

VOLUME-REGULATORY AMINO ACID TRANSPORT IN HEPATOCYTES FROM RAJA ERINACEA

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Many cell types have been shown to regulate their volume following cell swelling by activating a plasma membrane channel that allows taurine and perhaps other intracellular organic osmolytes to efflux from the cell. We demonstrated that skate hepatocytes also possess a swelling-activated osmolyte channel that is permeable to taurine, and have demonstrated that this channel is regulated by intracellular ATP (Ballatori et al., *Am. J. Physiol.* 267:G285-G291, 1994; Ballatori and Boyer, *Am. J. Physiol.* 262:G451-G460, 1992; Ballatori et al., *Mol. Pharmacol.* 48:472-476, 1995).

To further characterize the nature of the channel and its potential substrates, the present study compared volume-activated efflux of ¹⁴C-*taurine*, to that of ¹⁴C-*L-alanine*, ¹⁴C-*L-phenylalanine*, ¹⁴C-*methylaminoisobutyric acid* (MeAIB), ¹⁴C-*betaine*, ¹⁴C-*glycine*, ³H-*myoinositol* and ¹⁴C-*sorbitol*. Hepatocytes were isolated from male skates and were preloaded with radioisotope by incubating with 0.1 mM of the indicated compounds for 2 h at 15°C. Hepatocytes to be loaded with amino acids were incubated in medium that also contained 2 mM aminooxyacetic acid to inhibit pyridoxal phosphate-dependent enzymes. Cells were then washed to remove extracellular radioactivity, and hypotonicity was induced by diluting the cell suspensions either 40% or 50% with H₂O. Cellular ¹⁴C or ³H content at 10, 30 and 60 min after swelling was measured by scintillation spectrometry.

Cell swelling produced a marked activation of ¹⁴C-*taurine* efflux, with ~50% of the amino acid released after one hour of incubation in medium diluted 40% with water. *Betaine*, *glycine*, *MeAIB*, and *L-alanine* were released at rates comparable to *taurine* following cell swelling. In contrast, cell swelling produced minimal stimulation of *phenylalanine* efflux. Comparable findings have previously been reported in skate red blood cells (Haynes and Goldstein, *Am. J. Physiol.* 265:R173-R179, 1993). However, skate hepatocyte swelling produced only a small increase in *myoinositol* efflux (10-20% released after one hour) and an even smaller effect on *sorbitol* efflux (5-10% stimulation of efflux). However, there was considerable spontaneous release of radioisotope from ¹⁴C-*sorbitol*-loaded cells, under isosmotic conditions. It is possible that some of these compounds may have been metabolized by the hepatocytes, although this was not quantitated in the present study.

The present findings indicate that in addition to *taurine*, other organic osmolytes can be released by skate hepatocytes in response to cell swelling. In particular, small neutral amino acids such as *glycine*, *L-alanine*, and *betaine* are released; however, the larger and more bulky amino acid *phenylalanine* is not readily released. *Sorbitol* and *myoinositol* are also released at a relatively slow rate after cell swelling. Additional studies are needed to distinguish whether these organic osmolytes are released by a single multi-specific channel, or by distinct swelling-activated mechanisms. (Supported by the National Institute of Environmental Health Sciences (ES03828 and ES01247), and the National Institute of Diabetes and Digestive and Kidney Diseases (DK34989 and DK25636)).