EFFECTS OF CA++ IONOPHORE AND PHORBOL ESTER ON TAURINE EFFLUX FROM SKATE (RAJA ERINACEA) ERYTHROCYTES.

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Free amino acids (FAA) are present in high concentrations in the cells of many marine vertebrates and invertebrates where they function in cell volume regulation. In elasmobranchs, they counteract the osmotic effects of high salt concentrations in extracellular fluid (ECF). During environmental dilution, when the ECF osmolality falls, FAA are released from the cells to reduce the osmotic gradient between the intra and extracellular fluids. In the little skate, Raja erinacea, hypoosmotic stress, either in vivo or in vitro, causes an increased release of taurine and B-alanine, the two major free amino acids in skate erythrocytes.

In this study we investigated possible cellular mechanisms that might be involved in the volume regulatory response of taurine efflux to in vitro hypoosmotic stress in the RBC of the skate. Specifically, we tested the effects of two intracellular signals, elevated Ca++ and protein kinase C activation, on taurine release.

Blood was drawn from skates via a caudal vessel and centrifuged to remove plasma and buffy coat. Amino acid efflux from RBC was measured as described by Boyd and Goldstein (Comp. Biochem. Physiol. 60: 319, 1978.). The cells were resuspended in one volume (with respect to the original volume) of an elasmobranch incubation medium (EIM) consisting of 300mM NaCl, 5.2mM KCl, 2.7mM $MgSO_4$, 5.0mM $CaCl_2$, 15mM Tris-HCl (pH 7.5) and 370mM urea (940mOsm). This suspension was preincubated with ^{14}C-taurine at a concentration of 5uCi/3.5ml (0.05umol) suspension for 3 h. All incubations were conducted in air, in a shaking water bath at 15±1 C. After three hours, the cells were washed twice with EIM containing taurine to remove excess ^{14}C-taurine and resuspended to the original 3.5ml volume. For the experimental incubation, 0.3ml of the final RBC suspension was added to flasks containing 3.5ml EIM with 0.1mM taurine. Those cells tested for response to hypotonic environment were incubated in a hypotonic EIM (identical to control EIM except that the concentrations of NaCl and urea were decreased to 200mM and 250mM, respectively, at 650mOsm.) with 0.1mM taurine. The flasks were incubated for an additional hour and samples were taken at 0, 30, and 60min. The samples were centrifuged and the supernatants analyzed by scintillation counting to determine the amount of ^{14}C-taurine released during incubation. The remaining packed RBC were weighed and resuspended in 5% $HClO_4$. After a final high speed centrifugation, the $HClO_4$ extracts were assayed for ^{14}C-taurine remaining in the cells.

Taurine efflux was calculated from the amount of ^{14}C-taurine (per g RBC) appearing in the incubation medium and the specific activity of taurine in the RBC, expressed in terms of umol/g RBC: DPM in medium (per g RBC) divided by DPM/umol (in RBC) = umol in medium (per g RBC).

As shown in figure 1, taurine efflux increased linearly with time from a mean of 0.73 ± 0.12 umol/g at 30min to 1.37 ± 0.21 umol/g at 60min in 15 control samples. The efflux of taurine under hypoosmotic conditions showed a significant increase over the controls as expected. The means under these conditions were 1.64 ± 0.28 umol/g at 30min and 2.66 ± 0.44 umol/g at 60min

for n=4, roughly twice that of the controls. Addition of 0.1uM of the Ca++ ionophore A23187 also had a significant effect. It increased taurine release to 2.34 ± 0.72 umol/g at 30min and 2.71 ± 0.54 umol/g at 60min for n=6. To insure that the effect on taurine release by the Ca++ ionophore was not simply due to a disruption of ionic and electrical gradients, we tested the effect of ouabain as a control (not shown). By inhibiting Na+-K+ ATP-ase, ouabain disrupts the normal ionic gradients and electrical potential across the cell membrane. However, at 1.0 mM, ouabain had no effect on taurine release. Efflux at 30min was 1.04 ± 0.02 umol/g and 1.52 ± 0.33 umol/g at 60min for n=3.

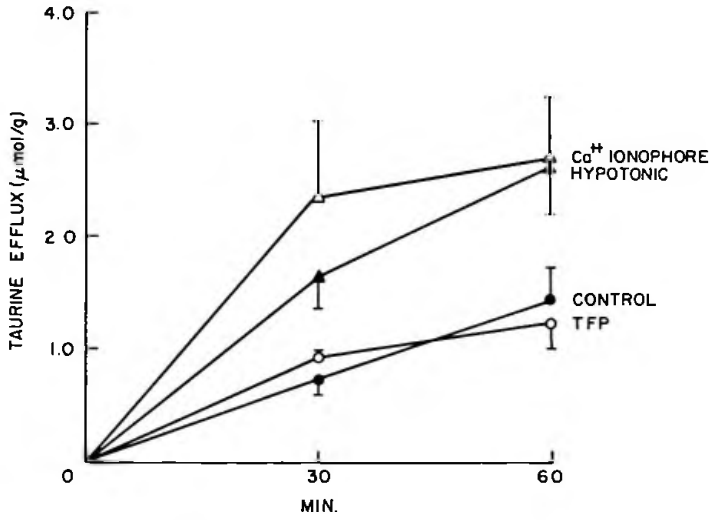

Figure 1. Effects of medium hypotonicity (650mOsm vs 940mOsm for control), Ca++ ionophore (A23187, 0.1uM) and calmodulin inhibitor trifluoperazine (TFP, 0.5-10uM) on taurine efflux from skate RBC.

The activation of taurine efflux by the Ca++ ionophore did not appear to operate via calmodulin. As shown in figure 1, addition of the calmodulin inhibitor, trifluoperazine (0.5-10uM), had no effect on taurine efflux. In addition, calmodulin inhibition did not block the stimulation of taurine release by hypotonic stress. The calmodulin inhibitor, compound 48/80 (50ug/ml), did not inhibit the increase in taurine efflux by hypotonicity (not shown). Efflux in hypotonic medium was 1.50 ± 0.47 umol/g at 30min, and 2.12 ± 0.47 umol/g at 60min (n=6). Efflux in hypotonic medium with compound 48/80 was 1.07 ± 0.63 umol/g at 30min and 2.25 ± 1.26 umol/g at 60min (n=3).

Figure 2 shows the effect of the phorbol ester, phorbol 12-myristate 13-acetate (TPA) at 0.1uM - 0.5uM, on the release of taurine. In the presence of this protein kinase C activator, efflux was significantly higher than that observed in both control and hypotonic samples. Efflux in the presence of the phorbol ester was 2.06 ± 0.61 umol/g and 4.03 ± 1.34 umol/g at 30 and 60min, respectively, for n=4, vs control values of 0.74 ± 0.13 umol/g and 1.31 ± 0.19 umol/g at 30 and 60min, respectively, for n=5.

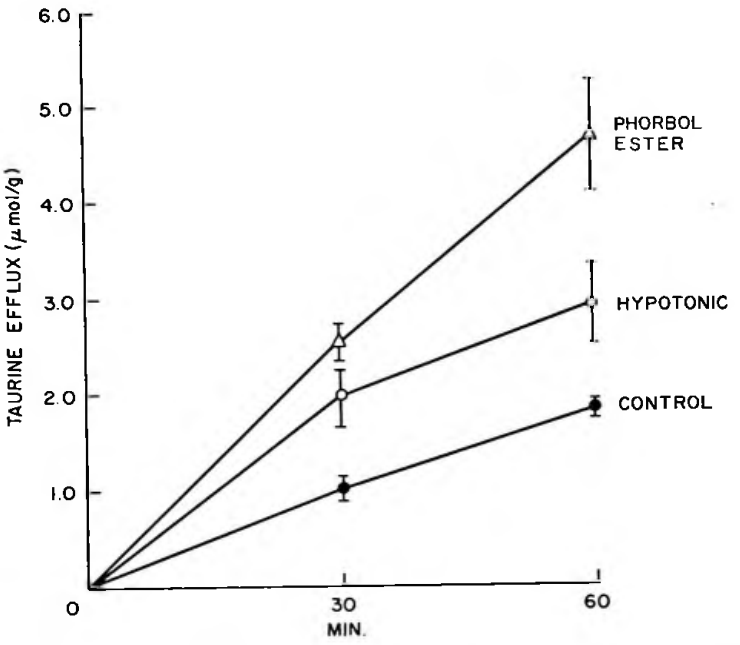

Figure 2. Stimulation of taurine efflux by phorbol ester (phorbol 12-myristate 13-acetate, 0.1-0.5uM.

In these experiments, a decrease in the extracellular osmolality increased taurine efflux in skate RBC. The Ca++ ionophore A23187, which is known to increase intracellular levels of free Ca++ ions, mimicked the effects of hypotonicity on taurine efflux. In Ehrlich ascites tumor cells, Ca++ and calmodulin appear to be involved in the taurine leak resulting from cell swelling or addition of Ca++ ionophore (Lambert, Molec. Physiol. 7: 323-332, 1985.). In skate RBC, Ca++ ionophore increased taurine release significantly. However, the release did not seem to involve calmodulin as a mediator, since the addition of trifluoperazine, a known inhibitor of calmodulin-regulated functions, exhibited no significant effect on basal taurine efflux, and compound 48/80, another calmodulin inhibitor, did not inhibit the stimulation of taurine efflux by hypoosmotic stress.

The experiments with the phorbol ester suggest that protein kinase C may be involved in the taurine release mechanism. It is possible that Ca++ and protein kinase C work in concert to regulate taurine release or that increased levels of Ca++ may activate the protein kinase C to increase taurine efflux. Future experiments will be concerned with the role of protein kinase C and Ca++ in the stimulation of taurine release during hypoosmotic stress.