PLASMA OSMOLARITY INCREASE DURING ADAPTATION TO SEA WATER OF FUNDULUS HETEROCLITUS AND THE INHIBITION OF THE OSMOTIC RESPONSE OF CHLORIDE CELLS BY THE CHANNEL BLOCKER DPC (N-PHENYLANTHRANILIC ACID)

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The rapid acclimation to higher salinities of Fundulus heteroclitus can be mimicked in the isolated opercular epithelium of this euryhaline fish by the addition of 50 mOsm of the non permeant mannitol to the basolateral side of the preparation mounted in Ussing type chambers. We have reported in this Bulletin (Zadunaisky, J.A. et al. 1992, Bull. MDIBL, 31,152-156) that this osmolarity increase produces a rapid and sustained stimulation of chloride secretion by the chloride cells. The increase produced by 50 mOsm mannitol is approximately 100% above the basal secretory rate, and it saturates between 200 and 300 mOsm. The stimulation occurs via the 2Cl-K-Na cotransporter and the Na/H exchanger, without intervention of the Cl/HCO_3 exchanger.

In spite of the existence in the literature of data for the Na and Cl content of plasma of Fundulus in sea water and fresh water (Pickford, G.E. and Grant, F.B., 1969, Trans. Conn. Acad. Arts. Sci, 43:25-70) there is no time course determination of the changes in osmolarity during the first days of transfer from fresh water to sea water. The data are of importance for these studies.

In order to produce these data, groups of Fundulus adapted to freshwater for 60 days, were transferred to sea water and plasma osmolarity measured up to 50 hours thereafter.

Fish were pithed, caudal vessels were cut, and the free flowing blood was collected into heparinized tubes. In order to obtain 50 ul of plasma for freezing point determinations, the plasma obtained from 7 specimens had to be pooled. The data are therefore a good representation of plasma osmolarity, however, it did not permit classical statistical analysis. Groups of 7 fish were then bled at 0, 2, 4, 5, 11, 22 and 50 hours after transfer to sea water.

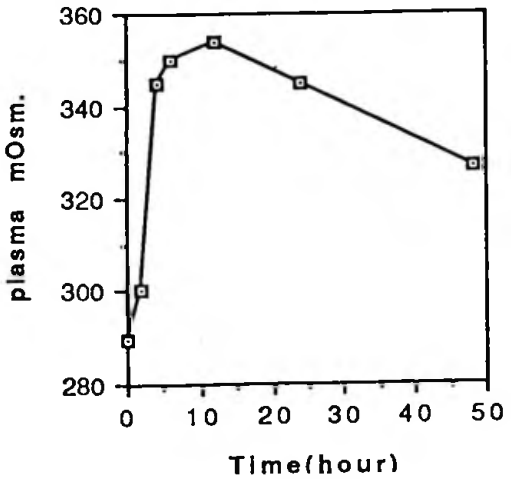

Fig. 1. Time course of plasma osmolarity Fundulus heteroclitus transfered at 0 time from fresh water to sea water. The values are the results of freezing point depression determination of plasma osmolarity pooling samples from 7 fish for each point. Note the peak at 11 hours and the gradual acclimation thereafter.

The results, shown in Fig. 1, indicate that an increase in total plasma osmolarity of 63 mOsm occurs and that it peaks at 10-11 hours. Thereafter the values drop slowly not reaching normal plasma levels in 50 hours.

The mechanism proposed for stimulation of chloride secretion by the reduction in volume of chloride cells, induced by the increased plasma osmolarity, involves the opening of chloride channels in the apical membrane of the Cl cells. In order to test this hypothesis, DPC (N-phenylanthranilic acid) was tested (Schiede, J.I., Zadunaisky, J.A., Amer. J. Physiol., 1988, 254: C519-C525) on the short circuit current of isolated preparations of opercular epithelia obtained from sea water adapted Fundulus heteroclitus. The results of 4 experiments indicate a reduction in the chloride current by the application of DPC at a concentration of 10^{-3} M. In order to test the blocking effect on the chloride channels, 50 mOsm of mannitol was administered to the basolateral side of the preparation when the inhibition by DPC reached 50% (average of 4 experiments 48.7%). The response to mannitol, normally an increase of approximately 100% in the chloride current was completely blocked by DPC. Therefore, chloride channels must be opened for the increase in chloride secretion to take place when the basolateral or plasma side of the chloride cells are exposed to higher osmolarities.

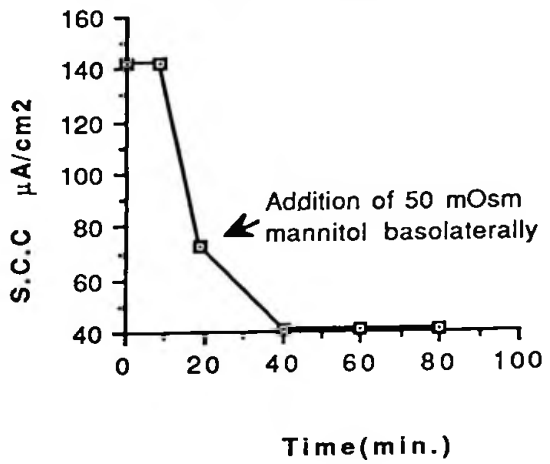

Fig. 2. Action of DPC on the chloride secretory current of opercular epithelia of Fundulus heteroclitus acclimated to s.w. before and after the addition of 50 mOsm of mannitol to the basolateral side of the preparation. Note the inhibitory effect of DPC on the short circuit current. At approximately 50% inhibition, mannitol was added. The expected increase in chloride secretion due to mannitol is not present.

These data, the time course of plasma osmolarity change during sea water adaptation of specimens of Fundulus heteroclitus, and the inhibition by a chloride channel blocker on the stimulation of chloride secretion produced by an osmolarity increase in the isolated opercular preparation of the same fish, give more strength to our contention that acclimation to salinities in euryhaline fish occur through cell volume regulation of the chloride cell of the gill (Zadunaisky, J.A. et al 1992, Bull. MDIBL, 31: 152-156).

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