MORPHOLOGICAL, FUNCTIONAL AND CHEMICAL CORRELATES OF FRESHWATER ADAPTATION IN ANGUILLA ROSTRATA

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After brief exposure to freshwater, seawater-adapted eels dramatically reduce the efflux of Na^+ and Cl^- across their gills although the activity of Na-K-ATPase in gill homogenates remains high at seawater levels. We wished a) to document the duration of the "freshwater turnoff" of chloride efflux by reimmersing eels in seawater after 24 hours of freshwater exposure; b) to see if the binding of ouabain to intact cells in gill filaments would parallel the enzyme activity in homogenates or the flux of ions in the intact fish; c) to investigate possible morphological correlates of this phenomenon using the technique of scanning electron microscopy of gill filaments.

Speciments of Anguilla rostrata weighing 100-300 g, trapped in freshwater, were adapted to seawater for 8 weeks before they were studied. The efflux of ^{36}Cl was measured by injecting 2 microcuries intraperitoneallyin a volume of 0.2 ml, allowing equilibrium for 30-60 minutes, determining the quantity of isotope appearing in the aerated bathing medium (1 liter of seawater at 16°C) at hourly intervals thereafter, and dividing by the specific activity of ^{36}Cl in plasma. The activity of Na-K-ATPase in gill filaments was determined by methods previously described.

For the study of ^3H ouabain binding by gill filaments, eels adapted to different saline concentrations were killed by decapitation. The gill arches were rapidly dissected, the filaments were cut from each arch and placed into oxygenated teleost Ringers solution containing 5 mM glucose and 5 mM potassium. The gill filaments were then incubated at 20°C for 15 min during which time the media was changed three times. Filaments were then transferred to flasks containing teleost Ringers with concentration of potassium of either zero or 40 mM, plus ^3H-ouabain (New England Nuclear), 4.78×10^{-9}M and incubated for 1 hour at 20°C. The gill filaments were then isolated by filtration over a nitrocellulose filter (pore diameter 0.45μ) and washed three times with 10 ml volumes of ouabain-free incubation media. The filaments were then transferred into pre-weighed vials, weighed to determine wet tissue weight, and then dissolved in tissue solubilizer (NCS). Bound ouabain was then measured by beta scintillation counting with a toluene cocktail (Spectrafluor) and corrected for sample quench. Extracellular fluid contamination was estimated with ^3H-inulin or ^{14}C-polyethylene glycol in identically processed tissue samples and was always less than 0.5%.

The surface ultrastructure of eel gill filaments was examined by scanning electron microscopy, as in the accompanying report by Hossler, Epstein and Karnaky.

As in previous studies (Bull. M.D.I.B.L., 15:31-32, 1975; 18:8-9, 1978) exposure of seawater-adapted eels to freshwater for brief periods of time (2 hrs) resulted in a fall in plasma clearance of chloride from an average of 5.3 ± 0.4 ml/hr/100g (n=4) to 1.9 ± 0.1 (n=4). While greatly reduced from seawater levels, chloride efflux did not reach the extremely low values seen in previous seasons after only 2 hours of freshwater exposure, probably because the initial period of adaptation to seawater was longer (8 weeks vs. 3-4 weeks) in the present series. After 24 hours of exposure to freshwater, chloride efflux was reduced to levels characterizing freshwater fish (Figure 1). Despite the fact that plasma clearance of salt was negligible, Na-KATPase activity in gill filaments remained high (Table 1).

The time course of disappearance of "freshwater turnoff" was studied by immersing SW eels in seawater for varying times after 24 hours of freshwater exposure (Figure 1). Chloride efflux rose gradually over 6-8 hours to the range usually seen in seawater-adapted fish. In most eels there was a rise in efflux between 1 and 3 hours after return to seawater but considerable variation was seen; in some cases chloride efflux rose well above the average seawater rate by 6 hours and in one fish the seawater rate was not attained until after 11 hours.

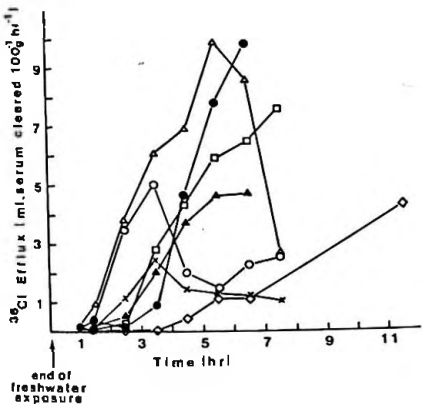

Figure 1. Plasma clearance of ^{36}Cl in individual SW eels that had been exposed to freshwater for 24 hrs., after they were reimmersed in seawater. The average plasma clearance of ^{36}Cl in 4 seawater-adapted fish was 5.2 ± 0.2 and in 4 freshwater-adapted eels 0.2 ± 0.1 ml/hr/100 g. Values are mean ± s.e.

Table 1

	Gill Na-K-ATPase μMPi/mg prot/hr	Ouabain binding to gill filaments moles x 10^{-15} per g wet wt		
		No K^+	40 mM K^+	Difference
FW	2.0 ± 0.3* (4)	14.9 ± 1.2*	6.4 ± 0.7	8.5 ± 0.7*
SW	12.5 ± 2.6 (4)	19.6 ± 0.9	6.9 ± 0.9	12.7 ± 0.9
24 hrs. FW turnoff	14.6 ± 1.3 (4)	23.9 ± 1.7*	10.3 ± 0.6*	13.6 ± 1.8

Values are mean ± s.e. (n).

*$p < 0.025$ when compared with each of the other two values in that vertical column.

The binding of radioactive ouabain to gill filaments was significantly inhibited by the presence of potassium in the incubation medium, as reported in other tissues (Table 1). The difference between binding in the presence and the absence of potassium may be taken as an index of ouabain bound specifically to Na-K-ATPase. Both total and specific ouabain binding to gill filaments of seawater eels was higher than in eels adapted to freshwater, as expected from the higher activity of Na-K-ATPase in homogenates of their gills. Ouabain binding remained high after 24 hrs of freshwater exposure, even though the rate of ion transport across the gill, as reflected in the plasma clearance of ^{36}Cl, fell to freshwater levels. To the degree that ouabain binding reflects the active sites of Na-K-ATPase in intact cells, these findings suggest that the phenomenon of "freshwater turnoff" is not a result of the inactivation of membrane Na-K-ATPase in chloride cells of the eel gill.

Scanning electron microscopy showed no obvious differences between the surface ultrastructure of the apical pits of chloride cells of freshwater adapted eels, seawater adapted eels, and seawater adapted eels exposed to freshwater for 24 hours. In this respect the Anguilla rostrata appears to differ from Mugil cephalus and Fundulus heteroclitus, as reported elsewhere in this Bulletin. Assisted by NIH grants 5S07 RR05764, 5RO1 GM24953, 18078, NSF grant PCM 77-01146, and by Alumni funds from Louisiana State University Medical Center.