Turnover of the H3 hydrogens of (2-^{13}C) glutamate and (2-^{13}C) glutamine during the cerebral metabolism of (1-^{13}C) glucose as detected by ^{13}C NMR.

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Synopsis

We report on the turnover of the H3 hydrogens of cerebral glutamate and glutamine as detected in the (2-^{13}C) resonances of these metabolites during (1-^{13}C) glucose infusion, in rats receiving 50% $^{2}H_2O$ in the drinking water for ten days. The time course of deuteration of the H3 hydrogens in (2-^{13}C) glutamate and (2-^{13}C) glutamine followed the sequence of events (2-^{13}C) glutamate → (2-^{13}C) glutamine → (2-^{13}C, 3-^{2}H) glutamine → (2-^{13}C, 3-^{2}H) glutamate → (2-^{13}C, 3-$^{2}H_2$) glutamine → (2-^{13}C, 3-$^{2}H_2$) glutamate, allowing to resolve in time the traffick of cerebral glutamate and glutamine between neurons and astrocytes.

Introduction

A variety of ^{13}C turnover experiments based on the administration of ^{13}C enriched substrates followed by dynamic acquisition of *in vivo* ^{13}C NMR spectra have allowed to explore cerebral metabolism in greater detail than previously possible with radioactive isotopes (1). In particular it has been possible to approach *in vivo* and *in vitro* the operation of the neuronal and glial tricarboxylic acid cycles and the intercellular glutamate-glutamine cycle in the brain of animals or humans (2). However, ^{13}C NMR methods are relatively slow providing only time averaged views of those metabolic processes occurring in faster time scales, a circumstance that has imposed a number of important assumptions. It would be then become useful to develop complementary methodologies with timescales approaching those of the more rapid metabolic processes. To this end we proposed recently the use of hydrogen turnover measurements (3). The hydrogen turnover method made it possible to monitor α-ketoglutarate/glutamate exchange in perfused liver and to follow the trafficking of α-ketoglutarate/glutamate skeletons through the mitochondrial and cytosolic compartments (4). Here, we report on the turnover of the H3 hydrogens of cerebral glutamate and glutamine, during (1-^{13}C) glucose infusions.

Experimental

Male Wistar rats (150-200g) placed in metabolic cages, fed *ad libitum* were provided with 50% $^{2}H_2O$ (or H_2O in controls) as drinking water for ten days. Drinking water, food intake, urine volume and fractional deuterium enrichment in the urine were monitored daily. After ten days of treatment, animals received a ninety minutes infusion of (1-^{13}C) glucose (8 μmol. min^{-1}.100g^{-1}) in the left jugular vein. The brains were funnel frozen and perchloric acid extracts prepared for high resolution ^{13}C NMR analyses. ^{13}C NMR spectra (11.9 Tesla, 22^{0}C, pH:7.2) obtained from extracts prepared at increasing times of (1-^{13}C) glucose infusion in deuterated rats depicted clearly detectable ^{2}H-^{13}C couplings and isotopic shifts. It was possible to resolve; (i) perprotonated (2-^{13}C) glutamate and glutamine singlet (55.44 and 54.93 ppm), (ii) (2-^{13}C, 3-$^{2}H_{proS}$) glutamate and glutamine shifted singlet (55.35 and 54.88 ppm) and (iii) (2-^{13}C, 3-$^{2}H_2$) glutamate and glutamine doubly shifted singlet (55.28 and 54.80 ppm). This allowed to determine the time courses of $H3_{proS}$ deuteration as caused by cytosolic isocitrate dehydrogenase (shifted singlet) and the $H3_{proS}$ and $H3_{proR}$ deuterations of glutamate and glutamine as caused by the tricarboxylic acid cycle (doubly shifted singlet). The ^{13}C turnover of the C2 carbon was investigated by monitoring the time course of increase in total C2 intensity as compared to the unchanged inositol C1,C3 resonance (73.18 ppm). The fractional ^{2}H enrichment in urine was determined along the ten days of treatment from ratio of the areas of $^{2}H_2O$ infrared band at 2500 cm^{-1} as compared to the area of the $^{1}H_2O$ band at 3355 cm^{-1}.

Results

After ten days of deuterium oxide treatment, $^{2}H_2O$ in the urine reached ca. 15.5 ± 5 %. Fig 1 illustrates the different turnover rates of ^{13}C (left panel) and ^{1}H (right panel) as observed in the C2 carbon of cerebral (2-^{13}C) glutamine. Similar results were obtained with (2-^{13}C) glutamate (not shown). Under these conditions, $^{2}H_2O$ treatment decreased the apparent first order rate constant of (2-^{13}C) glutamate or (2-^{13}C) glutamine production from glucose, revealing a small ^{2}H isotope effect on ^{13}C turnover. The time course of deuteration of the H3 hydrogens in (2-^{13}C) glutamate and (2-^{13}C) glutamine allowed to establish the following sequence of events: (2-^{13}C) glutamate → (2-^{13}C) glutamine → (2-^{13}C, 3-^{2}H) glutamine → (2-^{13}C, 3-^{2}H) glutamate → (2-^{13}C, 3-$^{2}H_2$) glutamine → (2-^{13}C, 3-$^{2}H_2$) glutamate.

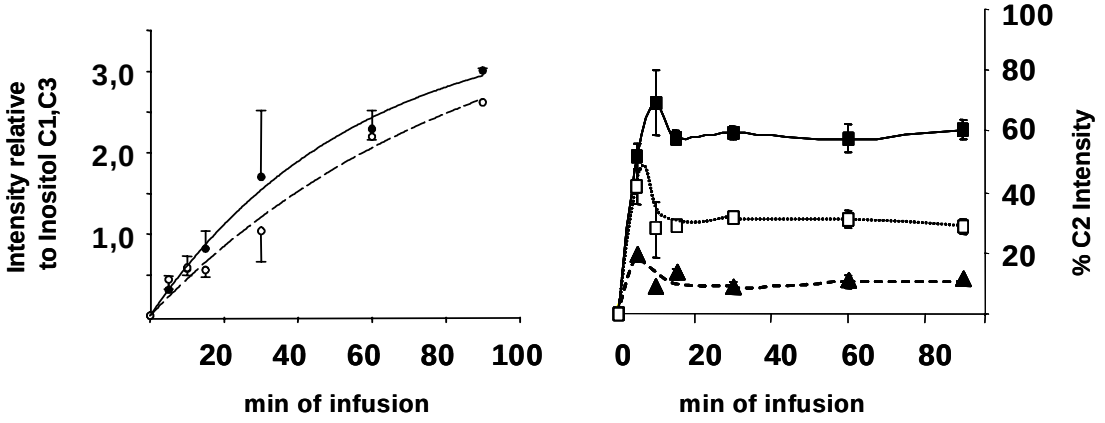

Figure 1. Left: Turnover of cerebral (2-^{13}C) glutamine in rats drinking tap water (filled circles) or 50% $^{2}H_2O$ (empty circles). Right: Turnover of H3 protonated and deuterated isotopomers of (2-^{13}C) glutamine (filled squares), (2-^{13}C, 3-^{2}H) glutamine (empty squares), (2-^{13}C. 3-$^{2}H_2$) glutamine (filled triangles). The results are shown as the mean±sd of three rat brains.

Discussion

A brief inspection of the A and B panels Fig. 1 reveals the different time scales of ^{13}C and ^{1}H turnover in (2-^{13}C) glutamine. Similar results were obtained for (2-^{13}C) glutamate (not shown). The faster timescale of ^{1}H turnover provides kinetic information on the sequence of deuterations occurring in a specified ^{13}C isotopomer once it has been formed. Our results reveal that the first event in cerebral (2-^{13}C) glutamate and glutamine turnover, is the formation of (2-^{13}C) glutamate, followed rapidly by the formation of (2-^{13}C) glutamine. Notably (2-^{13}C) glutamine is formed before than (2-^{13}C, 3-^{2}H) glutamate. Since glutamate is mainly produced in the neurons and glutamine in the glia, these observations reveal that transfer of neuronal glutamate to the glia occurs before it can be deuterated in the neuron. (2-^{13}C, 3-^{2}H) glutamine is formed soon after (2-^{13}C) glutamine, revealing a very fast activity of cytosolic isocitrate dehydrogensase in the astrocyte. Very soon after (2-^{13}C, 3-^{2}H) glutamine has been formed in the astrocyte (2-^{13}C, 3-^{2}H) glutamate is produced in the neuron, confirming again a fast transfer of glutamine from astrocyte to neuron. It becomes possible then to resolve kinetically the glutamate-glutamine cycle in the hydrogen turnover timescale. Interestingly, (2-^{13}C, 3-$^{2}H_2$) glutamine is produced earlier than (2-^{13}C, 3-$^{2}H_2$) glutamate, suggesting that (2-^{13}C, 3-$^{2}H_2$) glutamate is transferred to the astrocyte and amidated before it can be further metabolized in the neuron.

Acknowledgements

This work was supported in part by strategic group grant 2000-3 from the Community of Madrid to P.B. and grant SAF 2001-2045 to S.C. A.S. and M.B. are predoctoral Fellows from UNED. T.B:R is a predoctoral Fellow from the University of Coumbra, Portugal.

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