Dynamics of cerebral glucose analysed *in vivo* with a four-state conformational model

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Introduction: Glucose is the primary fuel required for brain function and its supply to the brain occurs through facilitative transporter proteins located in the blood-brain-barrier (BBB). Although steady-state transport models have been widely and reliably describe the glucose transport mechanism *in vivo*, such models assume a constant glucose consumption rate (CMR_{glc}). We now evaluated brain glucose dynamics by employing a four-state conformational model [1] that accounts for transport inhibition, and a dynamic method that allows distinguishing the parameters defining transport from CMR_{glc} [2].

Methods: Male Sprague-Dawley rats (n=6, 270±20 g) were prepared and maintained during the NMR experiment under α-chloralose anaesthesia as previously described [3]. After stable baseline of plasma glucose (G_{plasma}), glucose [20% (w/v) solution] was given as a bolus and then infused at a rate adjustable to the concomitantly measured plasma glucose concentrations to maintain stable glycaemia level. After at least 2 hours of hyperglycaemia, infusion was stopped. Continuous NMR measurements were performed during these glycaemia periods: baseline, step-function and decay (fig.1B). All experiments were carried out on an actively-shielded 9.4 T, 31 cm scanner (Varian/Magnex) using a homebuilt 10 mm ^{1}H quadrature surface coil. After shimming with FASTMAP [4], ^{1}H NMR spectra were acquired using SPECIAL [5] with TE of 2.8 ms and TR of 4 s. The volume of interest (120 µL) included cortical and hippocampal areas. Spectra were analysed with LCModel [6]. A temporal resolution of 5 minutes was sufficient to achieve CRLB lower than 20% for glucose at euglycaemia.

Glucose transport model: A four-state conformational model of glucose transport was used [1]. Brain glucose (G_{brain}) is thus described by the expression in Fig.1A, where T_{max} and K_t are the apparent maximum transport rate and half saturation constant, and K_{ii} represents the iso-inhibition constant for glucose transport. V_d is the volume for distribution of glucose in the brain (0.77 ml/g). The model was fitted to measured G_{brain} by minimization with the Levenberg-Marquat algorithm (Fig.1B) and fit quality was assessed by Monte-Carlo simulation, in which Gaussian noise with the same variance of fit results was added to the best fit. Since K_{ii}>>G_{brain} [1], the reversible model of transport [7] was also fitted to the data.

Table 1. Estimated kinetic parameters of glucose transport and consumption in the brain (mean±SE).

	Transport model	
	Conformational	Reversible
T_{max} (µmol/g/min)	1.10±0.16	1.01±0.19
K_t (mM)	0.93±0.43	0.72±0.39
K_{ii} (mM)	66.5±25.6	-
CMR_{glc} (µmol/g/min)	0.56±0.09	0.58±0.12

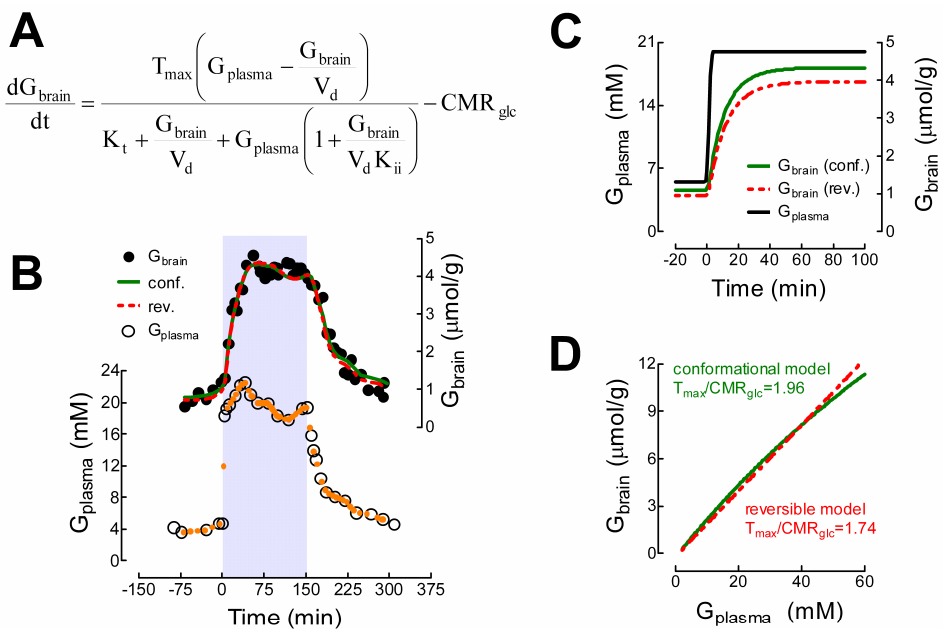

Figure 1. Panel A shows the expression defining the four-state conformation model. Note that with K_{ii}>>G_{brain} the expression represents the reversible model. Panel B shows the best fit of the conformational (green) and reversible (red) models to G_{brain} in a representative data set (one rat). G_{plasma} was interpolated for the time scale of G_{brain} (orange). The estimated parameters (table 1) were used to simulate G_{brain} for a given G_{plasma} function (C). Simulation of G_{brain} at steady-state G_{plasma} is shown in panel E.

Results and discussion: Kinetic parameters for glucose transport and utilization in the brain were similar for the two tested models (table 1). Simulations of glucose transport with these parameters confirmed similar G_{brain} for a given G_{plasma} (fig.1C,D), which was predicted to be 1.2 µmol/g at euglycaemia (5.5 mM). The uncertainty on the estimated parameters was lower for the reversible model by increasing the degrees of freedom in the absence of K_{ii}. In addition, we verified that K_{ii} largely exceeds G_{brain}. These results reinforce that the iso-inhibition term may be neglected from the model, suggesting fast isomerisation of the unloaded glucose carrier [1]. Therefore, we conclude that the reversible model [7] accurately describes the dynamics of glucose transport in the rat brain for G_{plasma} below 40 mM (fig 1D) and with Michaelis-Menten kinetics of glucose transport.

References: [1] Duarte *et al.* (2009) Front Neuroenerg 1:6. [2] Shestov *et al.* (2010) Proc Intl Soc Mag Reson Med 18:3321.[3] Duarte *et al.* (2009) J Neurochem 111:368. [4] Gruetter (1993) MRM 29:804. [5] Mlynárik *et al.* (2008) J Mag Reson 194:163. [6] Provencher (1993) MRM 30:672. [7] Gruetter *et al.*, J Neurochem 70:397.

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