VOLUME-ACTIVATED, ENERGY AND SULFHYDRYL-DEPENDENT TAURINE TRANSPORT IN HEPATOCYTES FROM THE LITTLE SKATE (RAJA ERINACEA)

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Taurine is involved in cell volume regulatory responses in tissues from a number of marine organisms, in mammalian cardiac, renal and brain tissues, and in Ehrlich ascites cells. This amino sulfonic acid is particularly important in the adjustment of intracellular osmotic pressure in marine invertebrates and fishes, where it is found in concentrations as high as 190 mM (Chen and Preston, Bull. Environ. Contam. Toxicol. 39:202, 1987). Our previous studies with isolated skate hepatocytes demonstrate that these cells exhibit a regulatory volume decrease (RVD) after swelling in hypotonic media, and that taurine is an important osmotic effector of the RVD (Ballatori et al., Bull. MDIBL 29:71-72, 1990). The present study examines the driving forces and intracellular signals for activation of volume-stimulated taurine transport using primary suspension cultures of skate hepatocytes (Smith et al., J. Exp. Zool. 241:291, 1987; Ballatori and Boyer, Am. J. Physiol. 254:R801, 1988). Freshly isolated hepatocytes were resuspended in elasmobranch Ringer, and ^{14}C-taurine fluxes measured by a rapid centrifugation procedure (Ballatori and Boyer, Am. J. Physiol. 254:R801, 1988). Intracellular water space was determined as the difference between the ^{3}HOH and ^{14}C-inulin distribution spaces.

Taurine efflux from skate hepatocytes was stimulated in media made hypotonic by addition of H_2O or removal of NaCl, as well as in cells swollen in isotonic media containing rapidly penetrating solutes (202 mM ethylene glycol or 202 mM additional urea substituted for 101 mM NaCl), suggesting that cell swelling rather than hypoosmolarity is the stimulus for the activation of taurine release. This volume-sensitive taurine transport was specific for this amino acid, as release of glutathione, ^{14}C-L-alanine and other alpha amino acids (eg., threonine, serine, glutamate, glutamine, glycine or valine) was unaffected by dilution with 40% H_2O. Addition of 50 mM taurine or hypotaurine to the incubation media also had no effect on volume-stimulated ^{14}C-taurine efflux, suggesting that the taurine concentration gradient across the plasma membrane is not the driving force for taurine efflux. Indeed, taurine was apparently released against its concentration gradient under these experimental conditions, suggesting an active, energy-dependent process. Volume-stimulated taurine transport was temperature sensitive, nearly completely blocked by metabolic inhibitors (2,4-dinitrophenol, KCN, sodium azide, oligomycin, CCCP, and antimycin A) and sulfhydryl-reactive reagents (N-ethylmaleimide, diamide, iodoacetate, t-butyl hydroperoxide, and mercury), but was unaffected by Ca^{++} ionophore, phorbol ester, dibutyryl-cAMP, vasopressin, or pretreatment with ouabain or furosemide. DIDS (0.5 mM) also produced a significant inhibition of volume-stimulated taurine efflux. Agents that inhibited taurine release (N-ethylmaleimide, diamide, $HgCl_2$, 2,4-dinitrophenol, and iodoacetate plus KCN) also diminished the regulatory volume decrease.

These findings suggest that transport of taurine out of skate hepatocytes is an energy and sulfhydryl-dependent process, that is activated during osmotic regulation of cell volume. The driving force for efflux is independent of the taurine concentration gradient across the skate hepatocyte plasma membrane. (Supported by National Institutes of Health Grants ES03828 DK39165, and DK34989).