Hormonal Effect on Na-K-ATPase Activity in the Gill of Japanese Eel, 
*Anguilla Japonica*, with Special Reference to Seawater Adaptation

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**Synopsis**

Na-K-activated adenosinetriphosphatase (Na-K-ATPase) has been implicated in the active excretion of sodium ions by the gill of teleosts living in sea water. In the eel, *Anguilla japonica*, the activity of this enzyme increased markedly one week after transfer from fresh water to sea water. Hypophysectomy resulted in a reduction of this adaptive increase and the impairment was restored by cortisol treatment. Cortisol injection into intact freshwater eels induced a rise in the branchial Na-K-ATPase activity. When seawater-adapted eels were returned to fresh water, the enzyme activity decreased gradually. Prolactin injection into hypophysectomized eels promoted this decrease. Injection of prolactin into intact or hypophysectomized seawater eels, however, failed to affect the enzyme activity. The significance of these results was discussed in relation to possible hormonal influences on seawater adaptation of the eel.

When eels are transferred from fresh water to sea water they begin to drink sea water and absorb it from the intestine together with monovalent ions. The excess Na and Cl ions are extruded by the gill against the concentration gradients. The amount of excretion from the gill of seawater eels is more than 10 times larger than that of active uptake by the gill of freshwater eels (Motais, 1967). In accordance with the increased transfer of the ions, the Na-K-activated adenosinetriphosphatase (Na-K-ATPase) activity in the eel gill increases markedly on transfer from fresh water to sea water (Kamiya and Utida, 1968; Utida et al., 1971). This enzyme has been implicated in the active sodium excretion; ouabain at a concentration of $10^{-5} \text{ M}$ inhibits the enzyme activity and also the sodium excretion by isolated gills from seawater eels (Kamiya, 1967; Kamiya and Utida, 1968). Furthermore, K ion is essential for the demonstration of Na-K-ATPase, and removal of this ion from the external sea water inhibits the sodium excretion by the gill as demonstrated *in vivo* (Maetz, 1969a) and *in vitro* experiments (Kamiya and Utida, 1968; Utida and Hirano, 1972).

Pituitary-interrenal axis seems to play an important role in the osmoregulation of teleosts in sea water; the plasma sodium concentration of eels adapted to sea water rises above normal after hypophysectomy (Butler, 1966) or interrenalectomy (Chan et al., 1967; Mayer et al., 1967). Hypophysectomy or interrenalectomy reduces the sodium exchange rate as well as the net sodium outflux in the eel gill, and ACTH or cortisol restores them to the normal level (Mayer et al., 1967; Maetz, 1969b). In contrast, prolactin favours freshwater life of teleosts; in hypophysectomized freshwater eels the extrarenal sodium outflux is higher than in intact eels, and prolactin treatment reduces the outflux to the
normal level (Maetz et al., 1967). Therefore, it is likely that these hormones have influence on the branchial Na-K-ATPase. The present investigation was carried out in order to know the effects of hypophysectomy and the administration of cortisol and prolactin on the Na-K-ATPase activity in the gill of eels adapted to fresh water or sea water.

Materials and Methods

Cultured Japanese eels, Anguilla japonica, were purchased from a commercial source. These fish, weighing about 200 g, were kept in fresh water aquaria at 20°C for a week prior to use. Hypophysectomized or sham-operated eels were kept in fresh water for a week after operation. They were then transferred to sea water and kept for a week or a month. Hypophysectomized fish were injected with cortisol-acetate (Sheroson F, Schering) at a dose of 1 mg per day for 5 or 7 days following transfer to sea water. The injections were given intraperitoneally in a volume of 0.2 ml. Controls were injected with the same volume of 0.9% NaCl solution. In another series of experiment intact freshwater eels were given 1 mg cortisol-hemisuccinate (Solu-Cortef, Upjohn) per day for 7 days.

For prolactin injection, eels adapted to sea water for a week were hypophysectomized in order to avoid possible antagonistic action of cortisol. They were kept in 30% sea water for 3-4 days after operation, in sea water for the following 7 days, and then transferred to fresh water. They received daily injections of bovine prolactin (Teikoku-zoki) for 7 days at a dose of 0.5 mg per day. The first injection was given while fish were in sea water, and the other six in fresh water. In other series of experiments both intact and hypophysectomized eels in sea water were injected with prolactin for 5 days. Controls were injected with 0.2 ml of 0.9% NaCl solution.

The fish were sacrificed on the day following the last injection. Gill filaments were dissected from the supporting gill arch and assayed for ATPase immediately, or stored at −20°C until assayed. Storage of gill filaments at −20°C caused a decrease by about 80% in Mg-ATPase and an increase by about 30% in Mg-Na-K-ATPase activity (unpublished observation). Procedures for the assay of ATPase were similar to those employed previously (Utida et al., 1971). Na-K-ATPase activity was calculated as the difference between Mg- and Mg-Na-K-ATPase activities.

Results

Effect of hypophysectomy and cortisol injection on Na-K-ATPase

As shown in Table 1, Na-K-ATPase activity in the gill of sham-operated eels increased markedly one week after transfer from fresh water to sea water. A similar increase after seawater adaptation was reported previously in the intact eel (Kamiya and Utida, 1968; Utida et al., 1971). The enzyme activity in hypophysectomized eels adapted to sea water for 7 days was significantly lower than that in sham-operated eels, although it was still higher than that in intact freshwater eels (P < 0.001). When cortisol was injected into hypophysectomized eels for 5 days, the enzyme activity was restored to the level of sham-operated fish (Expt I). This series of experiment was repeated using another group of eels (Expt II), and the development of Na-K-ATPase activity elicited by seawater adaptation was not so remarkable in both sham-operated and hypophysectomized eels as compared to those in the first experiment. However, cortisol treatment enhanced the enzyme activity significantly. When operated eels were kept in sea water for one month, no significant difference was observed between hypophysectomized and sham-operated eels (Expt III).

In intact eels maintained in fresh water, cortisol treatment induced a considerable increase in the branchial Na-K-ATPase activity (Expt IV).

Effect of prolactin on Na-K-ATPase

When seawater-adapted eels are returned to fresh water, the gill Na-K-ATPase activity decreases gradually and attains a freshwater level after one month (Utida et al., 1971). In the present study, seawater-adapted eels were hypophysectomized and transferred to fresh water. The enzyme activity was assayed after 7 days in fresh water. As shown in Table 2, daily injections of prolactin for 7 days pro-
### Table 1. Effect of hypophysectomy and cortisol injection on ATPase activity in eel gills

<table>
<thead>
<tr>
<th>No. of Expt</th>
<th>Adaptation</th>
<th>Treatment</th>
<th>No. of fish</th>
<th>ATPase activity(^a) ((\mu)moles P/mg protein per hr)</th>
<th>Na-K-ATPase</th>
<th>Mg-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fresh water</td>
<td>None</td>
<td>4</td>
<td>8.2 ± 1.1(^{b})</td>
<td>9.1 ± 0.9(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 week in</td>
<td>Sham-hypx</td>
<td>4</td>
<td>42.2 ± 2.7</td>
<td>8.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sea water</td>
<td>Hypx + saline</td>
<td>5</td>
<td>33.0 ± 2.6(^{*})</td>
<td>9.1 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypx + cortisol(^{c}) (5 days)</td>
<td>3</td>
<td>48.6 ± 2.0(^{**})</td>
<td>6.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1 week in</td>
<td>Sham-hypx</td>
<td>6</td>
<td>23.1 ± 2.3</td>
<td>17.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sea water</td>
<td>Hypx + saline</td>
<td>5</td>
<td>15.5 ± 1.6(^{*})</td>
<td>17.3 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypx + cortisol(^{c}) (7 days)</td>
<td>6</td>
<td>33.6 ± 4.1(^{**})</td>
<td>15.9 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 month in</td>
<td>Sham-hypx</td>
<td>4</td>
<td>37.0 ± 3.0</td>
<td>4.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sea water</td>
<td>Hypx</td>
<td>4</td>
<td>34.2 ± 6.4</td>
<td>5.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Fresh water</td>
<td>Saline</td>
<td>8</td>
<td>15.3 ± 1.1</td>
<td>17.1 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortisol(^{d})</td>
<td>10</td>
<td>25.9 ± 1.7(^{***})</td>
<td>15.1 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Assayed immediately after dissection of gill filaments.
\(^{b}\) Mean ± standard error.
\(^{c}\) Sheroson F was injected for 5 or 7 days at a dose of 1 mg per day.
\(^{d}\) Solu-Cortef was injected for 7 days at a dose of 1 mg per day.

\(^{*}\) \(P<0.05\) vs. sham-hypophysectomized controls.

\(^{**}\) \(P<0.01\) vs. saline-injected controls.

\(^{***}\) \(P<0.001\) vs. saline-injected controls.

### Table 2. Effect of prolactin injection on ATPase activity in eel gills

<table>
<thead>
<tr>
<th>No. of Expt</th>
<th>Adaptation</th>
<th>Treatment</th>
<th>No. of fish</th>
<th>ATPase activity(^a) ((\mu)moles P/mg protein per hr)</th>
<th>Na-K-ATPase</th>
<th>Mg-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 week in</td>
<td>Hypx + saline</td>
<td>5</td>
<td>49.9 ± 3.6(^{b})</td>
<td>1.5 ± 0.2(^{b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fresh water</td>
<td>Hypx + prolactin(^{e})</td>
<td>5</td>
<td>36.7 ± 3.7(^{*})</td>
<td>2.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1 week in</td>
<td>Hypx + saline</td>
<td>4</td>
<td>61.2 ± 2.9</td>
<td>3.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fresh water</td>
<td>Hypx + prolactin(^{e})</td>
<td>5</td>
<td>47.1 ± 2.7(^{**})</td>
<td>2.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypx + cortisol(^{d})</td>
<td>4</td>
<td>81.8 ± 10.8(^{***})</td>
<td>1.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Sea water</td>
<td>Saline</td>
<td>4</td>
<td>59.0 ± 5.9</td>
<td>1.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prolactin(^{e})</td>
<td>4</td>
<td>53.2 ± 3.8</td>
<td>1.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Sea water</td>
<td>Hypx + saline</td>
<td>6</td>
<td>71.4 ± 8.4</td>
<td>0.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypx + prolactin(^{d})</td>
<td>6</td>
<td>64.3 ± 4.2</td>
<td>1.1 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Assayed after storage of gill filaments at \(-20^\circ C\).
\(^{b}\) Mean ± standard error.
\(^{c}\) Bovine prolactin was injected for 7 days at a dose of 0.5 mg per day.
\(^{d}\) Sheroson F was injected for 7 days at a dose of 1 mg per day.
\(^{e}\) Bovine prolactin was injected for 5 days at a dose of 1 mg per day.
\(^{f}\) Bovine prolactin was injected for 5 days at a dose of 0.5 mg per day.

\(^{*}\) \(P<0.05\) vs. saline-injected controls.

\(^{**}\) \(P<0.01\) vs. saline-injected controls.

\(^{***}\) \(P<0.001\) vs. saline-injected controls.
duced a significant reduction in the gill Na-K-ATPase activity. On the contrary, in cortisol-injected eels the enzyme activity was significantly higher than that in saline-injected controls (Expt I, II). When prolactin was administered to intact or hypophysectomized eels maintained in sea water, no significant difference was observed between prolactin- and saline-injected animals (Expt III, IV).

Discussion

As shown in the present study, hypophysectomy in the eel resulted in a slower development of the gill Na-K-ATPase activity during the course of seawater adaptation, and cortisol restored it to the normal level. It is to be noted that a considerable increase of the enzyme activity was found even in hypophysectomized eels after transfer from fresh water to sea water. These findings seem to indicate that the adaptive increase in the gill Na-K-ATPase activity of the eel is, not solely but partly, under the control of the hypophysial-interrenal axis.

It has been shown that hypophysectomy in Fundulus heteroclitus adapted to sea water for several months reduces the branchial Na-K-ATPase activity and the cortisol treatment restores it to normal (Epstein et al., 1967; Pickford et al., 1970a). Similar results were reported recently by Milne et al. (1971) in Anguilla anguilla adapted to sea water for 2 weeks. These authors demonstrated that ACTH restores the branchial Na-K-ATPase activity of hypophysectomized eels to that of sham-operated fish. Therefore it is likely that ACTH-cortisol is responsible also for the maintenance of the high activity of the branchial Na-K-ATPase in sea water. However, in Anguilla japonica, when operated eels were adapted to sea water for one month, no significant difference was observed between hypophysectomized and sham-operated animals. Therefore, the dependency of the enzyme activity on the pituitary seems to be less remarkable in the cultured eel, Anguilla japonica, than in Anguilla anguilla and Fundulus heteroclitus.

It is of interest that cortisol induces an increase in the Na-K-ATPase activity in the gill of intact eels maintained in fresh water. The present result is in good agreement with that reported by Epstein et al. (1971) in Anguilla rostrata. These authors reported further that when freshwater yellow (non-migratory form) eels are treated with cortisol, their skin color turns to silver, resembling the silvering of migrating eels, and that they withstand the direct transfer to sea water, while untreated fish die from failure of readjustment of ionic regulation. In Oncorhynchus masou, the seawater-migratory type 'smolt' shows a higher activity of the gill Na-K-ATPase together with the interrenal hyperactivity as compared to the non-migratory type 'parr' of the same species (Utida and Hirano, 1972). It seems probable, therefore, that in the migratory stage of eels and salmons an increased secretion of cortisol may occur, which in turn induces a series of readjustments of ionic regulatory mechanisms, including an increase in the branchial Na-K-ATPase activity, in such a way as to prepare for seawater adaptation while fish are still in fresh water.

Prolactin has been shown to reduce the gill Na-K-ATPase activity of hypophysectomized Fundulus heteroclitus on transfer from sea water to fresh water (Pickford et al., 1970b). In Anguilla japonica prolactin has similar effect as shown in the present study. These results suggest that prolactin is involved in the adaptive reduction of the gill Na-K-ATPase activity on transfer of the euryhaline fish to fresh water.

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References


