TRIMETHYLAMINE OXIDE TRANSPORT BY SKATE (*Raja erinacea*) ERYTHROCYTES

Elizabeth Wilson and Leon Goldstein
Division of Biology and Medicine, Brown University, Providence, RI 02912

Trimethylamineoxide (TMAO) occurs in high concentrations in the tissues of marine animals. As an osmolyte, TMAO helps elasmobranchs to maintain an internal osmolarity that is similar to that of sea water. Nothing is known, however, about the transport of TMAO across cell membranes. The purpose of this study is to identify the mode(s) of TMAO transport by elasmobranch erythrocytes.

TMAO uptake in skate red blood cells (RBC) was measured using radiolabled 14C-TMAO. RBC were washed and suspended to 20% hematocrit using isotonic elasmobranch incubation medium (940 EIM [940 mOsm/kg H20] in mM: 300 NaCl, 5.2 KCl, 2.7 MgSO4, 5.0 CaC12, 15 Tris-HCl, 370 urea, pH=7.4). 0.25 mL of the RBC mixture was then added to a flask containing 1 ml of 940 EIM, 0.1 μCi 14C-TMAO and 0.1 mM TMAO. The sample was incubated in a shaking water bath (15°C) for 1h and an aliquot was withdrawn and centrifuged. The supernatant was aspirated, the RBC washed four times and extracted with perchloric acid. The sample was centrifuged and the supernatant analyzed for 14C-TMAO.

Under isotonic conditions, the 14C-TMAO uptake was 1.81 ± 0.04 nmol/g RBC•h (mean ± SE). Replacement of NaCl in the 940 EIM with LiCl significantly inhibited TMAO uptake (940 NaCl EIM: 2.61 ± 0.32 nmol/g RBC•h; 940 LiCl EIM: 1.60 ± 0.15 nmol/g RBC•h, n=4, P< .05). 2,4 dinitrophenol (2,4 DNP 0.2 mM) also inhibited TMAO uptake (940 NaCl EIM: 3.27 ± 0.17 nmol/g RBC•h; 940 NaCl EIM with 2,4 DNP: 1.28 ± 0.22 nmol/g RBC•h, n=4, P< .01). Swelling activated, Na+ independent (SANI) TMAO uptake was measured using hyptonic EIM (460 Li EIM) (100 mM LiCl, 250 mM urea). TMAO uptake was stimulated by cell swelling (940 LiCl EIM: 0.90 ± 0.04 nmol/g RBC•h; 460 LiCl EIM: 18.08 ± 1.13 nmol/g RBC•h), n=4, P< .01). SANI ^{14} C-TMAO uptake was self-inhibited by 43% when 25 mM cold TMAO was added to the incubation flask. When added in place of the cold TMAO, 25 mM taurine and 25 mM sucrose did not inhibit TMAO uptake. DIDS (0.1 mM), an inhibitor of the organic osmolyte channel, partially inhibited SANI TMAO uptake (460 LiCl EIM.: 18.08 ± 1.13 nmol/g RBC•h; 460 LiCl EIM with .1 mM DIDS: 12.27 ± 0.42 nmol/g RBC•h, n=4, P<.01). 1 mM quinine, another osmolyte channel blocker, also inhibited TMAO uptake (460 LiCl EIM.: 15.52 ± 1.20 nmol/g RBC•h; 460 LiCl EIM with quinine: 6.41 ± 0.55 nmol/g RBC•h, n=4, P<.02). The inhibition of TMAO uptake by these inhibitors suggests that an osmolyte channel identical or very similar to the taurine channel is involved in volume activated TMAO uptake.

The transport of TMAO across the cell membrane of skate erythrocytes occurs via two pathways. A Na+ cotransporter, inhibited by 2,4 DNP, is responsible for Na+ dependent uptake. Na+ independent uptake is volume activated, and inhibited by DIDS, and quinine. The Na+ independent TMAO uptake is likely to be via the volume activated organic osmolyte channel, band 3.

Supported by NSF Grant IBN9505567 (LG)