Kinetics of glucose transport across the surface membranes of single fast- and slow-twitch muscle fibres of the rat

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Glucose is an essential substrate for muscle metabolism. It is transported across the surface membranes by members of a family of specialized proteins, known as glucose transporters (GLUTs). Currently there is no simple technique for directly measuring the kinetics of glucose transport across the surface membranes of muscle fibres, because intracellular glucose can neither be directly measured nor realistically calculated with sufficient accuracy in whole body or whole muscle experiments. The aim of this study was to develop a technique for directly measuring the uptake of a fluorescent glucose analog (2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2-deoxyglucose, 2-NBDG (Yoshioka *et al.*, 1996)) across the surface membrane of single muscle fibres.

All experiments were approved by the La Trobe University Animal Ethics committee. Adult male rats (Long-Evans hooded) were killed by deep anesthesia with isoflurane (4% v:v) and the *extensor digitorum longus* (EDL, predominantly fast-twitch) and *soleus* muscles (predominantly slow-twitch) were rapidly dissected, blotted dry on filter paper and then were pinned in a Petri dish under paraffin oil at resting length. Segments of single muscle fibres with intact surface membranes were isolated under a dissecting microscope. The ends of the fibre segments were carefully tied with fine surgical thread (Decknatel 10.0) while still immersed in paraffin oil and the preparation was transferred to a well made out of Bipax epoxy resin on a glass cover slip filled with paraffin oil which was placed on the stage of a fluorescence microscope. A droplet of modified physiological saline (MPS; mM: NaCl, 145 when no glucose was present and 135 with glucose; KCl, 3; $MgCl_2$, 3.5; HEPES, 10; EGTA, 2 and glucose, 0 or 20) containing 50 μM 2-NBDG, was also placed under oil on the cover slip and the preparation was incubated for known periods of time in this droplet, followed by a brief wash in a MPS droplet without 2-NBDG . The preparation was then carefully placed on the bottom of the cover slip and the fluorescence signal (450-480nm excitation/ 500nm dichroic mirror/ 510-530nm emission) was measured from a 70μm region in the middle of the preparation. The fibre segment was then returned to the 2-NBDG containing droplet and the cycle was repeated. The integrity of the surface membrane in the preparations was checked by blocking the GLUTs with cytochalasin B and the concentration of the 2-NBDG in the preparation was calibrated by measuring the 2-NBDG signal from Triton X-100 treated single fibre segments exposed to droplets of relaxing solution (mM: K^+, 126; Na^+, 36; EGTA, 50; total ATP, 8; creatine phosphate (CP), 10; free Mg^{2+}, 1; HEPES, 90; pH 7.10; pCa (-log10 [Ca^{2+}]), <9.0) containing 2-NBDG. The 2-NBDG uptake curves could be well fitted by the expression A(1-exp(-kt)), where A is the final [2-NBDG] in the fibre, k is the rate constant and t is time and the rate of 2-NBDG uptake was calculated assuming a cylindrical shape of the fibre segment. Equilibrium was effectively reached in less than 2 min in both EDL and *soleus* fibres.

In the presence of 50 μM 2-NBDG and in the absence of glucose, the basal rate of 2-NBDG uptake was 8.7±2.5pmol/cm^2/s (n = 4) in the soleus and 5.3 ±1.3 pmol/cm^2/s (n=3) in the EDL fibres. Addition of 20 mM glucose to the 50 μM 2-NBDG MPS reduced the uptake rates of 2-NBDG by a factor of 3.5 in *soleus* fibres (2.5 ± 0.6 pmol/cm^2/s, n=3) and by half in EDL fibres (2.6 ±1.0 pmol/cm^2/s, n=3). This corresponds to an average GLUTs dissociation constant for glucose of about 7.9 and 12.6 mM for the *soleus* and EDL fibres, respectively. Using these values, the projected maximum rates of basal glucose transport in the soleus and EDL fibres is similar (69.2 *vs* 66.8 pmol/cm^2/s).

Yoshioka, K., Takahashi, H., Homma, T., Saito, M., Oh, K-B., Nemoto, Y., and Matsuoka., H. (1996). *Biochimica et Biophysica Acta* **1289**, 5-9.