Quantitative modelling of H3 hydrogen turnover in (2-^{13}C) glutamate and (2-^{13}C) glutamine during (1-^{13}C) glucose metabolism in the adult rat brain

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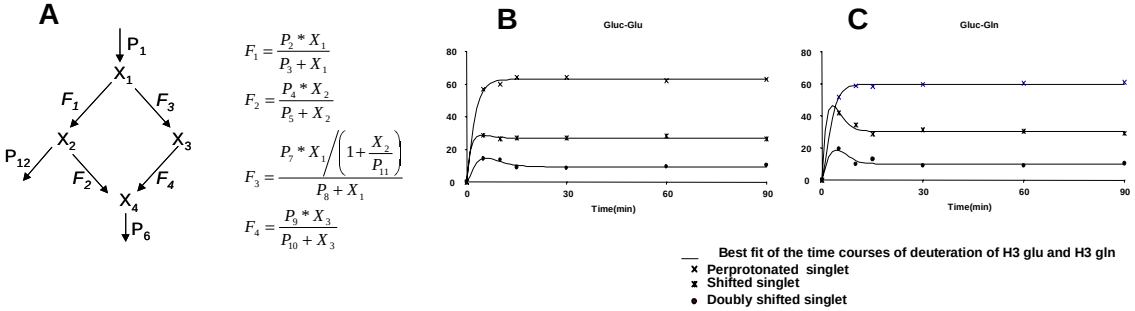
Introduction

^{13}C NMR measurements of carbon-13 turnover during the metabolism of ^{13}C labelled glucose have been used to investigate the neuronal and glial tricarboxylic acid cycle rates and the glutamine cycle, assuming single compartment kinetics and fast α-ketoglutarate/glutamate equilibration. More recently, we have shown that the faster timescale of (^{1}H, ^{2}H) ^{13}C NMR measurements allows us to resolve in time the corresponding cytosolic and mitochondrial components, a finding consistent with a slow α-ketoglutarate/glutamate exchange in the hydrogen turnover timescale. We have previously described quantitative interpretations for the kinetics of H3 deuteration in cerebral (2-^{13}C) glutamate and glutamine, during (2-^{13}C) acetate infusions, a primarily glial substrate (1). Here, we provide the first quantitative interpretation of the turnover of the H3 hydrogens of cerebral (2-^{13}C) glutamate and (2-^{13}C) glutamine during the metabolism of (1-^{13}C) glucose, a substrate metabolized mainly in the neuronal compartment of the adult rat brain.

Methods

Male Wistar rats (n=28, 150-200g) were placed in metabolic cages fed *ad libitum* received 50% $^{2}H_2O$ as drinking water ten days before the infusion of (1-^{13}C) glucose (32μmol.min^{-1}.100g^{-1}). During this period animals showed approximately a 20% reduction in water intake, but no other signs of toxicity. (1-^{13}C) glucose infusion in the right jugular vein was administered for 3, 5, 10, 15, 30, 60 and 90 minutes (n=4 in each timepoint), respectively. At the end of the infusion, the head of the animals was funnel frozen and extracted with perchloric acid for acid soluble metabolites. ^{13}C NMR spectra (11.9 Tesla, 22^{0}C, pH=7.2) of brain extracts depicted clearly detectable ^{2}H-^{13}C couplings and isotopic shifts. It was possible to obtain the complete time courses for the (i) perprotonated (2-^{13}C) glutamate and glutamine singlets (55.44 and 54.94 ppm). (ii) (2-^{13}C, 3-^{2}H) glutamate and glutamine shifted singlets (55.35 and 54.88 ppm) and (iii) (2-^{13}C, 3,3´-$^{2}H_2$) glutamate and glutamine doubly shifted singlets (55.28 and 54.78 ppm), respectively. To obtain quantitative values for the kinetic parameters describing H3 deuteration, we fitted the time courses to a Michaelis-Menten model of H3 turnover using MATLAB (The Mathworks, Natick, MA, USA) (Panel A). The model included Km´s (P_3, P_5, P_8 and P_{10}), Vmax (P_2, P_4, P_7, P_9) and Ki (P_{11}), of the enzymes or pathways inducing H3 exchange, as well as input (P_1) and output (P_6, P_{12}) fluxes to maintain steady state conditions. The model considers the time courses of (2-^{13}C) glutamate or glutamine (X_1), (2-^{13}C, 3-^{2}H) glutamate or glutamine (X_2), (2-^{13}C, 3´-^{2}H´) glutamate or glutamine (X_3) and (2-^{13}C, 3,3´-$^{2}H_2$) glutamate or glutamine (X_4).

Results



The data could only be fitted when considering two different sites for H3 and H3´ deuteration, respectively. Moreover, fittings improved significantly when considering that deuteration of H3, inhibited non competitively the deuteration of H3´. Although H3 or H3´deuteration cannot be distinguished by ^{13}C NMR, this inhibitory process causes the "burst" in the combined shifted singlet resonance (2-^{13}C, 3-^{2}H + 2-^{13}C, 3´-^{2}H´) and in the doubly shifted singlet (2-^{13}C, 3,3´-$^{2}H_2$), observed both in glutamate and glutamine (Panels B and C). This trend could not be simulated otherwise. The following results (Glu;Gln) were obtained: (**P_1** 0.43;0.54 **P_2** 1.84;3.56 **P_3** 2.27;4 **P_4** 3.66E-03;1.20E-03 **P_5** 0.56;0.41 **P_6** 0.27;0.81 **P_7** 0.61;1.27 **P_8** 0.027;0.048 **P_9** 0.243;0.289 **P_{10}** 0.44;0.266 **P_{11}** 9.38E-03;1.47E-02 **P_{12}** 1.8;2.3).

Discussion

Our results indicate that the two different (2-^{13}C) isotopomers of glutamate and glutamine deuterated in H3, have different metabolic fates. Whyle (2-^{13}C, 3-^{2}H) glutamate and glutamine are mainly extruded through P_{12} (F_1>>F_2 and F_3), the (2-^{13}C, 3´-^{2}H´) isotopomers are mainly oxydized in the tricarboxylic acid cycle through P_6 (F_4>>>F_3). On the other hand, present results confirm the slow exchange of α-ketoglutarate/glutamate between cytosol and mitochondria, because fast H3 or H3´ monodeuterations occurr mainly in the cytosol and slower H3 and H3´, double deuterations occur in the mitochondria. The present results provide the first quantitative interpretation for hydrogen turnover during (1-^{13}C) glucose metabolism in the adult, intact, rodent brain.

Bibliography

1. Sierra, A., Lopes da Fonseca, L. Ballesteros, P., Cerdán, S. (2004) Quantitative modelling of H3 hydrogen turnover in (2-^{13}C) glutamate and glutamin during (2-^{13}C) acetate metabolism in the adult rat brain. 22nd ESMRMB Meeting. Copenhaguen, Denmark. Presentation 103.