ACID PHOSPHATASE ACTIVITY
IN THE RAT NEUROHYPOPHYSIS DURING
INCREASED LEVELS OF GONADOTROPHIC HORMONES,
IN DIABETES INSIPIDUS (BRATTLEBORO STRAIN)
AND AFTER WATER LOADING

By
G. J. Boer, F. W. van Leeuwen, D. F. Swaab
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The acid phosphatase activity of the rat neurohypophysis was measured during increased gonadotrophic hormone levels, in diabetes insipidus (DI) (Brattleboro strain) and after water loading, i.e. conditions that interfere with the function of the hypothalamo-neurohypophyseal system (HNS). In addition determinations of tissue protein, lipid and DNA and of water metabolism were performed. Neurohypophyseal acid phosphatase activity expressed on a dry weight basis increased under all conditions. For gonadectomized females, Brattleboro rats and water loaded males an increased water metabolism was observed. The increased acid phosphatase activity is interpreted as being related to disposal of release residues during stimulation of the HNS (increased gonadotrophic hormone levels and DI) and to disposal of neurosecretory material during inhibition (water loading).

The neurosecretory process of the hypothalamo-neurohypophyseal system (HNS) comprises synthesis of the hormones vasopressin and oxytocin and their "carrier" proteins, the neurophysins, in the neurons of the supraoptic and paraventricular nuclei (SON and PVN), their transport within granules along the axons and their storage and release in the neurohypophysis.
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The neurosecretory process of the hypothalamo-neurohypophyseal system (HNS) comprises synthesis of the hormones vasopressin and oxytocin and their "carrier" proteins, the neurophysins, in the neurons of the supraoptic and paraventricular nuclei (SON and PVN), their transport within granules along the axons and their storage and release in the neurohypophysis.
Gonadectomy and administration of hormones

Using Hypnorm® (Philips-Duphar) anaesthesia (0.05 ml/100 g im), 77 females were ovariectomized (OV) and 31 males were castrated (CAS). Sham operations (SHAM) were performed by touching the testes via the incision. All operations were performed between 10 and 12 a.m. 14 days prior to sacrifice.

Starting on the day of operation, 37 ovariectomized females received arachis oil (Brocades, 0.1 ml d. d., sc) and 40 females 2 μg oestradiol benzoate (OeB) (Dienformon in arachis oil, 0.1 ml d. d., sc). During the last 2 days prior to sacrifice, one group of 8 ovariectomized, OeB-treated females also received 3 times 100 μg lutetinizing hormone (LH) sc (expressed in amounts equivalent to NIH-LH-S1). The preparation used, ovine NIH-LH-S16 (kindly supplied by the National Institute of Arthritis and Metabolic Disease), was administered in 0.5 ml of physiological saline. Other groups within the same experiment received saline only. All injections were given once daily at 9 a.m. and the animals were killed 2 h after the last injection. The OV/OeB group was considered as control group for the animals that were ovariectomized only and for those that received OeB and LH (Swaab 1970; Swaab & Jongkind 1970, 1971). This pre-treatment of the female control group is necessary to avoid the oestrous cycle that was found to influence HNS activity considerably (Heller 1959; Swaab & Jongkind 1970).

Water intake and urine production were measured in the 24 h period (9 a.m.–9 a.m.) before gonadectomy and before decapitation.

Groups of 8 rats of each experimental condition were used only for microchemical determination (see below).

Brattleboro rats

Brattleboro rats (14) were housed for 24 h (9 a.m.–9 a.m.) in metabolic cages in order to determine whether they were heterozygous (HET) or homozygous (HOM) for diabetes insipidus (DI) (Sokol & Valtin 1965; Swaab et al. 1973). In addition 12 male Wistar rats were kept for 24 h in metabolic cages.

Water loading

During 48 h 5 male rats received 10 ml tap water by means of a stomach tube every 3 h starting at 2 p.m. Controls had to swallow the tube but did not receive water. During the last 3 h the animals were placed in metabolic cages.

Histochemistry

Microchemical techniques were performed in order to have the advantage of obtaining different data from the same neurohypophysis. After decapitation, the pituitary was removed within 2 min and immediately frozen in Freon-12 (−150°C). The tissues were stored at −80°C. To obtain unthawed, unfixed, unstained and histologically well-defined posterior lobe transversal sections (16 μm), the procedure of Lowry (1953) was applied. From the lyophilized cryostat sections small samples were dissected out free hand. The samples were weighed on quartz-fibre balances (Lowry 1953). Pieces of 0.2–4.0 μg tissue were used for all assays.

Acid phosphatase activity was quantitatively determined as described by Jongkind (1969) using a-naphthyl acid phosphate as substrate. Protein was determined by the method of Lowry et al. (1951) modified by Boer & Jongkind (1974). Bovine serum albumin (Sigma) was used as a standard. Total lipid content was measured according to Lowry (1953) by weighing a tissue sample before and after alcohol-hexane extrac-
tion (Boer & Jongkind 1974). DNA content was determined according to Kissane & Robins (1958) using calf thymus (type V, Sigma) as the standard.

All chemicals came from Merck, except α-naphthyl acid phosphate (Sigma) and the Folin reagent for the protein determination (British Drug House).

Statistics

The significance of differences between the various groups was tested using Student's t-test. The effect of gonadectomy on water metabolism was assessed by expressing

Table 1.
Level of acid phosphatase activity, protein, lipid and DNA content1) in rat neurohypophysis 14 days after gonadectomy in male and female rats, after 3 days of LH administration in females, in heterozygous and homozygous Brattleboro rats and after water loading for 48 h.

<table>
<thead>
<tr>
<th>Experimental condition2)</th>
<th>No.3)</th>
<th>Acid phosphatase (mmol/μg dry weight/h)</th>
<th>Protein (% dry weight)</th>
<th>Total lipid (% dry weight)</th>
<th>DNA (ng/μg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>8</td>
<td>209 ± 4.84)</td>
<td>55.1 ± 0.8</td>
<td>33.2 ± 2.3</td>
<td>11.2 ± 0.38</td>
</tr>
<tr>
<td>CAS</td>
<td>8</td>
<td>238 ± 7.75)</td>
<td>56.8 ± 1.2</td>
<td>33.8 ± 2.3</td>
<td>11.6 ± 0.56</td>
</tr>
<tr>
<td>OV/OeB</td>
<td>8</td>
<td>181 ± 4.2)</td>
<td>55.3 ± 1.5</td>
<td>29.3 ± 1.3</td>
<td>10.5 ± 0.18</td>
</tr>
<tr>
<td>OV</td>
<td>8</td>
<td>198 ± 5.45)</td>
<td>55.6 ± 1.5</td>
<td>34.8 ± 2.35</td>
<td>11.0 ± 0.34</td>
</tr>
<tr>
<td>OV/OeB/LH</td>
<td>8</td>
<td>206 ± 5.15)</td>
<td>55.7 ± 1.7</td>
<td>32.8 ± 1.35</td>
<td>9.39 ± 0.195</td>
</tr>
</tbody>
</table>

Increased gonadotropic hormone levels

Brattleboro

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar (C)</td>
<td>12</td>
<td>322 ± 9.7)</td>
<td>55.9 ± 2.1</td>
<td>31.6 ± 0.62</td>
<td>11.1 ± 0.51</td>
</tr>
<tr>
<td>HET-DI</td>
<td>7</td>
<td>381 ± 265)</td>
<td>49.1 ± 1.35</td>
<td>35.0 ± 0.745</td>
<td>12.3 ± 0.73</td>
</tr>
<tr>
<td>HOM-DI</td>
<td>7</td>
<td>463 ± 175)</td>
<td>45.9 ± 2.055</td>
<td>38.2 ± 0.865</td>
<td>19.5 ± 1.455</td>
</tr>
</tbody>
</table>

Water loading

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5</td>
<td>353 ± 36)</td>
<td>60.9 ± 2.5</td>
<td>40.6 ± 3.8</td>
<td>9.58 ± 0.74</td>
</tr>
<tr>
<td>W</td>
<td>5</td>
<td>397 ± 15)</td>
<td>63.3 ± 2.1</td>
<td>32.5 ± 3.65</td>
<td>7.45 ± 0.625</td>
</tr>
</tbody>
</table>

1) All determinations were done in triplicate.
3) Number of animals.
4) Mean ± SEM.
5) Difference statistically significant at P ≤ 0.05 as compared with the control value.
6) Difference significant as compared with HET-DI value.
the results obtained before sacrifice as percentages of the corresponding values obtained the day before the operation. Data obtained from a control group were expressed in a similar way. Values of $P < 0.05$ were considered to be statistically significant.

**RESULTS**

*Microchemistry* (Table 1)

Neurohypophyseal acid phosphatase activity, expressed on dry weight basis, increased by 9 to 44% in all condition studied as compared to the appropriate controls. For water loading, however, this change was not statistically significant. No difference was found in tissue protein content except for the Brattleboro neurohypophyses in which a lower content was measured as compared to the data in Wistar rats. Total lipid content as percentage of dry weight remained unchanged in the neurohypophyses of castrated males, increased after ovariectomy, LH-treatment and in the Brattleboro rats, and decreased after water loading. Changes in an opposite direction were measured for DNA during water loading (−22%) and in diabetes insipidus (+76%), while during increased gonadotrophic hormone levels the only condition leading to a change was LH administration (decrease of 11%). With respect to the changes mentioned above, HET-DI rats consistently showed intermediate values between Wistar rats and HOM-DI rats.

*Water metabolism* (Table 2)

Because no dramatic changes in water metabolism after gonadectomy were expected, rather large groups were studied. Moreover, because of the considerable difference in basal water metabolism of each animal, the statistical significance of a change was calculated using the post-operative values expressed as a percentage of those in the pre-operative situation (see Materials and Methods).

Such calculation from the data of Table 2 demonstrated no change in water metabolism for castrated males ($P > 0.2$). On the contrary for ovariectomy a significant decrease in water metabolism was found. Calculations for urine production showed for the post-operative values (as percentage of the pre-operative data ($\pm$ sem)) a decrease from 153% ($\pm$ 15) in the OV/OeB (control) group to 97% ($\pm$ 8.1) in the OV group ($0.001 < P < 0.005$). Similar calculations for water intake showed a decrease ($P < 0.001$) from 122% ($\pm$ 7.3) to 92% ($\pm$ 3.7). In addition urine osmolality increased by 14% after ovariectomy from 87% ($\pm$ 5.4) to 99% ($\pm$ 4.1) of the pre-operative value ($0.05 < P < 0.10$).

The hydrated rats as well as the Brattleboro rats showed the expected increase in water metabolism.
Table 2.
Water metabolism of rats before and after 14 days of gonadectomy, of rats of the Brattleboro strain, heterozygous and homozygous for hereditary diabetes insipidus and of rats after 48 h overhydration.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>No. 2)</th>
<th>Water intake (ml/24 h/100 g body weight)</th>
<th>Urine production (ml/24 h/100 g body weight)</th>
<th>Urine osmolality (mOsm./kg)</th>
<th>Serum osmolality (mOsm./kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day before operation</td>
<td>Day before sacrifice</td>
<td>Day before operation</td>
<td>Day before sacrifice</td>
</tr>
<tr>
<td>Gonadectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM</td>
<td>23</td>
<td>9.72 ± 0.43(3)</td>
<td>8.68 ± 0.37</td>
<td>3.08 ± 0.24</td>
<td>2.74 ± 0.16</td>
</tr>
<tr>
<td>CAS</td>
<td>23</td>
<td>9.19 ± 0.49</td>
<td>7.71 ± 0.43</td>
<td>2.97 ± 0.22</td>
<td>2.66 ± 0.16</td>
</tr>
<tr>
<td>OV/OeB</td>
<td>24</td>
<td>8.93 ± 0.59</td>
<td>10.9 ± 0.65</td>
<td>3.13 ± 0.29</td>
<td>4.78 ± 0.47</td>
</tr>
<tr>
<td>OV</td>
<td>29</td>
<td>8.88 ± 0.47</td>
<td>8.19 ± 0.34(5)</td>
<td>3.21 ± 0.28</td>
<td>3.11 ± 0.26(5)</td>
</tr>
<tr>
<td>Brattleboro</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar (C)</td>
<td>12</td>
<td>-</td>
<td>7.64 ± 0.73</td>
<td>-</td>
<td>2.91 ± 0.21</td>
</tr>
<tr>
<td>HET-DI</td>
<td>7</td>
<td>-</td>
<td>7.10 ± 1.1</td>
<td>-</td>
<td>4.81 ± 0.46(5)</td>
</tr>
<tr>
<td>HOM-DI</td>
<td>7</td>
<td>-</td>
<td>53.8 ± 4.9(5)(6)</td>
<td>-</td>
<td>48.5 ± 5.2(5)(6)</td>
</tr>
<tr>
<td>Water loading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(1)</td>
<td>5</td>
<td>-</td>
<td>n. d.</td>
<td>-</td>
<td>n. d.</td>
</tr>
<tr>
<td>W</td>
<td>5</td>
<td>-</td>
<td>ca. 40</td>
<td>-</td>
<td>29.6 ± 5.3</td>
</tr>
</tbody>
</table>

1) For explanation of abbreviations see Materials and Methods section and legend of Table 1.
2) Number of animals.
3) Mean ± SEM.
4) Water intake and urine production were not detectable (n. d.) within the 3 h of metabolism cage housing; value of urine osmolality could only be given for one animal.
5) Difference statistically significant at $P \leq 0.05$ as compared with control value (for calculation within gonadectomy groups see Materials and Methods section).
6) Difference significant as compared with HET-DI value.
DISCUSSION

Various conditions are known to activate the rat HNS (for reviews see Ginsburg 1968; Swaab 1972). The increased secretion of neurohypophysial hormones during osmotic stress, parturition and lactation has consistently been found to be accompanied by an increased lysosomal enzyme activity (see Introduction). This increased enzyme capacity in the neurohypophysis is believed to be involved in the removal of release residues particularly since lysosomal enzymes capable of digesting such materials were found to be activated (Boer & Jongkind 1974).

After gonadectomy in male and female rats the acid phosphatase activity in the neurohypophysis expressed per dry weight increased (Table 1). The statistical significance disappeared however, if the activity was expressed on DNA basis ($0.2 < P < 0.4$). Expression per unit DNA is equivalent to the enzyme activity per whole neurohypophysis if the number of cells has not changed. This calculation has the advantage of being independent of changes in neurohypophyseal dry weight components by the experimental condition used (Boer & Jongkind 1974). After injection of LH the increase in activity expressed on DNA basis was found to be $28\% \quad (P < 0.001)$. Thus during endogenously or exogenously increased gonadotrophic hormone levels, conditions in which increased synthesis was observed in the magnocellular nuclei (Swaab 1972), a slight enzymatic response was found for the females only. Within the female groups (OV and OV/OeB/LH) an increased neurohypophysial lipid content (per DNA) appeared to be present which is in agreement with the electronmicroscopically observed pituicytic lipid accumulation (Zambrano & De Robertis 1968a). An increased cytoplasmic lipid content has been described also during the conditions of HNS activations mentioned before (Ortmann 1951; Malandra 1956; Krsulovic & Brückner 1969).

The female control group (OV/OeB) showed during the 14 days of the experiment a strong increase in water intake similar to that in normal female rats during the same period of development (Tarttelin & Gorski 1971). Compared to this increase the ovariectomized animals showed a decreased water metabolism which is also in agreement with Tarttelin & Gorski (1973) who reported in addition a restoration up to the control curve by daily injections of OeB. In view of these data, the observed increase in neurohypophysial lipid content and the slight increase of acid phosphatase activity during increased gonadotrophic hormone levels, a slightly increased hormone release seems to take place. This agrees with the influence of gonadotrophic hormones on water metabolism as has been suggested earlier (Lorraine & Matthew 1950; Swerdloff & Odell 1968; Gabe et al. 1968; Swaab 1972).

The diuresis of the homozygous Brattleboro rats (Table 2), appeared to be paralleled by an increased acid phosphatase activity. Expression of the enzyme
activity on DNA basis is not reliable in this condition, because the highly increased number of pituicytes (Scott 1968) will lead to an underestimation of the total activity (Boer & Jongkind 1974). Using data of posterior lobe wet weight (Van Wimersma Greidanus et al. 1974) and volume (Sokol & Valtin 1965), of the increased density of pituicytes (Scott 1968) and of the present DNA value (Table 1), the total dry weight of the homozygous neurohypophysis increased roughly twice as compared to that of the heterozygous rat. The acid phosphatase activity will therefore increase even about 2-fold more than the 44% mentioned in Table 1. This agrees fully with the supposed parallelism between lysosomal enzyme activity and hormone release within the neurohypophysis, since the homozygous Brattleboro's have morphological signs of a highly activated synthesis and extrusion of a presumably slightly modified and inactive vasopressin (Sokol & Valtin 1965; Scott 1968; Miller & Moses 1971; Kalimo & Rinne 1972; Swaab et al. 1973). Heterozygous rats always show intermediate values with respect to the different changes.

Water loading resulted in an obvious diuresis which lies in the range of the data for homozygous Brattleboro rats (Table 2). The neurohypophysial acid phosphatase activity on dry weight basis was found to increase by 12% (Table 1), and this increase was even more pronounced on DNA basis (+45%). The increased protein content expressed on DNA basis (+34%, $P < 0.001$) possibly reflects the increased amount of neurosecretory material in the neurohypophysis (Ortmann 1951; Soulairac & Soulairac 1964).

The present results during increased gonadotrophic hormone levels and in Brattleboro rats showed that neurohypophysial acid phosphatase activity is stimulated in two additional conditions of HNS activation. This is in favour of the postulate that the lysosomal response upon HNS stimuli is incorporated in the removal of the remaining release residues (Kurosumi et al. 1964; Whitaker & LaBella 1972; Boer & Jongkind 1974). The morphologically described lipid accumulation in the pituicytic cytoplasm for these conditions (Zambrano & De Robertis 1968a; Kalimo & Rinne 1972) has been confirmed microchemically (Table 1). This finding gives also evidence that the pituicytes might be incorporated in the disposal mechanism for the neurosecretory granule membranes (Kurosumi 1971; Boer & Jongkind 1974).

Acid phosphatase activity was however also activated during inhibition of the HNS by water loading. Although an isolated activation of acid phosphatase cannot be excluded, this might point to an additional and quite different lysosomal process in the neurohypophysis, namely that this enzyme response is related to autophagy of the excess of neurosecretory granules, i.e. to crinophagy as has been described for other endocrine systems (e.g. Farquhar 1971). Extensive axonal crinophagy has been reported when activation of the HNS was interrupted, i.e. cessation of lactation (Rufener 1973) or rehydration (Boudier 1974). In addition this phenomenon was seen more
frequently in normal rats than in water deprived animals (Whitaker & LaBella 1972).

During inhibition of HNS activity by water loading an increased lipid content was neither observed in the pituicytes (Ortmann 1951; Kurosumi et al. 1964) nor microchemically measured (on DNA basis, Table 1). The postulated crinophagy during hydration seems therefore to be a strictly intra-axonal phenomenon in which the pituicytes are not incorporated.

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