EVIDENCE FOR A Na^+/H^+ EXCHANGER IN THE CHLORIDE CELLS OF FUNDULUS HETEROCLITUS

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The opercular epithelium of Fundulus heteroclitus contains numerous chloride cells identical to the ones of the gills. When mounted in Ussing type chambers th cells transport chloride ions from the basolateral to the sea water side (Zadunaisky, 1984, Fish Physiol. Vol X b). The SCC is an indicator of this Cl^- secretion.

We have utilized the fact that the short circuit current follows closely the changes in intracullular pH to examine the presence of a Na/H exchanger in these cells. In the frog skin epithelium, intracellular pH measurements have indicated that the short circuit current is an excellent parameter for studies of cell pH (Harvey, B.J., et al, J.Gen. Physiol., Vol.92 pp 767-791, 1988). The short circuit current is decreased when the intracellular pH becomes acid and it increases as the pH becomes alkaline.

Opercular epithelia from sea water adapted Fundulus heteroclitus were mounted in Ussing chambers and bathed with teleost Ringer solution (see Zadunaisky et. al. MDIBL Bulletin, Vol. 30, 1991, pp 58-59), in which the bicarbonate was substituted with HEPES buffer. The medium was adjusted to pH 7.4.

The tests for the presence of Na^+/H^+ exchanger consisted of acidification with 15mM NH_4Cl added to the basolateral side. There is a transient increase in the SCC as the cell briefly undergoes alkalinization. The SCC then decreases to a minimum during the acidification, at which time the solution in the basolateral side is replaced with fresh Teleost ringer without NH_4Cl. This results in restoration of the normal intracellular pH. The current returns to its initial resting value as the intracellular pH becomes more alkaline. This method, introduced by Boron (boron W.F., DeWeer, F&P, J. Gen. Physiol. Vol. 67 pp 91-112, 1976) produces a sequence of intracellular pH displacements that activate the Na/H exchanger.

The addition of NH_4Cl to the apical side did not produce changes in the short circuit current, but when added to the basolateral side, a rapid drop in the current was observed. Therefore the additions of NH_4Cl and other drugs were performed only on the basolateral side of the preparation.

Amiloride, at concentrations of 10^{-2} and 10^{-3}M stopped or reduced the rate of recovery from the acid load. At 10^{-2} the remaining current was 20%, of controls in 7 experiements. These results were interpreted as a good indication that acidification had elicited the activation of the Na/H exchanger with subsequent alkalinization. This event was inhibitied by amiloride at high concentrations.

The rate of recovery from the NH_4Cl acid load was accelerated by several factors that were tested on the basis of their known effect on the Na/H exchanger in other organs or tissues (Grinstein, S. (Ed) Na/H Exchange, CRC Press, 1988).

Epidermal growth factor, at 10^{-5}M produced an acceleration of the recovery of 155% in 7 experiments. Phorbolester (PMA) at a concentration of 10^{-5}M produced a stiumulation of 165% in 7 experiments and IBMX at 10^{-4}M produced a stimulation of 216% in 8 experiments.

The sequence of events during the pH transients, the inhibition with amiloride at high concentrations, and the stimulation with EGF, PMA and IBMX permit us to conclude that a Na/H exchanger is present in the basolateral membranes of the chloride cells, which is activated by internal protons.

The rapid changes and transients in chloride secretion when small changes in pH are occuring is an indication that intracellular pH must be part of the control and signaling that these cells undergo in order to transport more or less salt. The transport, in turn maintains the homeostasis of the ion concentration, especially NaCl in the plasma of the fish. In fact, in another report in this issue of the Bulletin we find that changes in osmolarity activate the Na/H exchanger of the chloride cells.

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Abbreviations used: SCC - Short Circuit Current; EGF - Epidermal Growth Factor; IBMX - 3-Isobutyl-1-Methyl-Xanthine.