EFFECT OF HYPOTONICITY ON CHLORIDE TRANSPORT IN THE ISOLATED OPERCULAR EPITHELIUM OF FUNDULUS [illegible] EROCLITUS

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The rapid signal detected by Fundulus h. during acclimation to media of diverse salinity is apparently the total osmolarity of the plasma (Zadunaisky J.A. et al. 1992, Bull.MDIBL,31,152-156). For the transition from fresh water to sea water we concluded that the NaK2Cl cotransporter, and the Na/H exchanger were involved in this acclimation that occurs through cell volume regulation. Now we have examined the effects of hypotonicity on the isolated opercular epithelium. A reduction in osmolarity of the solutions bathing the isolated preparation was used as an experimental equivalent of the hypotonicity encountered when the fish moves from sea water to fresh water.

The isolated opercular preparation (see Zadunaisky, 1984, Fish Physiology, Vol. Xb) was mounted, voltage and short circuit current measured and after preliminary tests, the osmolarity of the solution bathing the basolateral side was reduced by replacement with dilute salt solution or plain distilled water. The short circuit current was reduced when the osmolarity was decreased in the basolateral side, no effect was found when it was reduced in the apical side, of these preparations obtained from sea water adapted Fundulus. Figure 1 shows the reduction in current for an average of 6 points for each of 6 osmolarities below the initial one of the isotonic solution. The values in Fig. 1, 6.3,12.5,25,50 and 100 indicate the reduction in miliosomoles produced on the basolateral side. It can be observed that a small reduction in osmolarity can produce a substantial change in chloride secretion.

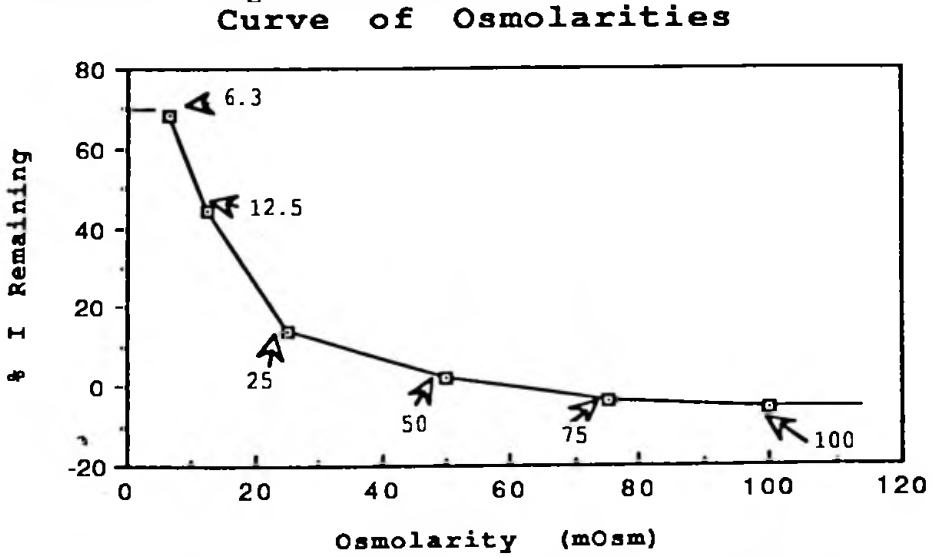

Fig. 1. Reduction in I (short circuit) at different hypotonic levels in the basolateral side of opercular epithelia. Each point is the mean of six experiments.

In order to understand which mechanisms are involved in the response to hypotonicity, the effect of 25 mOsm reduction in basolateral osmolarity was used as a standard test, while the preparation was treated with specific inhibitors of mechanisms existing in the cell membranes of the chloride cells. Thus it was found that 10^{-4} M DIDS partially inhibited the drop in chloride current produced by hypotonicity. In 5 experiments the controls dropped after 25 mOsm of hypotonicity to 13.7 % of the initial current while after DIDS they only dropped to 46.1. This implies that the Cl/HCO_3 exchanger of the basolateral membrane is of importance in the adaptation to lower salinities. In contrast the inhibition of the Na/H exchanger with amiloride 10^{-3}M and of the NaK2Cl cotransporter with furosemide 10^{-4}M did not modify significantly the response to hypotonicity. Because of the importance of K channels for cell volume regulation, we tested also quinine at a concentration 10^{-4}M on the preparation, alone and in combination with the effects of hypotonicity. We found that, in effect, quinine tended to reduce the current drop produced by hypotonicity indicating that part of the response involves the opening of a K channel. No effect was found of quinine on the response to mannitol in hypertonic concentrations, and we have to conclude that the activation of a K channel is involved only in the hypotonic response.

These results indicate that volume regulation with a vectorial response in chloride secretion occurs not only during adaptation to higher salinities but also during exposure to lower ones.

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