HEXOSE TRANSPORT BY FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) ERYTHROCYTES

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Glucose (Glc) uptake by flounder red blood cells (RBC) is a slow process, enhanced by anoxia and the Ca^{2+}-ionophore, A23187 (Booz et al., Bull. MDIBL 28: 47, 1989). This is true of many mammalian cells, where Glc uptake and metabolism are linked (Morgan and Whitfield, Curr. Topics Membr. Trans. 4: 255, 1973). Here we report studies characterizing this non-hormonal control of Glc uptake. Unless otherwise noted, values are mean ± SE for N fish.

A23187 induced Glc and 2-deoxyglucose (dGlc) uptake was nearly as great when medium Ca^{2+} was reduced to 10 µM (Table 1), suggesting that it was not acting by disrupting cell structure (Vann Bennett, Biochim. Biophys. Acta 988: 107, 1989). The calmodulin antagonist trifluoperazine (10 µM) did not block its effect. Ca^{2+}-enhanced uptake does not involve protein kinase C or a protease (Booz et al., loc cit). Hence, an effect of Ca^{2+} on either the carrier or putative regulatory site, as suggested for avian RBC (Bihler et al., Cell Calcium 3: 243, 1982), or on metabolism should be considered.

The 2 h dGlc uptake was on average 10.43 ± 2.79 (3) -fold more than that of Glc by the same cells, perhaps because it has higher affinity for the carrier, or enhances its own uptake by affecting metabolism, e.g., by depleting ATP.

Vanadate stimulates glycolysis in human RBC and also depletes them of 2,3-diphysphoglycerate (Ninfali et al., Arch. Biochem. Biophys. 226: 441, 1983). Anoxia should do the same in flounder RBC, because of the Pasteur effect and enhanced binding of 2,3-diphosphoglycerate by deoxygenated hemoglobin (Vann Bennett, loc cit). Vanadate (1 mM) did enhance Glc uptake as much as anoxia in the same cells, 5.83 ± 0.29 (4) and 5.91 ± 1.08 (4) -fold, respectively. Their effects were additive (2 fish). Hence, anoxia-enhanced uptake is likely not related to Na^+ pump activity, of which vanadate is a potent inhibitor (Nechat, Ann. Rev. Pharmacol. Toxicol. 24: 501, 1984).

N-Carbobenzoxy-glycyl-L-phenylalaninamide (CBZ) inhibits insulin enhanced, but not basal, Glc uptake by fat cells (Aiello et al., Biochem. 25: 3944, 1986). In flounder RBC, 1.5 mM CBZ inhibited 2 h Glc uptake with anoxia by 73 ± 3 % (4), while its effect on basal uptake was variable. At lower doses, CBZ may have enhanced basal uptake, since accumulation of Glc and its metabolites increased by as much as 100%. Alternatively, Glc oxidation may have been reduced; though, no effect was seen on basal oxidation of fat cells.

The findings with CBZ raise the possibility that basal and anoxia enhanced Glc uptake occur by different carriers, or two states of the same carrier. In addition, a re-examination of the role of metabolism in anoxia enhanced transport is warranted in light of the results with vanadate.

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TABLE 1. Effect of A23187 on 2 h hexose uptake (Media, hexose, 2 mM)

	Glucose uptake (mM)	2-Deoxyglucose uptake (mM)
No addition	0.18 ± 0.03	1.37 ± 0.07
10 µM A23187	1.20 ± 0.09*	2.09 ± 0.08*
Low Ca^{2+} Ringer's+	0.24 ± 0.04	1.63 ± 0.01
Low Ca^{2+} & 10 µM A23187	0.97 ± 0.04*	2.44 ± 0.10*

Mean ± SD, 3 measurements on cells of 1 fish. +Medium Ca^{2+} was reduced from 1.6 mM to 10 µM. *P <0.01 vs no A23187, same medium Ca^{2+} concentration.