A VOLUME-ACTIVATED TAURINE CHANNEL IN HEPATOCYTES FROM RAJA ERINACEA

N. Ballatori[1], T.W. Simmons[2], and J.L. Boyer[3]
[1]Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642
[2]Department of Biology, Indiana University of Pennsylvania, Indiana, PA 15705
[3]Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, CT 06510

Eukaryotic cells respond to an increase in intracellular volume by activating membrane transporters or enzymes that enable them to expel or eliminate specific solutes. In most cell types regulatory volume decrease (RVD) results in the release of both inorganic and organic solutes. Potassium and chloride are the major inorganic osmolytes, and are released either through separate volume-activated channels or by KCl cotransport systems. The major organic osmolytes are amino acids, polyols and methylamines. Although the transport systems involved in their release are not well understood, recent studies suggest that multispecific volume-activated channels could be involved (Banderali and Roy, Am. J. Physiol. 263:C1200-C1207, 1992; Haynes and Goldstein, Am. J. Physiol. 265:R173-R179, 1993; Kirk et al., J. Biol. Chem. 267:23475-23478, 1992). Volume-activated taurine, glucose, and uridine transport in flounder erythrocytes increases in parallel with increasing cell volume, and shows similar sensitivities to a number of anion channel blockers (Kirk et al., J. Biol. Chem. 267:23475-23478, 1992). Organic osmolyte transport in skate erythrocytes is also inhibited by DIDS and other anion transport blockers (Haynes and Goldstein, Am. J. Physiol. 265:R173-R179, 1993). In MDCK cells, Banderali and Roy (Am. J. Physiol. 263:C1200-C1207, 1992) have identified a volume-activated, outwardly rectifying anion channel that is permeable to chloride, aspartate, glutamate, and the anionic form of taurine (at pH 8.2).

Taurine is a major intracellular osmolyte of skate hepatocytes, and is selectively released from these cells following cell swelling (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). Our previous studies have demonstrated that volume-activated taurine efflux from skate hepatocytes is independent of Na^+, temperature sensitive, DIDS inhibitable, and nearly completely blocked by metabolic inhibitors and sulfhydryl reagents (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992), and is localized to the basolateral membrane of skate hepatocytes (Simmons et al., Bull. Mt. Desert Isl. Biol. Lab. 32:54-55, 1993). The present study examines whether volume-activated taurine efflux in these cells is mediated by a channel or a carrier, and determines further characteristics of the transport pathway.

Hepatocytes were isolated from male skates by a collagenase perfusion technique (Smith et al., J. Exp. Zool. 241:291-296, 1987). Immediately after isolation, cells were resuspended in elasmobranch Ringer's and incubated at 15°C in polypropylene tubes. Preloading with [^{14}C]taurine was achieved by incubating with 0.2 mM [^{14}C]taurine for 1.5-2 h at 15°C. The cells were then centrifuged at 250xg for 2 min, and resuspended in Ringer's solution also maintained at 15°C. This washing step was repeated to eliminate any residual extracellular [^{14}C]taurine. Within 5-10 min of the final resuspension, taurine efflux measurements were started. At time zero, hypotonicity was induced by diluting the cell suspension with 40% H_2O, or 40% Ringer's for control, and the ^{14}C remaining within cells was then measured over 90 min. The intracellular water space was determined as the difference between the ^3H_2O and [^{14}C]inulin distribution spaces.

Our previous studies demonstrated that exposure of isolated skate hepatocytes to hypotonic media activates both Na^+-independent taurine uptake and efflux transport mechanisms (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). To assess whether taurine transport is mediated by a carrier or a channel, we compared the dependency of these fluxes on transmembrane taurine concentration gradients. Rate coefficients of [^{14}C]taurine influx and efflux were measured over a 5 min interval after a 40% dilution with water, in the presence of either 0.1, 1, 10, 40 or 100 mM extracellular taurine. Rate coefficients for volume-stimulated [^{14}C]taurine uptake and efflux in isolated skate hepatocytes were similar (Table 1). Moreover, [^{14}C]taurine uptake and efflux were unaffected by extracellular taurine concentration, consistent with the opening of a volume-activated channel that allows [^{14}C]taurine to move down its concentration gradient. Rate coefficients were unaffected by extracellular taurine concentrations as high as 100 mM, providing further evidence for a channel.

Table 1. Rate coefficients for [^{14}C]taurine uptake and efflux during regulatory volume decrease.

$[Taurine]_{out}$, mM	k_{in}, min^{-1}	k_{out}, min^{-1}
0.1	0.0144±0.0026	0.0154±0.0032
1	0.0136±0.0027	0.0124±0.0014
10	0.0164±0.0014	0.0185±0.0056
40	0.0185±0.0014	0.0179±0.0065
100	0.0153±0.0014	0.0129±0.0023

Values are means ± SEM of 4 cell preparations. Rate coefficients were measured over a 5 min interval after a 40% dilution with water. For the efflux experiments, the cells were preloaded with [^{14}C]taurine by incubating with 0.2 mM [^{14}C]taurine for 1.5-2 h, then washed free of extracellular taurine, whereas uptake was measured in Na^+-free ($choline^+$) Ringer's to eliminate the contribution of the Na^+-dependent uptake component.

Volume-activated taurine efflux was previously shown to be inhibited by DIDS (0.5 mM), and nearly completely blocked by a number of metabolic inhibitors and sulfhydryl reagents (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). Table 2 shows that these same agents also blocked volume-activated [^{14}C]taurine uptake. As with efflux, uptake was nearly completely prevented by pretreatment of the cells with the metabolic inhibitors 2,4-dinitrophenol, antimycin A, and KCN plus iodoacetate, as well as the sulfhydryl-reactive agent N-ethylmaleimide, and the transport inhibitor DIDS (Table 2). In contrast, membrane depolarization in high K^+ media had no effect on volume-activated taurine flux (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992).

The role of intracellular ATP in volume-activated taurine transport was studied further by examining the effects of ATP depletion at different times after the hypotonic stimulus. Administration of 2,4-dinitrophenol, a highly membrane permeant metabolic inhibitor, nearly completely prevented any further release of taurine when added at different times after cell swelling, indicating that taurine release requires the continual presence of intracellular ATP. Similar effects were noted with two other metabolic inhibitors, antimycin A and the combination of KCN plus iodoacetate; however, the effects of these agents were not as immediate as seen with 2,4-dinitrophenol, most likely reflecting the relative ease with which antimycin A

and KCN plus iodoacetate enter cells and deplete energy stores. In contrast to these compounds, the sulfhydryl reagent N-ethylmaleimide was able to block volume-activated taurine transport only when cells were pretreated with the inhibitor for 10-30 min. The reason for this delayed effect is not clear, but may also reflect limited entry into the cells and interaction with target sites.

Table 2. Effects of metabolic inhibitors and DIDS on volume-stimulated taurine uptake in isolated skate hepatocytes.

	Concentration	% of control
Control		100
2,4-Dinitrophenol	0.5 mM	5±3*
Antimycin A	30 ug/ml	3±2*
Iodoacetate + KCN	1 mM each	30±4*
N-Ethylmaleimide	1 mM	9±4*
DIDS	0.5 mM	27±10*

Values are means ±SEM (n=4). Cells were resuspended in Choline-Ringer's to block Na^+-dependent taurine uptake, and were pretreated for 30 min with the indicated concentrations of metabolic or transport inhibitors, prior to the hypotonic stimulus (40% H_2O). Taurine uptake was measured from 1.5-6.5 min after addition of water, at an extracellular [^{14}C]taurine concentration of 10 mM. *Significantly different from control cells exposed to hypotonic media, $p<0.05$, using Student's t test.

Volume-activated taurine efflux was also immediately blocked when isotonicity was restored, and swollen hepatocytes rapidly returned to their normal volumes. This rapid volume activation and inactivation suggests that the signal transduction mechanisms that control channel function respond quickly and efficiently to changes in cell volume.

In summary, these findings demonstrate that taurine efflux from skate hepatocytes swollen in hypotonic media is mediated by a channel. This channel is localized to the basolateral membrane and appears to require the continual presence of intracellular ATP for its function. (Supported by National Institutes of Health Grants ES03828 and DK34989, and by a Pew Charitable Trusts Fellowship).