Metallothionein in male reproductive organs of adrenalectomized and hydrocortisone-treated Wistar rats

Neena Nair & R S Bedwal*
Cell Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302 004, India
Received 8 August 2003; revised 3 December 2003

Adrenalectomy resulted in an increase in metallothionein (MT) levels in testes, caput and cauda epididymis and prostate of rats but not in seminal vesicles where its levels decreased significantly. Inspite of administration of hydrocortisone, MT in testes, prostate (1.2 mg), caput (0.3 mg days 2, 8; 0.6 mg and 1.2 mg) and seminal vesicles (0.3 mg day 2, 4; 0.6 mg and 1.2 mg) remained increased. Thus adrenal insufficiency/hydrocortisone has no direct influence on MT levels. However, the increased levels of MT can be related to its ability to protect the cells from free radical damage caused by atrophy of reproductive tissues in adrenalectomised rats. Exogenously administered hydrocortisone to ADX rats resulted in return to ADX state as hydrocortisone metabolizes (half-life < 12 hr) and hence MT levels remained increased. The observations could provide a clue for the physiological functioning of the male reproductive tissue in a state of adrenal deprivation and hormonal supplementation.

Keywords: Adrenalectomy, Hydrocortisone, Metallothionein, Rat, Reproductive organ

Acute stress activates two principal components: hypothalamic-pituitary-adrenal (HPA) axis and sympatho-adrenomedullary system1,2. Adrenalectomy has been shown to abolish HPA axis induced suppression of gonadotropin3. However, influence on HPA axis is not always inhibitory but depends on the interaction of two neuroendocrine axes4. In vivo and in vitro studies on pituitary cells have revealed that glucocorticoid suppresses basal and/or gonadotropin-releasing hormone (GnRH) stimulated LH release5. Reports with testosterone are less consistent as increases6 and decreases7 have been observed. Glucocorticoid receptor like immunoreactivity (GR-LI) has been localized on Leydig cells, early pachytyene and zygotyone primary spermatocytes8, peritubular myoid cells, fibroblasts and basal cells of epididymis9 and prostate10. Glucocorticoids are the major regulators of metallothionein (MT)-1, II (particularly in some brain areas) and III11,12. Metallothionein, a ubiquitous polypeptide, cysteine rich low molecular weight intracellular protein of 6 kDa that has high affinity for divalent metals Cd²⁺ and Zn²⁺ (Hildebrand et al.16), plays an important role in (1) detoxification of heavy metals, (2) homeostatic regulation of essential metals and (3) cellular defense against reactive oxygen species or free oxygen radical16,18. Several in vivo and in vitro experiments such as radiation and high oxygen tension cause induction of MT synthesis18,19. MT's are reported to be present in high concentration in the nucleus and their excellent hydroxyl scavenging properties confer protection of DNA from hydroxyl radical attacks20.

The aim of the present study is to evaluate metallothionein status in adrenalectomy and hydrocortisone treated adrenalectomised male Wistar rats.

Materials and Methods
Colony bred male Wistar rats (120) weighing 270-332 g were maintained in a well-ventilated animal room (12: 12 hr L: D) in polypropylene cages with stainless steel grills.

Experimental Protocol
Rats were divided into following 6 groups: group 1: sham operated (SO), group 2: adrenalectomised (ADX), group 3: adrenalectomised control (ADX-C), group 4: adrenalectomised + hydrocortisone (0.3 mg), group 5: adrenalectomised + hydrocortisone (0.6 mg), group 6: adrenalectomised + hydrocortisone (1.2 mg).

Sham operations and bilateral adrenalectomies were performed under light ether anaesthesia by dorsal approach21. The peritoneum and dermis were sutured by cat gut thread, while ethicon silk thread (No. 30) was used for epidermis. Rats had free access to standard food (Aashirwad Feed Ltd., Chandigarh, India) and 0.9% NaCl solution ad libitum. Prior to commencement of the experiments, the rats (adrenalectomized) were left for a recovery period of

*Correspondent author:
Phone : 0141-2711158
7 days. Hydrocortisone (total dosages of 0.3, 0.6 and 1.2 mg/animal) was then administered sc (as fractionated) for 2, 4, 8 and 16 days in olive oil whereas the ADX-C (bilaterally adrenalectomised and left for a recovery period of 7 days) were administered with vehicle i.e. olive oil only. The animals were autopsied under light ether anaesthesia after 24 hr of the last injection. Tissues viz., testes, caput and cauda epididymis, prostate and seminal vesicles were excised, trimmed off of extraneous tissues and weighed on an electronic balance.

MT was estimated by Onaska and Cherian22 and Scheuhammer and Cherian22. Briefly, MT in the samples (cytosolic fraction of various male reproductive organs) was saturated by addition of Cd and the excess of Cd and other Cd-bound proteins were removed by binding with rat red blood hemolysate, heat treatment and precipitation while Cd-bound MT, being heat stable, remained in the supernatant and was estimated as Cd by Scheuhammer and Cherian23. Metallothionein (MT's, with mol. wt. 6-7 kDa, 61-83 amino acids of which cysteine residues) bind

### Table 1-Metallothionein (µg/MT/g wet weight) estimation in adrenalectomized, adrenalectomized + hydrocortisone treated male Wistar rats

[Values are mean ± SE]

<table>
<thead>
<tr>
<th>Days</th>
<th>Sham-operated</th>
<th>Adrenalectomy</th>
<th>ADX-C</th>
<th>ADX + 0.3 mg</th>
<th>ADX + 0.6 mg</th>
<th>ADX + 1.2 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>12.43 ± 0.14</td>
<td>67.1 ± 0.14Aa</td>
<td>9.42 ± 0.22</td>
<td>20.87 ± 0.34Bd</td>
<td>29.72 ± 0.84Bd</td>
<td>51.03 ± 0.21Bd</td>
</tr>
<tr>
<td>Caput</td>
<td>16.73 ± 0.14</td>
<td>71.01 ± 0.82Aa</td>
<td>10.22 ± 0.14</td>
<td>17.31 ± 0.11Bd</td>
<td>48.71 ± 0.21Bd</td>
<td>50.36 ± 0.11Bd</td>
</tr>
<tr>
<td>Cauda</td>
<td>38.14 ± 0.14</td>
<td>61.86 ± 0.86Aa</td>
<td>5.74 ± 0.30</td>
<td>9.28 ± 0.04Bd</td>
<td>15.07 ± 0.16Bd</td>
<td>38.97 ± 0.17Bd</td>
</tr>
<tr>
<td>Prostate</td>
<td>14.36 ± 0.12</td>
<td>34.46 ± 0.41Aa</td>
<td>22.15 ± 0.56</td>
<td>13.09 ± 0.13Bd</td>
<td>21.58 ± 0.86Bd</td>
<td>31.83 ± 0.11Bd</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>67.31 ± 0.01</td>
<td>13.39 ± 0.41Aa</td>
<td>7.97 ± 0.15</td>
<td>11.74 ± 0.34Bd</td>
<td>18.33 ± 0.10Bd</td>
<td>19.62 ± 0.20Bd</td>
</tr>
<tr>
<td>Testes</td>
<td>14.59 ± 0.53</td>
<td>79.8 ± 0.94Aa</td>
<td>14.49 ± 1.49</td>
<td>40.40 ± 2.27Bd</td>
<td>44.85 ± 0.17Bd</td>
<td>55.20 ± 0.53Bd</td>
</tr>
<tr>
<td>Caput</td>
<td>17.41 ± 0.56</td>
<td>72.02 ± 6.30Aa</td>
<td>18.88 ± 0.40</td>
<td>15.44 ± 1.50Bd</td>
<td>45.54 ± 0.46Bd</td>
<td>48.27 ± 0.21Bd</td>
</tr>
<tr>
<td>Cauda</td>
<td>39.30 ± 0.12</td>
<td>61.34 ± 6.60Aa</td>
<td>14.30 ± 0.15</td>
<td>10.41 ± 0.11Bd</td>
<td>24.17 ± 0.60Bd</td>
<td>47.92 ± 0.91Bd</td>
</tr>
<tr>
<td>Prostate</td>
<td>18.45 ± 0.12</td>
<td>36.69 ± 0.94Aa</td>
<td>22.18 ± 0.14</td>
<td>14.01 ± 0.11Bd</td>
<td>21.81 ± 0.13Bd</td>
<td>37.41 ± 0.10Bd</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>56.75 ± 0.69</td>
<td>12.21 ± 0.53Aa</td>
<td>7.13 ± 0.24</td>
<td>13.14 ± 0.35Bd</td>
<td>22.11 ± 0.30Bd</td>
<td>19.62 ± 0.20Bd</td>
</tr>
<tr>
<td>Testes</td>
<td>16.38 ± 0.57</td>
<td>80.92 ± 0.22Aa</td>
<td>24.27 ± 0.11</td>
<td>51.24 ± 4.44Bd</td>
<td>55.73 ± 0.75Bd</td>
<td>57.34 ± 0.14Bd</td>
</tr>
<tr>
<td>Caput</td>
<td>44.00 ± 0.01</td>
<td>73.18 ± 6.10Aa</td>
<td>10.58 ± 0.22</td>
<td>13.21 ± 0.11Bd</td>
<td>35.41 ± 0.11Bd</td>
<td>39.16 ± 0.37Bd</td>
</tr>
<tr>
<td>Cauda</td>
<td>40.10 ± 0.11</td>
<td>74.37 ± 0.18Aa</td>
<td>16.23 ± 0.39</td>
<td>11.29 ± 0.15Bd</td>
<td>29.86 ± 0.10Bd</td>
<td>49.47 ± 0.15Bd</td>
</tr>
<tr>
<td>Prostate</td>
<td>28.26 ± 0.95</td>
<td>67.23 ± 3.1Aa</td>
<td>29.48 ± 0.11</td>
<td>14.06 ± 0.15Bd</td>
<td>24.21 ± 0.10Bd</td>
<td>39.10 ± 0.87Bd</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>18.49 ± 0.28</td>
<td>7.55 ± 0.16Aa</td>
<td>15.59 ± 0.09</td>
<td>15.30 ± 0.15Bd</td>
<td>48.83 ± 0.14Bd</td>
<td>49.08 ± 0.83Bd</td>
</tr>
<tr>
<td>Testes</td>
<td>19.31 ± 0.13</td>
<td>24.83 ± 0.29Aa</td>
<td>28.74 ± 0.26</td>
<td>60.88 ± 2.44Bb</td>
<td>61.65 ± 0.25Bd</td>
<td>67.28 ± 0.11Bd</td>
</tr>
<tr>
<td>Caput</td>
<td>53.71 ± 0.14</td>
<td>76.45 ± 6.02Aa</td>
<td>12.54 ± 0.18</td>
<td>12.10 ± 0.18Bb</td>
<td>20.31 ± 0.41Bb</td>
<td>24.46 ± 0.11Bd</td>
</tr>
<tr>
<td>Cauda</td>
<td>40.18 ± 0.14</td>
<td>83.63 ± 2.04Aa</td>
<td>24.35 ± 0.95</td>
<td>27.84 ± 0.25Bb</td>
<td>36.19 ± 0.15Bb</td>
<td>51.99 ± 0.12Bd</td>
</tr>
<tr>
<td>Prostate</td>
<td>37.39 ± 0.43</td>
<td>70.04 ± 3.09Aa</td>
<td>41.09 ± 0.13</td>
<td>15.74 ± 0.11Bb</td>
<td>30.18 ± 0.10Bb</td>
<td>47.185 ± 0.63Bd</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>17.34 ± 0.20</td>
<td>7.55 ± 0.21Aa</td>
<td>27.88 ± 0.46</td>
<td>18.74 ± 0.32Bb</td>
<td>67.05 ± 0.54Bd</td>
<td>71.17 ± 0.11Bd</td>
</tr>
</tbody>
</table>

A = sham operated vs adrenalectomy; B = ADX-C vs adrenalectomy + hydrocortisone
P values: * non-significant; † < 0.05; ‡ < 0.01; †† < 0.001

**Statistical analysis** — Student's t test was used for comparison of means.

**Results**

The results are presented in Table 1.

Metallothionein concentration generally increased in the tissues after adrenalectomy (group 2). Highly significant (P < 0.001) increase was evident on all days in testes, prostate (except on day 8 where the increase was non-significant), caput epididymis (except on day 16 where increase was significant and P < 0.01), and cauda epididymis (except on day 4 where increase was almost significant P < 0.05). On the other hand, a highly significant decrease was noticed in seminal vesicles of group 2 animals.

Hydrocortisone administration to adrenalectomised rats revealed an increase in metallothionein concentration of testes of group 4, 5 and 6, caput and cauda epididymis of group 5 and 6, prostate and seminal vesicles of group 4, 5 and 6. However, prostate MT of group 4 and 5 exhibited a decrease (Table 1).

**Discussion**

Metallothionein (MT's, with mol. wt. 6-7 kDa, 61-68 amino acids of with 20 as cysteine residues) bind
seven atoms of zinc or 12 atoms of copper/MT molecule under physiological conditions through thiolate bonds and act as intracellular “sink” to trap electrophiles, alkylating agents and free radicals. MT mRNAs and MT’s have been detected in testis, epididymis, prostate and seminal vesicles. In seminiferous tubes they have been localized in spermatocytes, spermatids, Sertoli cells and Leydig cells and have both specific and non-specific binding sites on the spermatozoal membranes. MT mRNA and MT have been reported to decrease in orchidectomized rats while administration of testosterone to orchidectomized rats restored both MT mRNA and MT thus indicating the dependence of MT mRNA and MT’s on testosterone in male reproductive organs. Similarly administration of glucocorticoids, production of cytokines such as IL-1 and 6 and INF in acute phase response induces MT and accumulates cellular zinc. Adrenalectomy has been reported to cause degenerative changes/atrophy in male reproductive organs (viz. testes, epididymis, prostate and seminal vesicles). Increased MT levels exhibit sensitivity to oxidative stress. As indicated by the present study the increased levels reflect oxidized state of metal thiolate clusters which could scavenge deleterious oxygen radicals. Administration of agents like chloroform, paraquat, H2O2 and radiations that induce oxidative stress or ROS and inflammatory stress caused by interleukin/cytokinin and interferon stimulated leukocytes for protection of host cells, lead to an increase in MT levels in several organs. Thus, there are ample reasons to suspect that MT’s may be involved in protection against oxidative damage caused by degeneration or atrophied conditions of reproductive tissues due to lowered testosterone level in adrenalectomized rats. However, decrease in seminal vesicle could not be explained.

Administration of different doses of hydrocortisone (i.e., 0.3, 0.6 and 1.2 mg/ml) did not reveal a consistent pattern of MT, yet in general a high level was estimated in male reproductive organs of rats. Oral administration of prednisone (synthetic cortisol) at a mean dose of 17.5 mg/day for an average of 6.9 years reduced serum testosterone level by 33% and free testosterone level by 40% (Reid et al.) while MacAdams et al. exhibited a decrease of 50% in testosterone level. Administration of hydrocortisone at these levels in the present study was insufficient, hence the animals returned to adrenalectomized state and thus degeneration persisted. As a homeostatic mediator, MT being an important antioxidant, increase in MT level reflects a general adaptation against oxidative stress.

Conclusively, the present study indicates that adrenalectomy increases MT concentrations in all the tissues of male reproductive organs except seminal vesicles. With administration of hydrocortisone (at 0.3, 0.6 and 1.2 mg/ml) MT remained unchanged in different tissues of male reproductive organs. Thus adrenal insufficiency (adrenalectomy) or administration of hydrocortisone has no direct influence on MT levels. However, the increased levels of MT can be related to (1) increased demands of zinc thionein by male reproductive organs and (2) to provide protection against oxidative damage caused by atrophy of reproductive tissue due to lowered testosterone levels in adrenalectomized rats. Moreover, exogenously administered hydrocortisone was metabolized within 12 hr (half life < 12 hr) and the animals returned to adrenalectomized state and thus MT levels, as was noticed in adrenalectomized state remained elevated.

Acknowledgement

Neena Nair thanks CSIR, New Delhi for the award of Pool Officer (SRA) and financial assistance.

References