HEXOSE TRANSPORT BY DOGFISH (SQUALUS ACANTHIAS) AND FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) ERYTHROCYTES

George W. Booz*, Arnost Kleinzeller**, and John A. Payne***

*Dept. of Pharmacology, Thomas Jefferson University, Phila., PA 19107
**Dept. of Physiology, University of Pennsylvania, Phila., PA 19104
***Dept. of Zoology, University of Florida, Gainesville, FL 32611

The possible coupling of glucose (Glc) uptake by flounder and dogfish RBC to metabolism was investigated by assessing the effect of anoxia (1 mM KCN and N2). Hexose uptake was followed radioisotopically (Ellory and Young, eds., Red Cell Membranes- A Methodological Approach, New York: Academic Press, 1982, pp 127-129).

Glc entered flounder RBC at a slow, linear rate over 5h. With 2 mM, the apparent cell level at 2h was 0.33 ± 0.11 mM (3) [mean ± SE]. Anoxia increased the 2h uptake to 1.27 ± 0.22 mM (3), and this additional uptake was inhibited 96% by 50 µM phloretin. The enhanced uptake was due to an increase in Vmax (0.21 vs. 1.27 µmol/ml/h), with little change in Km (2.0 vs. 2.9 mM).

As with other cells (Bihler et al., Biochim. Biophys. Acta 821: 30-36, 1985), the divalent cation ionophore A23187 (10 µM) also enhanced Glc uptake at 1h: 0.87 ± 0.03 mM vs. 0.04 ± 0.01 mM (3 determinations/1 fish). Leupeptin (0.2 mM), an inhibitor of Ca2+ dependent, extralysosomal proteases, did not block this effect. The A23187 effect likely did not involve protein kinase C, since it was not enhanced by the phorbol ester, TPA. In contrast to its action in adipocytes (Martz, et al., J. Biol. Chem. 261: 13606-13609, 1986), TPA (1 µM) by itself had little effect on uptake.

Even with 2h anoxia, apparent cell Glc levels were less than in the medium (0.5-25 mM), suggesting that uptake remained rate limiting for metabolism. This was confirmed with 2 mM 2-deoxy-Glc. While anoxia enhanced the uptake of 2-deoxy-Glc by 250% at 1h, all of it was phosphorylated. By 5h, for both control and anoxia, only some 10% was present as free sugar.

No uptake of 5 mM Glc or 2-deoxy-Glc by shark RBC was seen at 1h (4 fish). Insulin had no effect. With 1 mM Glc, anoxia increased the cell Glc at 1h to 0.16 and 0.08 mM (2 fish); at 4h, 0.73 mM (0.08 mM control). Phloretin (50 µM) inhibited this uptake 85%.

For flounder and shark, 5 mM 3-O-methyl-Glc uptake was rapid, reaching a steady state of less than 50% of the medium level by 20 min. For flounder, prior anoxia (1h) enhanced uptake with anoxia 70% at 1 h. In contrast, uptake by shark RBC was reduced from 2.25 ± 0.25 mM (7) to 0.45 ± 0.10 mM (5); for both, uptake was directly proportional to concentration (0.5-25 mM). Phloretin also reduced uptake (60% with 0.5 mM), but only at levels which interact with lipids of the RBC membrane (Jennings and Solomon, J. Gen. Physiol. 67: 381-397, 1976). A high affinity (0.1 µM) system for 3-O-methyl-Glc uptake was not seen. Rapid 3-O-methyl-Glc uptake may represent passive diffusion, as described for avian RBC (Wood and Morgan, J. Biol. Chem. 244: 1451-1460, 1969), or binding to the outer cell surface. When cells were made anoxic for 5 h, carrier-mediated uptake of 1 mM 3-O-methyl-Glc was seen. Phloretin (50 µM) and 20 mM 3-O-methyl-Glc inhibited this uptake 75 and 100%, respectively.

In conclusion, Glc uptake by flounder and dogfish RBC is coupled to its metabolism. Because uptake is so slow, these cells could be useful in defining the events which link Glc transport and metabolism in many mammalian cells.

[G.B. was a recipient of a Summer Fellowship of the American Heart Association- Maine and an MDIBL student scholarship.]