A REDUCTION IN CHLORIDE SECRETION BY LOWERED OSMOLARITY IN CHLORIDE CELLS OF *FUNDULUS HETEROCLITUS*

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The rapid signal perceived by the chloride cells of the gills of euryhaline fish during the transition from fresh to seawater is due to the increased osmolarity of the plasma (Zadunaisky et al. J. Mem. Biol. 143:207, 1995; Zadunaisky Kidney Int. 49:1563-1567, 1996). This study was conducted to test if the reverse is true, that is if the reduction in plasma osmolarity produces reduction in chloride secretion. Isolated opercular epithelia of *Fundulus heteroclitus*, adapted to seawater, were mounted in Ussing chambers. The chloride current was recorded while gradually reducing the osmolarity of the basolateral side by simple dilution.

The results indicate that a substantial drop (more than 70 %) in chloride current occurs with a reduction of 12.5 mOsm of the basolateral osmolarity. With a reduction in osmolarity of 25 mOsm, the current reaches zero and partially recovers to reach a steady state at 5-10 % above zero. Because of the possibility that the reduction of chloride concentration in the basolateral side could be responsible for the change and not the lowering of osmolarity, two curves were obtained: one of the effect of osmolarity in small decrements, and the other of decreased chloride concentration at constant osmolarity when chloride has been substituted by methyl sulfate. The drop in chloride current was significantly faster when the osmolarity was reduced than when the chloride concentration was dropped. This indicates that the reduction in chloride secretion with slightly lower osmolarity in the basolateral side is not due to reductions in chloride concentration.

To test which membrane proteins involved in cell volume regulation were important for the effect produced by swelling of the chloride cells due to reduced osmolarity, preparations were treated first with specific inhibitors of the Cl/HCO_3 exchanger and the Na/H exchanger. It was found that in the presence of DIDS at 10^{-4} M, the reduction in current produced by a decrease in osmolarity of 25 mOsm was completely blocked. On the contrary, use of amiloride at 10^{-2} M did not inhibit the reduction in chloride secretion in similar circumstances. This indicates that the Cl/HCO_3 exchanger is crucial for the response to hypotonicity and the Na/H exchanger is not. It is interesting to note that in the case of increased osmolarity with mannitol, the Na/H exchanger was essential for the chloride current increase and the Cl/HCO_3 exchanger was not. Use of quinidine 10^{-5} M reduced the response of the current, indicating that perhaps opening of a K channel in the basolateral side is part of the response to hypotonicity and deserves further experimentation.

In summary, the rapid signal for activation or reduction of chloride secretion in chloride cells is the change in plasma osmolarity, probably both in the transition from fresh to seawater and vice versa.

Supported by NIH grant EY 1340. Michael Balla was a fellow of the National Science Foundation REU program #93-22221 to MDIBL. This fellowship is gratefully acknowledged.