COMPARISON OF TAURINE CHANNEL ACTIVATION IN SKATE (*RAJA ERINACEA*) RED BLOOD CELLS AND RAT RENAL MEDULLARY CELLS

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Taurine is one of the organic compounds used in skate red blood cells and in mammalian inner medullary collecting duct (IMCD) cells as an osmolyte in volume regulation. During regulatory volume decrease following the initial swelling of the cells in hypotonic media, taurine channels which mediate rapid taurine efflux from the cells are activated. The mode of activation of these channels is still a matter of debate. Recently, Wittels et al. (Am. J. Physiol. 279:R69-R76, 2000) described that in skate red blood cells hypoosmotic swelling caused a large increase in taurine efflux whereas swelling induced by treatment with isoosmotic non-electrolytes produced a much smaller stimulation. This result raised some doubt as to the exclusive role of intracellular ionic strength as the sole trigger for channel opening. We therefore investigated whether a similar effect can be observed in a mammalian system that also uses taurine as an organic osmolyte.

IMCD cells were isolated from the white papillary tissue of rat kidneys as described recently (Stokes et al., Am. J. Physiol. 253:F251-F262, 1987) in a 600 mosm/l HEPES-Ringer solution composed of 268 mM NaCl, 16 mM HEPES-free acid, 16 mM HEPES-Na salt, 5 mM D-glucose, 3.2 mM KCl, 2.5 mM $CaCl_2$, 1.8 mM KH_2PO_4, 1.8 mM $MgSO_4$, pH 7.4. The tissue was minced and the pieces were incubated under agitation with air for 75 min in the same buffer containing 0.2% collagenase, 0.2% hyaluronidase and 0.001% DNAse at 37°C. A series of low-speed differential centrifugations followed. The final pellet contained IMCD cells at a purity of > 95%. Viability of the cells subjected to different experimental protocols was determined by measuring the loss of cytosolic lactate dehydrogenase (LDH) from the cells. LDH activity was determined at room temperature as the amount of pyruvate consumed, by continously monitoring the decrease in absorbance due to oxidation of NADH at 340 mm. None of the experimental manipulations described below had a significant impact on cell membrane integrity.

Taurine efflux from IMCD cells exposed to hypoosmotic media (300 mosmol/l by removal of NaCl) was 33.3±10.6% of total cellular content in 5 minutes, about 70% of which could be inhibited by 0.5 mM SITS (4-acetamido-4´-isothiocyanatostilbene-2,2´-disulfonic acid). Thus, a strong reproducible activation of taurine channels was observed. When IMCD cells were exposed to isoosmotic media in which part of the NaCl had been replaced by diethylurea or ethylene glycol taurine efflux was 25.1±6.4% and 37.5±15.2%, respectively. Again, about 70% of the efflux could be inhibited by SITS. Therefore, in contrast to skate red blood cells, the amount of taurine released under the various experimental conditions from IMCD cells was not significantly different.

If one assumes that in IMCD cells similar changes in cell volume and intracellular ionic strength occur (measurements are underway) as those found in skate red blood cells these results suggest a parallelism betweeen changes in intracellular ionic strength and the degree of activation of taurine channels in IMCD cells. In future experiments it has to be established whether this also holds for other organic osmolytes and how changes in intracellular ionic strength can activate the different signal transduction pathways that have

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