2-1

Glucose Transporter Isoforms GLUT1 and GLUT3 Transport Dehydroascorbic Acid.

Oran Kwon*, Steven C. Rumsey#, and Mark Levine#.
Korean Food and Drug Administration and # Molecular and Clinical Nutrition Section, NIDDK, NIH

Ascorbate (AA) is transported across cellular membranes by two distinct mechanisms. Ascorbate itself is transported by a sodium-dependent saturable transporter which has not been isolated. Ascorbate outside cells can be oxidized to dehydroascorbic acid (DHA), which is transported by a different mechanism. Once within cells, dehydroascorbic acid is immediately reduced to ascorbate by both chemical and protein mediated processes. DHA is structurally similar to glucose. Therefore, DHA entry has been proposed to be mediated by glucose transporters. To characterize DHA and AA transport, the *Xenopus laevis* oocyte expression system was used and transport of DHA and AA via glucose transporter isoforms GLUT1-5 and SGLT1 were examined. mRNA coding for the individual proteins was injected into oocytes and concentration-dependent DHA and AA transport activity was assessed. Radiolabeled 2-deoxyglucose (DG), fructose, or glucose uptake performed within the same experiment was a positive control for transporter activity. [^{14}C] DHA transport in GLUT1 and GLUT3 expressing oocytes was over 100-fold greater than control sham-injected oocytes. DHA trnasport by oocytes expressing GLUT2, GLUT5, and SGLT1 did not differ from sham-injected controls. GLUT4 transport of DHA was only 2-4 fold above control. Uptake of radiolabeled sugars for the different transporters was in the range expected. Ascorbate transport by GLUT1-5 and SGLT1 was not different from sham-injected controls(<1 pmol/oocyte/10-min incubation). Identity of all cDNA clones was confirmed by restriction fragment mapping and cDNAs of GLUT1 and GLUT3 were further verified by polymerase chain reaction. To determine DHA transport kinetics internal reduction of DHA to AA must be complete and efflux of DHA should not occur. Under conditions of incomplete reduction, reduction rather than transport becomes limiting. Consistent with these observations, DHA efflux occurred only when reduction was incomplete. At all concentrations of DHA, HPLC analysis confirmed 100% reduction of DHA to AA within oocytes. Kinetic parameters were calculated using best-fit analysis and Eadie-Hofstee transformation. The apparent K_m was 1.1 $\pm$ 0.2 mM and V_{max} was 108 pmol/min/oocyte for GLUT1, and apparent K_m was 1.7 $\pm$ 0.3 mM with V_{max} of 241 pmol/min/oocyte for GLUT3. DHA transport activity in both GLUT1 and GLUT3 was inhibited by 2-DG, D-glucose, and 3-O-methylglucose among

other hexoses while fructose and L-glucose showed no inhibition. Inhibition by the endofacial inhibitor, cytochalasin B, was non-competitive and inhibition by the exofacial inhibitor, 4,6-O-ethylidene-a-glucose, was competitive. Expressed mutant constructs of GLUT1 and GLUT3 did not transport DHA. DHA and 2-DG uptake by Chinese hamster ovary cells overexpressing either GLUT1 or GLUT3 was increased 2-8 fold over control cells. These studies suggest GLUT1 and GLUT3 isoforms are the specific glucose transporter isoforms which mediated DHA transportand subsequent accumulation