

**RAPID ACTIVATION OF GILL Na<sup>+</sup>,K<sup>+</sup>-ATPase  
IN THE EURYHALINE TELEOST *Fundulus heteroclitus***

Juan Miguel Mancera  
Departamento de Biología Animal,  
Facultad de Ciencias del Mar, Universidad de Cádiz,  
11510 Puerto Real, Cádiz, Spain,  
E-mail: juanmiguel.mancera@uca.es

Stephen D. McCormick  
Conte Anadromous Fish Research Center,  
Biological Resources Division, USGS,  
P.O. Box 796, Turners Falls, MA 01376, USA, E-mail:  
mccormick@umext.umass.edu

**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

*Fundulus heteroclitus* is an estuarine euryhaline fish with the capacity to live in a wide range of environmental salinities. In its intertidal habitat the fish is subjected to large and often rapid changes in environmental salinity. Acclimation to changing environmental salinity requires pre-existing mechanisms and the ability to respond to changing conditions. Activation of gill chloride cells and regulation of gill Na<sup>+</sup>,K<sup>+</sup>-ATPase are very important for the acclimation of fish from fresh water to seawater (Wood and Marshall, 1994). In *Fundulus heteroclitus* an increase in gill Na<sup>+</sup>,K<sup>+</sup>-ATPase activity occurs 2-3 days after transfer from hypoosmotic to hyperosmotic conditions (Jacob and Taylor, 1983). In addition, a more rapid activation of gill Na<sup>+</sup>,K<sup>+</sup>-ATPase have been reported (Towle et al., 1977). However, in the same species Marshall et al. ('99) did not observe any change in this activity following exposure to seawater. The physiological significance and cellular mechanism for the rapid increase in Na<sup>+</sup>,K<sup>+</sup>-ATPase activity observed in the gill chloride cell is not clear. The aim of this study was to analyze the potential mechanisms of rapid activation of gill Na<sup>+</sup>,K<sup>+</sup>-ATPase in the euryhaline teleost *Fundulus heteroclitus*.

## Materials and methods

*Fundulus heteroclitus* were transferred from low salinity water (LSW, 0.1 ppt salinity) to SW (35 ppt) and sampled for gill biopsy at 0 h (LSW-acclimated fish), 3 h, 6 h, 12 h, 24 h, 3 d and 7 d after transfer. In a second experiment fish were transferred and sampled at 1 h, 2 h, 3 h and 6 h. The rapid activation *in vitro* was examined using different osmolalities (300, 500, 600 and 800 mosm/kg) in gill organ culture. Samples were taken at 0 h, 3 h and 6 h of culture. In addition, the influence of several inhibitors (actinomycin D, cycloheximide and bumetanide at doses of  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M) was analyzed in gill culture in hyperosmotic medium (600 mosm/kg) to determine the mechanisms involved in rapid activation of gill  $\text{Na}^+, \text{K}^+$ -ATPase.  $\text{Na}^+, \text{K}^+$ -ATPase activities were determined using the microassay method of McCormick (1993). The method of McCormick and Bern (1989) was used to culture primary gill filaments. Significant differences among groups were tested by one-way ANOVA followed by Student-Newman-Keuls multiple comparison test. Two-way ANOVA and Student-Newman-Keuls multiple comparison test were used to test the significance of time and detergent treatment. Results were considered significantly different when  $p < 0.05$ .

## Results

Exposure of *Fundulus heteroclitus* to SW induced a rise in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity 3 h after transfer. After 12 h the values dropped to initial levels but showed a second significant increase 3 d after transfer. The absence of detergent in the enzyme assay resulted in lower values of gill  $\text{Na}^+, \text{K}^+$ -ATPase and the rapid increase after transfer to SW was not observed (Figure 1).  $\text{Na}^+, \text{K}^+$ -ATPase activity of gill filaments *in vitro* for 3 h increased proportionally to the osmolality of the culture medium (600 mosm/kg > 500 mosm/kg > 300 mosm/kg). Osmolality of 800 mosm/kg resulted in lower gill  $\text{Na}^+, \text{K}^+$ -ATPase activity relative to 600 mosm/kg. Increasing medium osmolality to 600 mosm/kg with mannitol also increased gill  $\text{Na}^+, \text{K}^+$ -ATPase. Cycloheximide inhibited the increase in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity observed in hyperosmotic medium in a dose-dependent manner ( $10^{-4}$  M >  $10^{-5}$  M >  $10^{-6}$  M). Actinomycin D or bumetanide in the culture (doses of  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M) did not affect gill  $\text{Na}^+, \text{K}^+$ -ATPase (Figure 2). Injection of fish with actinomycin D prior to gill organ culture, however, prevented the increase in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity in hyperosmotic media.

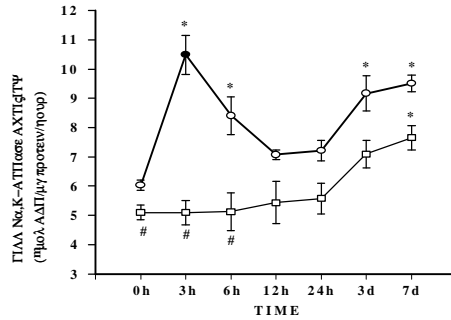


Figure 1. Changes in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity after transfer of *Fundulus heteroclitus* from LSW (0 h) to SW (35 ppt salinity). Gill  $\text{Na}^+, \text{K}^+$ -ATPase activity was measured with detergent (0.1 % deoxycholic acid; circle) and without detergent (square). Each point represents mean  $\pm$  standard error (n=6-7 fish). \* indicates significant difference from time 0 ( $p < 0.05$ , two-way ANOVA test and Student-Newman-Keuls multiple comparison test). # indicates significant difference between groups at the same time ( $p < 0.05$ , t- test).

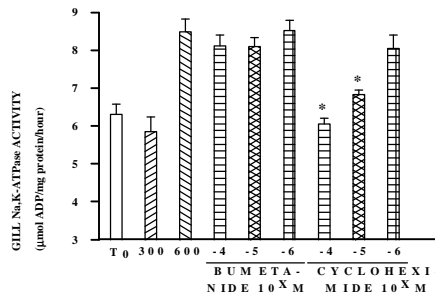


Figure 2. Effect of bumetanide and cycloheximide on gill  $\text{Na}^+, \text{K}^+$ -ATPase activity after 3 h in culture using hyperosmotic medium (600 mosm/kg). Values are means  $\pm$  standard error (n=4-5). Asterisk indicates significant difference compared to hyperosmotic control medium ( $p < 0.05$ , one-way ANOVA test and Student-Newman-Keuls multiple comparison test).

## Conclusions

The results of the present papers show a rapid and transitory increase in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity during first hours after transfer of *Fundulus heteroclitus* from LSW to SW. Similar results are obtained *in vitro* using a gill culture system after increasing medium osmolality. The origin of this increase in gill  $\text{Na}^+, \text{K}^+$ -ATPase activity could involve modifications of pump catalytic subunits, changes in the subcellular distribution of pump units or increase in translational or post-translational kinetics. The results obtained with actinomycin D and cycloheximide suggests that the rapid activation of gill  $\text{Na}^+, \text{K}^+$ -ATPase activity in *Fundulus heteroclitus* is dependent on transcriptional and translational processes.

## References

- Jacob, W.F. and M.H. Taylor. 1983. The time course of seawater acclimation in *Fundulus heteroclitus* L. J. Exp. Zool. 228: 33-39
- Marshall, W.S., Emberley, T.R., Singer, T.D., Bryson, S.E. and S.D. McCormick. 1999. Time course of salinity adaptation in a strongly euryhaline estuarine teleost, *Fundulus heteroclitus*: a multivariable approach. J. Exp. Biol. 202: 1535-1544
- McCormick, S.D. 1993. Methods for non-lethal gill biopsy and measurement of  $\text{Na}^+, \text{K}^+$ -ATPase activity. Can. J. Fish Aquat. Sci. 50: 656-658
- McCormick, S.D. and H.A. Bern. 1989. *In vitro* stimulation of  $\text{Na}^+ - \text{K}^+$ -ATPase activity and ouabain binding by cortisol in coho salmon gill. Am. J. Physiol. 256: R707-R715
- Towle, D.W., Gilman, M.E. and J.D. Hempel. 1977. Rapid modulation of gill  $\text{Na}^+ + \text{K}^+$ -dependent ATPase activity during rapid acclimation of the killifish *Fundulus heteroclitus* to salinity change. J. Exp. Zool. 202: 179-186
- Wood, C.M. and W.S. Marshall. 1994. Ion balance, acid-base regulation, and chloride cell function in the common killifish, *Fundulus heteroclitus*- A euryhaline estuarine teleost. Estuaries 17: 34-52

