased by treatment with progesterone in gonadectomized rats. Recently Khandwekar et al. (4) demonstrated that castration in rats decreased the ascorbic acid and dehydroascorbic acid contents with simultaneous increase in the diketogulonic acid content of liver. They also observed that the degradation of ascorbic acid was enhanced in castrated rats. Testicular secretions were suggested to be the responsible factor in the maintenance of higher tissue levels of ascorbic acid as well as the higher activities of biosynthetic enzymes in rats by Stubbs et al. (5).

This communication, therefore, deals with a detailed study of ascorbic acid synthesis and degradation in castrated and normal rats when administered with male sex hormones, namely testosterone and dehydroepiandrosterone.

MATERIALS AND METHODS

D-Glucurone-δ-lactone and L-gulono-γ-lactone were obtained from Sigma Chemical Co., and 2, 3-diketogulonate was prepared by the method of Kagawa (6). Testosterone and Dehydroepiandrosterone were purchased from V. P. Chest Institute, Delhi. All the chemicals were of analytical grade.

Male albino rats (100–125 g body wt) were maintained on a stock laboratory diet (7) and distributed into six groups. Bilateral castration was carried out for group II, III and IV rats, while rats of group I served as sham-operated controls. Rats of group III and IV were administered testosterone 5 mg/day in 0.1 ml of corn oil and ethanol mixture (8:2) and with dehydroepiandrosterone 5 mg/day in the same solution respectively. The rats of other groups were also administered 0.1 ml of the corn oil-ethanol mixture for proper control. The experiments were carried out for two weeks. Rats were killed by decapitation and blood was collected in oxalate tubes for total ascorbic acid estimation. Liver and kidney were removed, rinsed in ice cold water and blotted dry. Part of the tissue was homogenized in 9 volumes of isotonic sucrose. The homogenate was centrifuged at 10,000 × g at 0°C for 20 min to obtain tissue extract free of heavy particles and the supernate thus obtained was again centrifuged at 100,000 × g at 0°C for 1 hr to yield microsomes and soluble fractions. Different estimations were carried out as follows.

Ascorbic acid, dehydroascorbic acid and diketogulonic acid were estimated by the method of Roe et al. (8) in liver, blood and urine (24 hr urine samples collected in 10 percent oxalic acid).

Ascorbic acid-Synthesizing enzymes D-gulucurono-δ-lactone hydrolase, (EC 3.1.1.19), L-Gulono-γ-lactone hydrolase (EC 3.1.1.18) and L-gulono-δ-lactone oxidase (EC 1.1.3.8) were estimated by the method of Salomon and Stubbs (9–10).

Ascorbic acid-degrading enzymes dehydroascorbatase and 2, 3-diketoalde-
Table 1. Ascorbic acid (AA), dehydroascorbic acid (DHA), diketogulonic acid (DKA) contents of liver, urine and blood in rats. Values are expressed as mean ± SEM of six rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Urine</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (mg/100 g fresh liver)</td>
<td>DHA (mg/100 ml urine)</td>
<td>DKA (mg/100 ml blood)</td>
</tr>
<tr>
<td>I Sham operated</td>
<td>26.5±0.66</td>
<td>4.1±0.09</td>
<td>1.3±0.05</td>
</tr>
<tr>
<td>II Castrated</td>
<td>17.6±0.45</td>
<td>2.3±0.16</td>
<td>8.2±0.78</td>
</tr>
<tr>
<td>III Castrated + test</td>
<td>24.2±0.42*</td>
<td>3.5±0.08*</td>
<td>3.2±0.46*</td>
</tr>
<tr>
<td>IV Castrated + dehydropi-</td>
<td>18.4±0.16</td>
<td>2.9±0.85</td>
<td>7.9±0.59</td>
</tr>
<tr>
<td>Normal</td>
<td>27.86±1.89</td>
<td>5.69±0.35</td>
<td>1.82±0.09</td>
</tr>
<tr>
<td>VI Normal + testosterone</td>
<td>25.71±1.77</td>
<td>5.89±0.35</td>
<td>1.79±0.05</td>
</tr>
</tbody>
</table>

* p < 0.001 as compared to group II using student's t-test.
nate decarboxylase were estimated by the method of Kagawa (6, 11). Protein was determined by the method of Lowry (12). The details of these estimations and respective enzyme units were the same as described earlier (13).

RESULTS

The significant alterations in the metabolites of ascorbic acid in urine and liver of castrated rats reported in Table 1 are in accordance with our earlier communication (4). It is also evident from Table 1 that testosterone administration to castrated rats resulted in significant restoration ($p<0.001$) of the hepatic ascorbic acid metabolites to the level of the sham control rats. The urinary contents of ascorbic acid metabolites showed no significant alterations. However, dehydroepiandrosterone administration failed to modify the altered ascorbic acid meta-

Table 2. Effect of testosterone on the activities of some ascorbic acid-synthesizing enzymes in liver of castrated rats. Values are expressed as mean ± SEM of six rats. Respective enzyme activities are denoted in text.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D-Glucuronolactone hydrolase</th>
<th>L-Gulono-δ-lactone hydrolase Specific activity</th>
<th>L-Gulono-γ-lactone oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham controls</td>
<td>952±28</td>
<td>1,398±35</td>
<td>1.383±0.005</td>
</tr>
<tr>
<td>II Castrated</td>
<td>760±24</td>
<td>1,348±27</td>
<td>0.902±0.011</td>
</tr>
<tr>
<td>III Castrated+ testosterone</td>
<td>830±16**</td>
<td>1,355±22</td>
<td>1.211±0.012*</td>
</tr>
<tr>
<td>V Normal</td>
<td>966±39</td>
<td>1,385±39</td>
<td>1.281±0.004</td>
</tr>
<tr>
<td>VI Normal+ testosterone</td>
<td>948±42</td>
<td>1,379±50</td>
<td>1.266±0.007</td>
</tr>
</tbody>
</table>

* $p<0.001$ as compared to group II using student’s t-test.
** $p<0.05$ as compared to group II using student’s t-test.

Table 3. Effect of testosterone on the activities of ascorbic acid-degrading enzymes in liver and kidney of castrated rats. Values are expressed as mean ± SEM of six rats. Respective enzyme activities are denoted in text.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dehydroascorbate</td>
<td>2,3-Diketoaldonate decarboxylase Specific activity</td>
</tr>
<tr>
<td>I Sham controls</td>
<td>0.105±0.009</td>
<td>11.52±0.50</td>
</tr>
<tr>
<td>II Castrated</td>
<td>0.259±0.012</td>
<td>11.59±0.55</td>
</tr>
<tr>
<td>III Castrated+ testosterone</td>
<td>0.195±0.015*</td>
<td>11.56±0.51</td>
</tr>
<tr>
<td>V Normal</td>
<td>0.108±0.005</td>
<td>11.69±0.58</td>
</tr>
<tr>
<td>VI Normal+ testosterone</td>
<td>0.107±0.004</td>
<td>11.40±0.65</td>
</tr>
</tbody>
</table>

* $p<0.001$ as compared to group II using student’s t-test.
EFFECT OF MALE SEX HORMONES IN RATS

It is evident from Table 2 that two out of the three ascorbic acid synthesizing enzymes, namely D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase, were conspicuously decreased in castrated rats. The activity of the hepatic L-gulono-γ-lactone hydrolase was found to be unaffected by castration. Testosterone was found to increase the activities of D-glucurono-δ-lactone hydrolase (p<0.05) and L-gulono-γ-lactone oxidase (p<0.001), when administered to castrated rats (Table 2). It was also observed that testosterone caused significant decrease (p<0.001) in the activities of renal and hepatic dehydroascorbatase which is increased in castration. Exogenous administration of testosterone to normal rats did not show any effect on either ascorbic acid synthesizing or degrading enzymes as evidenced from all the three tables.

DISCUSSION

Recently Khandwekar et al. (4) have reported alterations in the metabolites of ascorbic acid in castrated rats. It is evident from our present results that such alterations of ascorbic acid metabolites in castration were restored to normal levels by testosterone administration. It is also clear that dehydroepiandrosterone the other androgenic hormone did not have any significant effect on ascorbic acid metabolism in castrated rats. The alterations in the ascorbic acid metabolism in necrotic rats were recently found to be restored by orabolin (ethylestrenol), a synthetic androgen (14). Both testosterone and orabolin have in common an OH group at C-17. On the other hand androsterone and dehydroepiandrosterone have a keto group at C-17. It is therefore felt that the functional OH group at C-17 in androgens might have some role in ascorbic acid metabolism. This view is in slight disagreement with the earlier suggestion that functional groups as C-3 and C-17 in androgens are apparently not essential for biological activity (15).

Stubbs et al. (5) had earlier reported a significant decrease of all the four hepatic biosynthetic enzymes of ascorbic acid in castrated rats. Our present study, however, suggests that L-gulono-γ-lactone hydrolase is not at all affected by castration, although the activity of other biosynthetic enzymes is found to be decreased. Continuous testosterone administration to castrated rats for two weeks was found to restore to a significant extent the decreased activity of the hepatic D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase. It is difficult at present to suggest any possible reasons for the discrepancy noted in the activity of L-gulono-γ-lactone hydrolase in castration. It may however be noted that whereas Stubbs et al. (5) used matured male rats, we have used young male rats for the experimental purpose. It may also be mentioned here that out of the four biosynthetic enzymes of ascorbic acid, somatotropin was found to control only L-gulono-γ-lactone hydrolase in rats (16). This however does not extensively suggest that somatotropin regulates solely the above enzyme in rats of all ages. It may be
probable that the control of this enzyme in young rats is rendered by somato-
tropin and in matured rats the responsibility is assumed by testosterone. At the
present juncture the above suggestion needs to be examined carefully.

Dehydroascorbatase one of the two degrading enzymes of ascorbic acid was
found to be increased significantly in castrated animals, which is in accordance
with our earlier report (4). Testosterone administration to castrated rats was
found to bring about significant decrease in this enzyme activity both in liver and
kidney. A similar restoration effect of hepatic dehydroascorbatase activity by
another anabolic hormone, namely orabolin in necrotic rats has already been
reported by Banerjee et al. (14).

Exogenous administration of testosterone propionate in cockerels for 4
days was found to decrease the renal and hepatic ascorbate (17). We could not,
however, detect any significant alteration in the ascorbic acid metabolism in
normal rats treated with testosterone continuously for 2 weeks.

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