ION TRANSPORT IN RED CELLS OF ATLANTIC MACKEREL (SCOMBER SCOMBRUS): EFFECT OF CATECHOLAMINES AND VOLUME PERTURBATIONS

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Cell volume and pH regulatory systems in the red cells of many fish have been shown to play a significant role in adaptational strategies used by these animals to cope with environmental changes. Most of the work in this field has been on red cells from freshwater adapted rainbow trout (Oncorhynchus mykiss). In these cells, binding of catecholamines to plasma membrane β_1–adrenoceptors activates an amiloride–sensitive Na/H exchange that helps maintain an alkaline internal pH in the face of stress–induced extracellular acidosis. An alkaline cytosol promotes the high hemoglobin–oxygen affinity necessary for efficient uptake at the gill, particularly under hypoxic conditions. A high internal pH also prevents an acid–induced unloading of oxygen from hemoglobin (the Root effect). One consequence of this response, however, is red cell swelling secondary to net uptake of Na via Na/H exchange .

In the saltwater environment, marine teleosts face serious osmoregulatory challenges. The ever-present tendency for salt loading is circumvented by a delicately balanced relationship between seawater imbibition (Smith, Quart. Rev. Biol. 7:1-26, 1932) and salt extrusion at the gill (Evans, in Fish Physiology, Ed. by Hoar and Randall, Vol. XB, Chapter 8, 1984). Any disturbance in this relationship along with continued drinking can produce a marked increase in plasma Na and Cl. We have described just such an event in stressed Atlantic mackerel (Scomber scombrus) (Starke and McManus, Bull. MDIBL, 28:17–19, 1989). Following capture (hook and line) and confinement to live cars, there was a marked rise in plasma osmolality due primarily to an increase in Na and Cl. Presumably, salt extrusion at the gill was inhibited by stress-related factors. Surprisingly, in reponse to this hypertonic challenge, the red cells did not shrink, but maintained constant volume by taking up a precise osmotic equivalent of both ions. After a week of confinement, both extracellular osmolality and intracellular Na returned to normal. In view of the work on rainbow trout cited above, it appears likely that the mechanism of net salt uptake by the red cells during the stress response was catecholamine activation of Na/H exchange. The purpose of the experiments reported here was to test this assumption in vitro, and to explore further the volume regulatory characteristics of mackerel red cells.

Mackerel caught in Frenchman Bay were confined to live cars immersed in seawater. After the stress response was established (24-48 hours), blood was drawn from the caudal vein using a heparinized syringe. A gauze sponge soaked in seawater and wrapped round the gills prevented suffocation. The red cells and plasma were separated by centrifugation and washed 3 times in an iced NaCl solution isosmotic with the hypertonic plasma (470 mOsm/kg). Resuspended in a similar hypertonic Ringer to a final hematocrit of about 15%, the cells were incubated overnight at 4°C to achieve steady states with respect to ions and water. The Ringer contained (mM): 2.5 KCl, 0.75 $CaCl_2$, 5 glucose, 1 $MgCl_2$, 10 TMA-TES (TMA = tetramethylammonium; TES = (N-Tris [hydroxymethyl] methyl-2-aminoethane-sulfonic acid, titrated to pH 7.7 at 12°C with TMA hydroxide), and sufficient NaCl to bring the final osmolality to 470 mOsm/kg. After washing and resuspending the cells in this same Ringer, all subsequent incubations were performed at 12°C.

Osmolality was determined using a Wescor vapor-pressure osmometer (5100B). Na and K were determined on perchloric acid (3.6% PCA/1.5 mM $CsNO_3$) extracts of packed cells using an IL flame photometer. Cl was measured with a Radiometer CMT-10 chloridometer. Ion and water content of the cells was calculated from these data in combination with wet weight/dry weight cell water determinations (Schmidt and McManus, J. Gen. Physiol. 70: 59-79, 1977). To study Na/H exchange directly, net proton efflux from DIDS-treated cells into unbuffered media was assayed

by monitoring external pH changes as previously described (Payne and McManus, Bull. MDIBL, 28: 57–59, 1989).

Addition of norepinephrine (10^{-5} M) to the incubation medium promoted cell swelling during the first hour associated with a marked accumulation of Na. After the first hour, the increase ceased and volume remained stable for six hours. Ouabain had no effect on either the rate or magnitude of swelling, although it did change the relative proportions of Na and K in the cells. The sum of Na and K contents at any given point in time, however, was not altered by the presence of ouabain.

To confirm that this effect was due to catecholamine activation of Na/H exchange, net proton efflux was monitored directly by the method referred to above. Before hormone was applied, the unbuffered medium acidified slowly at a rate of –0.05 pH units/minute. For the first 30–40 seconds after addition of 1 μM isoproterenol, there was little change in rate of acidification, but then an abrupt increase to –0.35 pH units/minute occurred. It is interesting to note that we observed a similar delay in onset of proton efflux after activation of Na/H exchange by the phorbol ester, PMA, in shark red cells (Payne and McManus, op. cit.), as did Grinstein in PMA–stimulated lymphocytes (PNAS, 82: 1429–1433, 1985). In contrast to these results, as well as those reported by Motais in experiments on trout red cells (Progress in Cell Research. 1: 179-193, 1990), we were unable to show any effect of phorbol esters in this system. This could be related to the intracellular pH, since Motais et al. (op. cit.) showed that PMA stimulation of Na influx occurred only when internal pH was below 7.6, whereas isoproterenol was effective up to pH 8.0. Therefore, a complete study as a function of internal pH will be necessary before a definite conclusion can be reached concerning the effect of PMA on these cells. Amiloride (0.5 mM) promptly blocked acidification of the external medium, further supporting the conclusion that the catecholamine activated Na/H exchange.

Cell shrinkage also stimulated external acidification. After recording a similar control rate of –0.05 pH units/minute, medium osmolality was abruptly increased to 650 mOsm/kg by addition of a small volume of concentrated NaCl. The rate doubled to –0.1 pH units/minute, but again only after a 30–40 second time delay. Amiloride also blocked this process. When the osmotic increase was produced by adding concentrated KCl, there was no change in the control rate of acidification, but subsequent addition of concentrated NaCl (with no change in final osmolality) produced an increased rate of acidification <u>without</u> a time delay. Thus, the net efflux of protons required external Na, but the time delay was related only to the hypertonic stimulus itself. These experiments confirm that both cell shrinkage and catecholamine addition stimulate Na/H exchange in mackerel red cells.

Swelling induced ion transport was examined by observing net K loss from cells incubated in a hypotonic medium. Since intracellular Na was already elevated due to the hypertonic stress response, net loss of cell water could also occur by the well known dehydrating capability of the Na pump (Clark et al. Biochim. Biophys. Acta 646: 422-432, 1981). To avoid this complication, cells were depleted of Na by overnight incubation in a Na free, high K medium (470 mOsm/kg), then washed and reincubated in the presence of ouabain in a Na–free hypotonic medium (200 mOsm/kg) in which Na was replaced by TMA. One batch of cells was washed with a solution in which Cl was replaced by sulfamate, a permeant anion that has been shown to have little effect on the volume and pH of red cells (Payne, Lytle and McManus. Am J. Physiol. 259: C819–C827, 1990). They were then re-incubated in a similar Cl–free hypotonic medium. The swollen cells showed a net loss of K sensitive to bumetanide (10^{-3} M) and inhibited by removal of Cl, suggesting the presence of swelling induced [K–Cl] cotransport.

When norepinephrine (10^{-5} M) was added to cells suspended in the hypotonic medium, net K efflux was inhibited about 40%, which cannot be related its effect on Na/H exchange since there

was no Na present, either in the cells or the medium. We have shown that catecholamine, or cyclic–AMP, addition directly inhibits [K–Cl] cotransport in the duck red cell (Starke and McManus, J. Gen. Physiol. 92: 42a-43a, 1988). If that is also the case in the mackerel, then there may be a coordinated regulation between the swelling and shrinkage induced pathways — agents which stimulate one coordinately inhibit the other — similar to the effect we have previously demonstrated in both duck (Starke and McManus, op. cit.) and dog (Parker et al. J. Gen. Physiol. 96:1141-1152, 1990) cells. Further experiments will be required to confirm this conclusion.

The fish red cell is a useful model in this regard since it manifests [K–Cl] cotransport like many other red cells, has a shrinkage–induced Na/H exchange like the dog cell, but is hormonally regulated like [Na–K–2Cl] cotransport in the duck cell. Thus, it offers the investigator a unique opportunity to explore the interaction between cell volume dependent and hormonally controlled transport systems that share no ions in common, and therefore probably represent distinct gene products that are regulated by a common mechanism.

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