DIVALENT METALS MODULATE THE OSMOREGULATORY TAURINE EFFLUX PATHWAY IN SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Cell swelling in a variety of different cell types activates plasma membrane channels that mediate the efflux of intracellular organic osmolytes, including the amino acid taurine. We previously characterized a swelling-activated channel in skate hepatocytes, and demonstrated that this channel is regulated by intracellular ATP (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992; Ballatori et al., Mol. Pharmacol. 48:472-476, 1995; Jackson et al., Am. J. Physiol. 270:C57-C66, 1996), and inhibited by divalent inorganic mercury, $HgCl_2$, (Ballatori et al., Toxicol. Appl. Pharmacol. 95:279, 1988; Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992; Ballatori and Boyer, Toxicol. Appl. Pharmacol. 140:404, 1996).

The mechanism by which $HgCl_2$ inhibits the channel is not yet known; however, our recent studies indicate that the effects of mercury are not explained by changes in intracellular ATP levels or ATP/ADP ratios (Ballatori and Boyer, Toxicol. Appl. Pharmacol. 140:404, 1996). Mercuric chloride inhibited swelling-stimulated [^{14}C]taurine efflux in isolated skate hepatocytes at concentrations (20-40 μM) that had no effect on intracellular ATP levels or ATP/ADP ratios. In order to examine whether other metals also affect channel function, the present study measured volume-activated [^{14}C]taurine efflux in isolated skate hepatocytes pretreated for 30 min with increasing concentrations of monovalent, divalent, and trivalent metal ions. Cellular ATP levels were measured to confirm that the effects of the metals were not due to changes in intracellular ATP levels, which would indirectly affect channel activation. ATP was measured by the method of Hill et al. (Methods Enzymol. 148:132-141, 1987). Methods for cell isolation and taurine efflux were as described by Ballatori and Boyer (Am. J. Physiol. 262:G451-G460, 1992).

Several of the divalent metals tested significantly inhibited taurine efflux without affecting cellular ATP levels, including zinc (1 mM; 49% of control), nickel (10 mM; 59%), cobalt (10 mM; 66%), manganese (10 mM; 47%), calcium (25 mM; 77%) and magnesium (25 mM; 66%). Strontium (10 mM), barium (10 mM), and cadmium (1 mM) had only minimal effects. In contrast to the divalent metals, the monovalent cations cesium, rubidium and potassium (25 mM each), and the trivalent cations lanthanum and gadolinium (1 mM each) had no effect on taurine efflux. Higher concentrations of lanthanum and gadolinium were toxic to the cells. Because lanthanum and gadolinium have been shown to inhibit stretch-activated channels at micromolar concentrations, the swelling-activated taurine channels may be distinct from stretch-activated channels. When volume regulation was assessed by video-microscopic analysis in single cells (Fletcher et al., MDIBL Bull. 35:62, 1996), 10 mM nickel (n=7), 10 mM manganese (n=9) and 1 mM zinc (n= 10) all delayed the volume response to hypotonic stress. Hepatocyte swelling was slightly greater after addition of these metals, and there was a delay of several minutes before a volume regulatory response was observed. Thereafter the response was similar to the control cells. The present study indicates that several divalent metals alter cell volume regulation and inhibit swelling-stimulated taurine efflux, whereas monovalent and trivalent metals had no effect. Inhibition of taurine efflux was observed at metal concentrations that had no effect on cellular ATP levels, indicating a distinct mechanism of action. (Supported by ES03828, ES01247, ES06484, DK34989, DK25636, and DK48823, and by the NSF Young Scholars Program, ESI9452682).