TAURINE TRANSPORT AND RED CELL VOLUME IN HAGFISH (MYXINE GLUTINOSA)

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The mechanism of volume-activated (VA) taurine release from hypotonically stressed cells has not been definitively established, although studies using inhibitors of the anion exchanger, band 3, have suggested that this protein is involved in the response. Since the RBC of hagfish are deficient in band 3 (Ellory et al. J. Exp. Biol. 129:377, 1987), we examined the effects of hypoosmotic exposure on taurine transport and cell volume in these RBC.

Blood was collected from skates and hagfish through a caudal vessel using a heparinized syringe. RBC were separated by centrifugation, washed with isosmotic incubation media, and resuspended in appropriate media for use in experiments. To determine the amount of band 3 in hagfish compared with skate RBC, cell membranes were prepared by high speed centrifugation and were incubated with $^3H_2$DIDS, which binds with high affinity to band 3. After 30 min membranes were washed free of unbound $^3H_2$DIDS, and the proteins separated by SDS-PAGE. Radioactivity in the various membrane proteins was quantified by liquid scintillation counting of digested gel slices. Skate RBC showed two peaks for bound $^3H_2$DIDS. The major peak, representing 95% of the total radioactivity, was located on band 3. The remainder of the bound $^3H_2$DIDS was found in a small peak, most likely representing glycophorin. In contrast, hagfish RBC membranes showed only one small peak of bound $^3H_2$DIDS, containing less than 10% of the radioactivity found in skate RBC, and all localized to band 3. Additional evidence for low level of band 3 in hagfish RBC was found in sulfate transport studies. We used sulfate uptake to determine band 3 activity. We added 0.2ml of 20% RBC suspension to 2.5ml of either isosmotic or hypoosmotic incubation medium containing luCi $^{35}SO_4$. The solutions were incubated at 15° C in a shaking bath and samples were collected at several timepoints. Hagfish RBC showed only 9% the amount of sulfate uptake of skate RBC at 60 min.

We next examined the effect of band 3 deficiency on VA taurine transport in hagfish RBC. When hagfish RBC are exposed to medium approximately 1/2 isosmotic, taurine efflux is not significantly increased from the isosmotic control at 60 min, and it is only 13% of that in skate RBC under the same conditions. In addition, hagfish cell volume increases immediately to 180% of control upon exposure to hypoosmotic medium, with no significant RVD over 1h period. Skate RBC under the same conditions reduce cell volume by 16% in one hour. The finding that VA taurine efflux and RVD are greatly reduced in the band 3-deficient hagfish RBC supports our hypothesis that band 3 is involved in VA taurine release.

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