Some Effects of Mammalian Neurohypophyseal Hormones on Metabolism and Active Transport of Sodium by the Isolated Toad Bladder*

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The active transport of sodium by the isolated toad bladder has been previously demonstrated (1). The present study concerns the stimulation of active sodium transport in this preparation by neurohypophyseal hormones and the changes in oxygen consumption, tissue glycogen content, and lactic acid production which are associated with the hormonal effect.

METHODS

The preparation of tissue, the composition of the incubating media, and the analytical techniques used in this study have all been reported in previous publications from this laboratory (1-3). We are indebted to Professor V. duVigneaud for the generous supplies of the two purified neurohypophyseal hormones used in this study, oxytocin (ON-5) and arginine-vasopressin (AVN-3). The commercial vasopressin used was pitressin (Parke, Davis and Company).

RESULTS

Hormonal Stimulation of Sodium Transport—In a previous study (1) it was shown that mammalian neurohypophyseal hormones regularly increased the short circuit current through the isolated toad bladder. Table I demonstrates that this increase in current is entirely accounted for by an increase in active sodium transport. In seven experiments of four 30-minute periods each, the unidirectional sodium fluxes were measured simultaneously with Na\(^{22}\) and Na\(^{4}\) together with the short circuit current according to the technique of Ussing and Zerahn (4). When the smaller flux of sodium from the serosal to mucosal surface is subtracted from the larger value in the opposite direction (mucosal to serosal) the net sodium flux is obtained. When this figure for sodium reabsorption is compared with the simultaneously measured short circuit current and both values are expressed in the same units, excellent agreement is obtained. This correspondence in the toad bladder between electrical current and net sodium flux had been previously demonstrated in the absence of hormone (1). From Table I it is evident that the same good agreement holds after hormonal treatment as well. Hence the hormone stimulates active sodium transport and all the electrical activity of the membrane is quantitatively accounted for by sodium transport. Ussing and Zerahn (4) previously had shown similar results for the isolated frog skin.

Although the active sodium transport is stimulated by hormone an effect on the passive flux (serosal to mucosal) could not be detected. Both in the experiments shown in Table I and in an earlier group of measurements a spontaneous and apparently unavoidable variability was observed in the small values of the passive flux between successive 30-minute periods. Although the absolute magnitude of this spontaneous variability was small it still was sufficient to preclude detection of a significant hormonal effect on the passive flux.

The stimulatory effect of the hormone on the permeability to sodium in the mucosal-to-serosal direction tended to diminish with time in most experiments. Thus the permeability in the fourth period was found to be slightly less than during the third period in most instances. The effect on the short circuit current similarly was not sustained but decreased with time.

Hormonal Stimulation of Oxygen Consumption—Table II shows the effect of neurohypophyseal hormones on the rate of oxygen consumption by the toad bladder. Oxygen consumption was measured in a Warburg apparatus for three consecutive hours in 10 paired experiments. After an initial control hour, hormone was tipped from the side arm into one of the two paired flasks and in every experiment this resulted in a higher rate of oxygen consumption during the subsequent 2 hours of observation. The control tissue showed no regular change in oxygen consumption with time. When the change in oxygen consumption from the first to the second hour of measurement was analyzed statistically, a significant increase was found when hormone was added but no significant change occurred in the absence of hormone.

This stimulatory action of the preparations of neurohypophysial hormones on oxygen consumption was found to depend upon the presence of sodium as indicated in Table III. When a sodium-free magnesium or choline Ringer solution was used the stimulatory effect was still was sufficient to preclude detection of a significant hormonal effect on the passive flux.

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Hormonal Stimulation of Glycerolysis—Table IV, A shows that aerobic glycolysis, as measured by the disappearance of glycogen and the production of lactic acid, was also stimulated by neurohypophysial hormones. Twelve experiments were performed with paired bladder halves; one-half was incubated in the presence of neurohypophysial hormones and the other half in the
TABLE I
Comparison of sodium flux and short circuit current through isolated toad bladder with and without neurohypophyseal hormone

Figures are the mean values obtained during two successive 30-minute periods before and two successive 30-minutes after addition of 0.05 ml (1.0 unit) of commercial vasopressin to the 15.0 ml of Ringer's solution bathing the serosal surface of the bladder in seven experiments. The area of chamber was 7.07 cm² and the average dry weight of tissue was 0.98 mg per cm².

<table>
<thead>
<tr>
<th>Hormone</th>
<th>No. of 30-min periods</th>
<th>Mean Na flux</th>
<th>Mean short circuit current</th>
<th>S.E. mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M → S</td>
<td>S → M</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14</td>
<td>24.4</td>
<td>3.1</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>34.8</td>
<td>4.2</td>
<td>31.6</td>
</tr>
</tbody>
</table>

* M → S, mucosal to serosal; S → M, serosal to mucosal.

TABLE II
Effect of neurohypophyseal hormone on QO₂ of toad bladder in sodium Ringer solution

Ten experiments on 10 paired bladder halves.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Mean difference (hours 3 - 1)</th>
<th>S.E. mean difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With hormone*</td>
<td></td>
<td>±0.03</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Hormone added at end of first hour; 0.2 unit of oxytocin used in 8 experiments and 0.055 unit of arginine-vasopressin, in remainder. QO₂ = μl O₂ per mg dry tissue per hr.

TABLE III
Effect of neurohypophyseal hormone on QO₂ of toad bladder in sodium-free Ringer solutions

No significant difference found between successive hourly QO₂ values.

<table>
<thead>
<tr>
<th>Hours</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Magnesium Ringer (8 paired experiments)</td>
<td>0.05</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Hormone added*</td>
<td>1.11</td>
<td>1.16</td>
<td>1.13</td>
</tr>
<tr>
<td>B. Choline Ringer (11 paired experiments)</td>
<td>1.05</td>
<td>0.93</td>
<td>0.90</td>
</tr>
<tr>
<td>Hormone added*</td>
<td>1.13</td>
<td>1.08</td>
<td>1.07</td>
</tr>
</tbody>
</table>

* Hormone added at end of first hour; either 0.2 or 0.4 unit of oxytocin used. QO₂ = μl O₂ per mg dry tissue per hr.

When experiments identical with those shown in Table IV, A were performed in a sodium-free Ringer solution there was no significant effect of the hormone on glycogen disappearance or lactate production, as shown in Table IV, B.

The short circuit current, and hence presumably the active transport of sodium by the isolated toad bladder, is also stimulated by neurohypophysial hormones under aerobic conditions (1). An attempt was therefore made to determine whether the stimulation of sodium transport anaerobically by the hormones was also associated with an increased rate of glycolysis. Table IV, C shows the effects of neurohypophysial hormones on lactate formation and glycogen disappearance in 21 paired experiments. In contrast to the clear cut effects of the hormone on these measurements under aerobic conditions (Table IV, A) no significant effect on glycogen breakdown is seen although a small but significant effect on lactate formation is noted. It is possible that a small effect of the hormone on glycolysis may be obscured against the background of the high rates of glycolysis occurring anaerobically in the absence of hormone and by the considerable variability in the glycogen content from one portion of tissue to the next.
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DISCUSSION

Ussing and Zerahn (4) had previously demonstrated a stimulation of the short circuit current and of active sodium transport in the frog skin by mammalian neurohypophyseal hormones. Stimulation of the short circuit current of the isolated toad bladder by neurohypophyseal hormones had also previously been reported (1). The equality of net sodium reabsorption and short circuit current after hormone indicates that this increased electrical activity is quantitatively accounted for by the increase of active sodium transport. No other ion of the medium besides the sodium ion appears to be actively transported across the membrane either in the resting state or after hormonal stimulation.

An action of the hormone in these studies could only be demonstrated definitely on the movement of sodium in the direction of active transport. In spite of a careful attempt to observe an action of the hormone on the passive flux of sodium from the serosal-to-mucosal sides, the spontaneous variability from period to period in the low value for the passive flux made it impossible to discern such an effect of hormone. In this sense we have been unable to confirm the contention of Ussing and Zerahn that the effect of the hormone on the sodium flux in the two directions is proportionately the same. On the other hand, our results also do not permit us to deny this contention.

The present results indicate that the increase in metabolism by the toad bladder induced by neurohypophyseal hormones is entirely secondary to the action of the latter to increase the active transport of sodium. The metabolic effects of the hormones on oxygen consumption, glycogen utilization, and lactate production failed to be elicited when sodium was omitted from the incubating medium. This dependence of energy metabolism on the presence of sodium is consistent with our previous findings (2) that the rate of energy metabolism of toad bladder in the absence of neurohypophyseal hormones is also dependent upon the presence of sodium ions. In these experiments the oxygen consumption was first measured at zero sodium transport when no sodium was present in the medium. This value was then compared with the increased rate of oxygen consumption obtained after addition of sodium to the medium, when active sodium transport was occurring, and a ratio of sodium transported to the increase in oxygen consumed was calculated.

From the increment in oxygen consumption and in sodium transport after addition of hormone one may estimate the efficiency of the transport process. From Table II an increase of 0.49 μl or 0.022 μmole of O₂ per hour per mg dry weight of tissue is obtained. From Table I the increase in active sodium transport resulting from hormone was approximately 11 μamps per 0.98 mg dry weight or 0.42 μeq of sodium per mg dry weight per hour. Hence on the average some 19 sodium ions are transported per molecule of oxygen consumed. Although this calculation utilizes mean figures separately determined for oxygen consumption and sodium transport, the result is in good agreement with those of Zerahn (7) and of Leaf and Renshaw (8) for isolated frog skin and of Leaf et al. (2) for isolated toad bladder. Zerahn (7) compared the increments of oxygen consumption and sodium transport after the addition of sodium to the medium bathing isolated frog skin and obtained values of 16 to 20 sodium ions transported per molecule of oxygen consumed. A similar comparison made for the toad bladder yielded an average figure of 17 sodium ions transported per molecule of oxygen consumed (2). When the increments of sodium transport and oxygen consumption by isolated frog skin after hormonal stimulation were compared a mean figure of 18 sodium ions transported per molecule of oxygen consumed was obtained (8). Although there is undoubtedly considerable error in such an estimation, the conclusion seems justified that more than one sodium ion can be transported per high energy phosphate bond synthesized and that the efficiency of sodium transport is similar if measured through the range of zero to resting sodium transport rates or from resting to hormonally stimulated transport rates.

Table II shows that no decrease in the rate of oxygen consumption occurred even during the second hour following the addition of hormones to the incubating medium. By contrast a significant drop (p <0.05) in the rate of sodium transport had already occurred in the second half hour after addition of hormone. In spite of administering the excessively large amounts of hormone purposely used in the present study the hormonal stimulation of sodium transport is rarely sustained even for 1 hour. On the other hand the increased passive permeability of the membrane to urea (6) and water produced by this amount of neurohypophyseal hormones is usually sustained for several hours. This evidence of a persistent action of the hormone on the membrane suggests that the decline in the stimulated rate of sodium transport results from exhaustion of some intermediate necessary for the active sodium transport system. That oxygen consumption remains high while sodium transport declines indicates decreasing efficiency for sodium transport with time and suggests some "uncoupling" of energy metabolism and the sodium transport system, although the possibility that an oxygen debt is incurred when the hormone is first administered cannot be excluded.

SUMMARY

1. Measurements have been made of the effects of mammalian neurohypophyseal hormones on the rates of active sodium transport, oxygen consumption, glycogen utilization and lactic acid production by the isolated urinary bladder of the toad, Bufo marinus.

2. The hormones specifically stimulate the active transport of sodium from the mucosal to serosal surfaces of the membrane. No effect of the hormones on the passive movement of sodium in the opposite direction was detectable though a large spontaneous variability makes uncertain a possible action of the hormone on the passive flux.

3. The increased rate of sodium transport stimulated by neurohypophyseal hormones was associated with an increased rate of energy metabolism by the tissue. That the stimulation of energy metabolism was entirely secondary to the hormonal effect on active sodium transport was indicated by the lack of an effect of the hormones on the energy metabolism of the tissue in the absence of sodium in the incubating medium.

4. Calculations of the ratio of sodium ions transported per oxygen molecule consumed from the increments in each that followed addition of neurohypophyseal hormones yield a mean figure of 19. The results indicate a decreasing efficiency of sodium transport with time after the addition of hormone.

1 Unpublished results of R. M. Haye and A. Leaf.
REFERENCES

Some Effects of Mammalian Neurohypophyseal Hormones on Metabolism and Active Transport of Sodium by the Isolated Toad Bladder
Alexander Leaf and Eleanor Dempsey


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