# TAURINE TRANSPORT AND VOLUME REGULATION IN HEPATOCYTES OF THE LITTLE SKATE (RAJA ERINACEA)

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Taurine, an important intracellular osmolyte in both invertebrate and vertebrate species, was present in relatively high concentrations (217±65 umol/g protein; n=8, ±SD; or approximately 65 mM) in hepatocytes from the elasmobranch Raja erinacea. To examine the role of this amino acid in skate hepatocyte volume regulation, transmembrane fluxes of taurine were measured under basal conditions and during regulatory volume decrease (RVD) induced by decreasing extracellular osmolarity by dilution (Ballatori et al., Toxicol. Appl. Pharmacol. 95:279, 1988). Hepatocytes were isolated from male skates by a collagenase perfusion technique, as previously described (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 Ringers, and $^{14}$C-taurine, $^{14}$C-L-alanine and $^{86}Rb^+$ 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 $^{3}H_2O$ and $^{14}$C-inulin distribution spaces.

Under isosmotic conditions, the high intracellular taurine levels were maintained by an active $Na^+$-dependent uptake process and a slow $Na^+$-independent efflux. Kinetic studies suggested the presence of two saturable $Na^+$-dependent taurine uptake systems (apparent Km(taurine)=0.089±0.055 and 4.47±0.98 mM, and Vmax=0.19±0.14 and 1.65±0.84 nmol·ul$^{-1}$·15 min$^{-1}$, for high and low affinity components, respectively, n=4), as well as a $Na^+$-independent uptake system. $^{14}$C-Taurine uptake was diminished by replacement of $Cl^-$ with $NO_3^-$, and almost completely abolished by replacement of $Na^+$ with choline$^+$. With these ion replacements, Hill plots revealed that the transport system operated with an apparent $Na^+/Cl^-$/taurine coupling ratio of 2:2:1 and exhibited apparent Kms for $Na^+$ and $Cl^-$ of 110 and 155 mM, respectively. Uptake was inhibited by other beta-amino acids, but not by alpha-amino acids, taurocholate or DIDS (0.5 mM).

Efflux of $^{14}$C-taurine under isosmotic conditions was relatively slow (~10% in 2h), and was unaffected by replacement of $Na^+$ with choline$^+$ or $K^+$, by replacing $Cl^-$ with $NO_3^-$, nor by the addition of 50 mM unlabeled taurine to the extracellular media, suggesting that taurine efflux is not an exchange system. In contrast, efflux was markedly stimulated in hypotonic media. Cell suspensions diluted with either 0, 30, 40 or 50% $H_2O$ released 11, 24, 41 and 65% of intracellular $^{14}$C-taurine over a two hour period, respectively. The time course of taurine release paralleled the volume recovery after swelling cells in hypotonic media: RVD and taurine release were both quite high during the first 20 min after dilution, then decreased and approached baseline rates by 40 min. Volume-stimulated taurine efflux was unaffected by replacement of $Na^+$ with choline$^+$ or $K^+$, and was only slightly diminished by replacing $Cl^-$ with $NO_3^-$. Addition of 50 mM taurine or hypotaurine to the extracellular media also had no effect on volume-stimulated $^{14}$C-taurine efflux. Swelling-induced efflux was specific for taurine, as release of

intracellular $^{86}Rb^+$, $K^+$, glutathione, $^{14}C$-L-alanine and other alpha-amino acids (e.g., threonine, serine, glutamate, glutamine, glycine or valine) was unaffected by dilution with 40% $H_2O$. In addition, volume-stimulated $^{14}C$-taurine release was temperature-sensitive, partially inhibited by treatment with KCN (0.25 mM) or DIDS (0.5 mM), and nearly completely blocked by 0.5 mM 2,4-dinitrophenol, but was unaffected by pretreatment of the hepatocytes with ouabain (2 mM).

These findings suggest that functionally distinct pathways mediate taurine uptake and efflux in skate hepatocytes. Uptake is largely $Na^+$-dependent and requires $Cl^-$ for maximal activity. The efflux pathway is an energy-dependent process which is activated during osmotic regulation of cell volume. In contrast with findings in other vertebrate species, the RVD in skate hepatocytes is essentially independent of $K^+$ fluxes, and appears to result from release of organic osmolytes, including taurine. (Supported by National Institutes of Health Grants ES03828, DK39165, and DK34989).