VOLUME ACTIVATED TRIMETHYLAMINE OXIDE (TMAO) EFFLUX IN RED BLOOD CELLS OF SPINY DOGFISH (SQUALUS ACANTHIAS)

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Volume regulation is a fundamental property of most cells. In the red blood cells of skates (*Raja erinacea*), cell swelling activates the release of small organic compounds (e.g. taurine) out of the cell which induces a water loss that compensates for the swelling. Previous studies have shown that cell swelling activates tyrosine kinases, p72syk and p56lyn, which are thought to play a role in the opening of the volume-activated osmolyte channel. Dogfish (*Squalus acanthias*), another elasmobranch, maintains osmotic balance with the marine environment by retaining extracellular TMAO, along with urea and NaCl. High intracellular TMAO concentrations suggests that the osmolyte functions in cell volume regulation in the dogfish. Preliminary studies have shown that the uptake of ^{14}C-TMAO occurs via a sodium independent and a sodium dependent pathway in dogfish red blood cells and the efflux of TMAO is stimulated by hypotonic volume expansion (Wilson and Goldstein, J. Exp. Zool., vol. 284, pp 605-609, 1999). The purpose of the present study was to determine whether volume-activated TMAO efflux occurs via the swelling activated osmolyte channel.

Dogfish erythrocytes were isolated and resuspended to 20% hematocrit in either isotonic (940 elasmobranch incubation media or EIM), hypotonic (460 EIM), isosmotic non-electrolytes (ethylene glycol or diethyl urea) or isosmotic electrolyte (ammonium chloride), and the samples were placed in a 15^{o} shaking water bath for designated time periods. Aliquots of the samples were taken (in duplicate, where n=3-12), washed, centrifuged and the supernatant was separated from the pellet. The red blood cell pellets were lysed, centrifuged and the supernatant was used to determine intracellular TMAO or electrolyte concentrations. For taurine efflux experiments, erythrocytes were pre-incubated in 940 EIM with ^{3}H-taurine for 1 h, washed, brought to 20% hematocrit in one of the above media, and the experiment proceeded as described above. Aliquots of the supernatant were placed in scintillation vials and analyzed by scintillation counting. For inhibitor studies, agents were present in the incubation medium during the experiment, but piceatannol was present in incubation medium during pre-incubation and during the experiment.

When the dogfish red blood cells were incubated in hypotonic and isosmotic non-electrolytes (ethylene glycol and diethyl urea), the cells swelled (1.52 to 1.65 times the control) and there was a decrease in intracellular electrolytes (28% to 30% decrease). Effluxes of TMAO and taurine increased 35-40% and 16-25%, respectively, within 30 min. Tyrosine kinases, p72syk and p60lyn, were also activated to levels significantly higher than control. However, none of these effects occured when isosmotic electrolyte (ammonium chloride) was used. Pharmacological blockers (DIDS, niflumic acid and quinine) of the volume-activated osmolyte channel significantly inhibited TMAO and taurine effluxes. Tyrosine kinase inhibitor, AG18, significantly blocked both TMAO and taurine effluxes but piceatannol, another tyrosine kinase inhibitor, inhibited taurine efflux but not TMAO efflux. Our findings suggest that TMAO efflux in dogfish RBC occurs via a volume-activated pathway similar to that reported for taurine in other fish RBC and requires a change in intracellular electrolyte concentration as well as cell swelling. Volume activation of tyrosine kinases accompany and possibly regulate the activation of TMAO efflux under these conditions. Supported by NSF grant IBN-9974350 (to L.G.).
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