Sodium- potassium-activated adenosine triphosphatase and osmotic regulation by fishes

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SODIUM POTASSIUM-ACTIVATED adenosine triphosphatase (Na- K-ATPase) is thought to play a key role in the active reciprocal transfer of sodium and potassium across the plasma membrane of individual cells (34) and there is increasing evidence that it is intimately involved in the secretion of sodium across epithelial membranes, as in the kidney (21), the gill (7, 20), or the avian salt gland (3, 9, 12, 16). The specific activity of Na- K-ATPase in gill and kidney tissue of the euryhaline teleost, Fundulus heteroclitus, has been shown to change in an adaptive way when the fish is transferred from seawater to freshwater (7, 8). It seemed of interest to determine how the amount of this enzyme in sodium-transporting organs of other marine vertebrates might be influenced by variations in the kind of osmotic stress the animals were subjected to, and by consequent variations in the quantity of sodium transported. Several species of stenohaline and euryhaline fish were studied. In addition, the adaptive responses of gill, intestine, and kidney of Anguilla rostrata to transfer from fresh to salt water were measured.

METHODS

Specimens of marine teleosts, including the sea raven (Hemitripterus americanus), longhorned sculpin (Myoxocephalus octodecimspinosus), killifish (Fundulus heteroclitus), and a marine elasmobranch, the spiny dogfish (Squalus acanthias) were supplied by the collecting crew of The Mount Desert Island Biological Laboratory. The fish were maintained for several days in running seawater tanks or in marine live-cars. Smallmouthed bass (Micropterus dolomieu) and shiners (Notopterus) were caught in a nearby lake and maintained in running tap-water tanks. Freshwater eels (yellow Anguilla rostrata) were trapped in a freshwater lake and were either kept in a submerged live-car in this lake or transferred to running-water tanks of controlled salinity. All fish were starved for several days before use.

The fish were immobilized by section of the midbrain and spinal cord with a scalpel. The gills, kidneys, and intestine were removed and extraneous soft tissue was trimmed off the gills and kidneys. Gill filaments, intestinal mucosa, and kidney tissue of the euryhaline teleost, Fundulus heteroclitus, were either kept in a submerged live-car in this lake or transferred to running-water tanks of controlled salinity. The specific activity of Na- K-ATPase in gill and kidney tissue of the euryhaline teleost, Fundulus heteroclitus, has been shown to change in an adaptive way when the fish is transferred from seawater to freshwater (7, 8). It seemed of interest to determine how the amount of this enzyme in sodium-transporting organs of other marine vertebrates might be influenced by variations in the kind of osmotic stress the animals were subjected to, and by consequent variations in the quantity of sodium transported. Several species of stenohaline and euryhaline fish were studied. In addition, the adaptive responses of gill, intestine, and kidney of Anguilla rostrata to transfer from fresh to salt water were measured.

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tissues this was shown to be equal to the amount of ATP breakdown inhibited by \(10^{-4}\) M ouabain. Breakdown of ATP in the medium containing no potassium is referred to as Mg-ATPase or residual ATPase. The protein content of the tissue homogenate was determined by the method of Lowry (23) using crystalline bovine albumin as standard.

RESULTS

ATPase activity of gills of freshwater and seawater teleosts and of spiny dogfish. (Table 1, Fig. 1). The specific activity of Na-K-ATPase in gill filaments of freshwater teleosts like the smallmouthed lake bass (\(M.\) dolomieu), yellow cichlid (\(A.\) rostrata), and lake minnow (\(N.\) triceps) was considerably lower than in the gills of seawater species like the flounder (\(P.\) americanus), killifish (\(F.\) heteroclitus), sea raven (\(H.\) americanus), longhorned sculpin (\(M.\) octodecimspinosus), and goosefish (\(I.\) americanus) Values for gills of freshwater fish ranged from 1 to 6 U/mg protein and in saltwater teleosts from 9.4 to 21 U/mg protein. The saltwater elasmobranch fishes, the gill filaments contain a considerable amount of the stenohaline seawater teleost \(H.\) hemieuli (13.1 \(\pm\) 0.6). The kidney of freshwater eels, however, contained about habituated to saltwater (10-5 \(\pm\) 0.5), and higher than in levels found in the winter flounder, \(P.\) americanus, \(M.\) doloieui (21.0 \(\pm\) 2.0) was significantly higher K-ATPase activity of the freshwater bass kidney iM. doloieui.

The epithelium was scraped from the gill plates with a sharp scalpel; the low value for Na-K-ATPase activity in the epithelium was included in the gill material from \(S.\) acanthias, since the supporting tissue, i.e., the epithelium. Less extraneous tissue was included in the gill material from \(S.\) acanthias, since the epithelium was scraped from the gill plates with a sharp scalpel; the low value for Na-K-ATPase activity in the clasmobranch gill is therefore particularly significant.

ATPase activity in kidneys and rectal gland. (Table 2). Na-K-ATPase activity of the freshwater bass kidney \(M.\) doloieui (21.0 \(\pm\) 2.0 U/mg protein) was significantly higher than levels found in the winter flounder, \(P.\) americanus, habituated to saltwater (10.5 \(\pm\) 2.0), and higher than in the stenohaline seawater teleost \(H.\) hemieuli (13.1 \(\pm\) 0.6). The kidney of freshwater eels, however, contained about the same amount of Na-K-ATPase as did seawater flounders.

An interesting finding was the high activity of Na-K-ATPase in the agglomerular kidneys of two goosefish (24.6 and 27.0 U/mg protein), in spite of the fact that reabsorption of glomerular filtrate is clearly not one of the functions of this kidney. Both Na-K ATPase and residual ATPase of the agglomerular kidneys of \(L.\) rorato were comparable to values found in the glomerular kidneys of the freshwater bass, \(M.\) dolomieu.

The activity of Na-K-ATPase in whole homogenates of the rectal gland of \(S.\) acanthias averaged 45 U/mg protein, which was higher than that of any other tissue measured. A comparably high concentration of Na-K-ATPase in the rectal gland of clasmobranchs was reported previously by Bonting (2).

Adaptive changes in Na-K-ATPase in \(A.\) rostrata. (Table 3). Yellow eels trapped in fresh lake water (sodium concentration less than 5 mEq/liter) were kept in half-strength seawater for 2 days and then in full-strength running seawater. After acclimation to seawater for 2-3 weeks, the specific activity of Na-K-ATPase in gill filaments approximately doubled, increasing from 6.0 \(\pm\) 0.6 U/mg protein in freshwater to 11.4 \(\pm\) 1.1 in saltwater. The residual ATPase of the gill was unchanged.

Intestinal Na-K-ATPase was also increased in eels adapted to seawater, rising to 17.8 \(\pm\) 1.8 U/mg protein.

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**Table 1. ATPase in the gills of marine and freshwater fish**

<table>
<thead>
<tr>
<th>Species</th>
<th>Na-ATPase</th>
<th>Mg-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater teleosts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notropis</td>
<td>15.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Micropterus dolomieu</td>
<td>15.4 (\pm) 0.6</td>
<td>4.4 (\pm) 0.9</td>
</tr>
<tr>
<td>Anguilla rostrata</td>
<td>11.5 (\pm) 0.6</td>
<td>6.0 (\pm) 0.6</td>
</tr>
<tr>
<td>Marine elasmomarsh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squalus acanthias</td>
<td>14.3 (\pm) 1.1</td>
<td>2.7 (\pm) 0.4</td>
</tr>
<tr>
<td>Marine teleosts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myoxochoius octodecimpinosus</td>
<td>7.6</td>
<td>9.4</td>
</tr>
<tr>
<td>Fundulus heteroclitus</td>
<td>24.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Hemirepterus americanus</td>
<td>10.8 (\pm) 1.3</td>
<td>13.8 (\pm) 1.4</td>
</tr>
<tr>
<td>Pseudopleuronectes americanus</td>
<td>15.9 (\pm) 1.6</td>
<td>16.0 (\pm) 1.9</td>
</tr>
<tr>
<td>Lophius americanus</td>
<td>20.5</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Values are means \(\pm\)SEM where more than 1 observation. N = no. of observations.

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**Table 2. ATPase activity in kidneys and rectal gland**

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Na-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micropterus dolomieu</td>
<td>6 Kidney</td>
<td>26.3 (\pm) 2.1</td>
</tr>
<tr>
<td>Anguilla rostrata</td>
<td>6 Kidney</td>
<td>17.1 (\pm) 1.5</td>
</tr>
<tr>
<td>Marine fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemirepterus americanus</td>
<td>3 Kidney</td>
<td>19.5 (\pm) 3.1</td>
</tr>
<tr>
<td>Pseudopleuronectes americanus</td>
<td>5 Kidney</td>
<td>18.8 (\pm) 1.5</td>
</tr>
<tr>
<td>Lophius americanus</td>
<td>2 Kidney</td>
<td>24.6</td>
</tr>
<tr>
<td>Squalus acanthias</td>
<td>2 Rectal</td>
<td>40.9</td>
</tr>
</tbody>
</table>

Values are means \(\pm\)SEM. N = no. of observations.
from an average value of 9.0 ± 1.3 in lake water. There was no significant change in residual ATPase.

Unlike the enzyme of gill and intestine, the Na-K-ATPase of eel kidneys was essentially unaltered by 9 weeks of adaptation to salt water. The level in kidneys of freshwater eels was 12.7 ± 1.6 and in kidneys of eels accustomed to saltwater, 15.8 ± 2.4 U/mg protein.

**DISCUSSION**

The comparative physiology of salt and water homeostasis in fishes has long fascinated biologists interested in the mechanism of sodium transport, because the demands of the external environment are so stringent and the evolutionary adjustments arrived at so varied (30, 35). Freshwater teleosts maintain a serum sodium of about 140-160 mEq/liter by reducing outflux of sodium to a minimum through gill and kidney, by active inward transport of a small amount of sodium by the gills, and by excreting large quantities of water through the kidneys (24, 30). Renal excretion of water is accomplished by ample glomerular filtration, (varying from 0.5 ml/kg per min in freshwater F. kansae (11) to 0.1 ml/kg per min in S. gairdneri (17)). About half the filtered water is reabsorbed and almost all of the filtered sodium. Active reabsorption of sodium by renal tubules is therefore essential for survival of the freshwater glomerular teleost. Inward transport of sodium by the gill in freshwater is negligible compared with the quantities of sodium excreted by the gills in seawater. In freshwater, urine, active transport of sodium by the gill is low, and by the kidney high.

In saltwater glomerular teleosts, in which the serum sodium usually varies from 180 to 200 mEq/liter compared to a concentration of sodium in seawater of about 450 mEq/liter, these positions are reversed. With the possible exception of certain species of eel, glomerular filtration rate is quite low (e.g., 0.01 ml/kg per min in longhomed sculpin M. octodecimspinosus (13)) and the amount of sodium available for tubular reabsorption must decline commensurately. Urine volume is negligible except under artificial conditions of “laboratory diuresis” (13, 14). In order to maintain its body volume in the hypertonic sea, glomerular teleosts drink large quantities of seawater (varying from 7 to 40% of body wt/day (25, 30, 35)). Sodium contained in the ingested water must be excreted via the gills against a chemical gradient (30). In addition, the gills must excrete sodium that enters the body by inward diffusion across the gill surface (24). Thus, in seawater, transport of sodium by the gills is high, and by the kidney low.

The specific activity of Na-K-ATPase in the organs of freshwater and saltwater eels appears to be roughly proportional to the level of sodium transport demanded by the environment and the species. The Na-K-ATPase activity of the rectal gland of the shark was extremely high, commensurate with the role of the gland in active secretion of sodium (4, 5). Activity was high in the gills of saltwater teleosts, much lower in gill tissue of freshwater species, and negligible in gill epithelium of the dogfish shark. On the other hand, Na-K-ATPase activity was almost twice as high in the kidneys of freshwater bass, Micropterus, as in kidneys of saltwater sea raven (Hemitripterus) and flounder (Pseudopleuronectes). These findings are reminiscent of analogous changes in Na-K-ATPase of gill and kidney that occur when P. heteroclitus is transferred from salt to freshwater (7, 8).

The changes in enzymatic activity in intestine and gill of A. rostrata when the eels were transferred from freshwater to saltwater have particular significance since they represent adaptive adjustments within a single species and also because the physiological responses of eels to environments of varying salinities have been especially well studied. The drinking rate of freshwater A. anguilla is 135 ml/hr per 100 g and more than doubles to 325 in eels adapted to seawater for at least a week (25). At the same time the capacity of the intestine to absorb sodium and water is increased by about the same magnitude (33). Similar changes are found in the intestine of the Japanese cultured eel A. japonica (29, 37) when the animal is transferred to seawater. The present experiments indicate that this increase in transport is paralleled by an increase in the specific activity of Na-K-ATPase in intestinal mucosa.

The response of intestinal transport capacity is blocked by hypophysectomy (13), and is said to be associated with an increase in intestinal alkaline phosphatase (36). Hypophysectomy is also known to interfere with osmoregulation in the European eel, A. anguilla (6). It is not yet clear whether hypophysectomy also reduces the concentration of Na-K-ATPase in intestine and gill of the eel adapted to seawater.

The outflux of sodium through the gills of A. anguilla is 5–10 times higher in eels adapted to seawater than in animals accustomed to freshwater (24, 26). Adrenalectomy interferes with this adaptation and cortisol injected over a period of 24 hr rapidly restores it (27). Large and rapid changes in isotopic flux across the gills of Anguilla cannot, however, be automatically equated with active transport, since in this species a large portion of the increased flux in seawater is accounted for by exchange diffusion (28). Isolated gills of seawater-adapted eels take up sodium from a,

**TABLE 3. Adaptive changes in Na-K-ATPase in Anguilla rostrata**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Environment</th>
<th>N</th>
<th>ATPase Specific Activity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mg-ATPase</td>
<td>Na-K-ATPase</td>
</tr>
<tr>
<td>Gill</td>
<td>Fresh</td>
<td>6</td>
<td>11.5 ± 0.6</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Gill</td>
<td>Salt</td>
<td>5</td>
<td>11.4 ± 0.7</td>
<td>11.4 ± 1.1</td>
</tr>
<tr>
<td>Intestine</td>
<td>Fresh</td>
<td>5</td>
<td>20.9 ± 4.7</td>
<td>9.0 ± 1.3</td>
</tr>
<tr>
<td>Intestine</td>
<td>Salt</td>
<td>6</td>
<td>29.9 ± 3.6</td>
<td>17.8 ± 1.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>Fresh</td>
<td>6</td>
<td>17.1 ± 1.5</td>
<td>12.7 ± 1.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>Salt</td>
<td>3</td>
<td>14.4 ± 1.2</td>
<td>13.8 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ±SEM. N = no. of observations.
hypertonic solution more slowly than gills from freshwater animals and this has been ascribed to an enhancement of active extrusion of sodium by gill epithelium, though changes in permeability are not excluded by the data (19, 37). Recently Kamiya and Utida (20) have documented a fourfold increase in Na- K-ATPase activity of a sodium iodide-treated microsomal preparation of the gills of A. japonica, 7 days after transfer from fresh- to seawater. Similar changes have been found by Motais (personal communication) but not by Kirschner (22) in A. anguilla. The present studies confirm and extend these observations in another species. Because in our experiments the specific activity of Na- K-ATPase rose in the whole homogenate of the entire gill, it is unlikely that the chief effect of transferring the eels from fresh- to saltwater was merely to change the ease with which plasma membranes of gill epithelial cells could be fragmented into “microsomal” size. It is not possible from the present experiments to decide whether the increase in enzyme activity represents a generalized increase in the concentration of the enzyme in all cells, or a proliferation of the “chloride” cells thought by some to be concerned with ion transport (18, 31).

Although the ATPase activity of gill and intestinal changes when eels were moved from freshwater to seawater, the ATPase activity of the kidneys did not. In this connection it should be pointed out that there may be a species variation in the changes in renal function that occur when eels are adapted to freshwater or seawater. In European silver eels (A. anguilla) acclimated to seawater for 10 days, the glomerular filtration rate is one-fourth of the value obtained in freshwater eels (32). On the other hand, Oide and Utida (29) reported that when Japanese eels (A. japonica) were adapted to seawater, the glomerular filtration rate, which had fallen immediately after transfer into seawater, recovered by 10 days to the level found in freshwater eels. Measurements of filtration rate in A. rostrata are not available, but if this species resembles A. japonica rather than A. anguilla, the reabsorptive work of the kidneys would not be greatly different in seawater than in freshwater. The specific activity of Na- K-ATPase in kidneys of F. heteroclitus rises when these fish are transferred from seawater to freshwater (8) in a way consistent with the increase in tubular reabsorption of sodium documented in F. kansae, a closely related species (11).

It seems likely that ATPase activity in fish kidney is controlled in part by factors in addition to the work of reabsorbing sodium from the glomerular filtrate. An unexpected finding was the relatively high concentration of Na- K-ATPase in the agglomerular kidney of the goosefish, L. americanus. Obviously, the activity of the enzyme cannot in this case be correlated with reabsorption of sodium from a glomerular filtrate. The urine of freshly caught Lophius is very low in sodium, though the sodium concentration increases as urine flow is augmented during laboratory diuresis associated with increased drinking (13, 14). Sulfate and magnesium are secreted into the urine, which serves as the chief route for the elimination of inorganic divalent ions absorbed from the intestine (1). The mechanism of formation of urine in agglomerular fish is not known. It seems possible that Na- K-ATPase is involved in the reabsorption of sodium by tubular cells from a precursor fluid, or even, conceivably, in the process of secretion of divalent ions and organic bases into the urine.

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REFERENCES


18. JOSUKA, K. Chloride excreting and mucous-secreting cells in the