In vivo ^{13}C NMR measurement of activity-dependent malate-aspartate shuttle flux in the brain

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Introduction

Carbon-13 NMR spectroscopy can be used to measure a number a metabolic fluxes in the brain (1), including glial and neuronal oxidative glucose consumption, glutamate-glutamine cycle and pyruvate carboxylase (2). The apparent exchange rate between 2-oxoglutarate and glutamate, Vx, has been proposed to reflect the malate-aspartate shuttle and thus depend on cellular energy status (2,3). The goal of this study was to measure changes in malate-aspartate shuttle under different levels of brain activity.

Methods

All experiments were performed on a 9.4T/31 cm horizontal bore magnet (Magnex/Varian). Male Sprague-Dawley rats were infused with [1,6-$^{13}C_2$]glucose under light α-chloralose (25 mg/kg/h i.v., n=5) and under morphine sulfate (25mg/kg/h i.p., n=5) anesthesia. The rate of ^{13}C incorporation into brain metabolites was measured from a 400 µl volume with a temporal resolution of 5 minutes using ^{13}C-detected semi-adiabatic DEPT combined with ^{1}H localization (5). Time series of ^{13}C spectra were quantified automatically with LCModel adapted for ^{13}C NMR (6). Following *in vivo* measurements, brains were funnel-frozen and extracts were analyzed by high-resolution NMR to determine the isotopic enrichment of metabolites. Blood glucose isotopic enrichment was determined using GC-MS. Time courses of ^{13}C label incorporation into glutamate C4, C3, C2, aspartate C2, C3 and glutamine C4, C3, C2 were fitted to a two-TCA cycle model to derive quantitative metabolic fluxes (2).

Results and Discussion

The excellent sensitivity and spectral resolution of ^{13}C NMR spectra allowed separate resolved detection of glutamate C4, C3, C2, glutamine C4, C3, C3, aspartate C3, C2 and glucose C6 (Fig. 1). Incorporation of ^{13}C label into glutamate was noticeably faster under morphine than α-chloralose (Fig. 2), consistent with a higher metabolic activity under morphine anesthesia (7). The fit of the model to the average time courses (Fig. 2) yielded random residuals with an average scatter of 0.3 µmol/g. V_x was clearly dependent on brain activity (Fig. 3) and strongly correlated with the rate of neuronal glucose consumption (r=0.99, p=0.01, not shown).

We conclude that under physiological conditions the exchange rate between 2-oxoglutarate and glutamate across the mitochondrial membrane is tightly regulated by the malate-aspartate shuttle and thus dependent on brain activity.

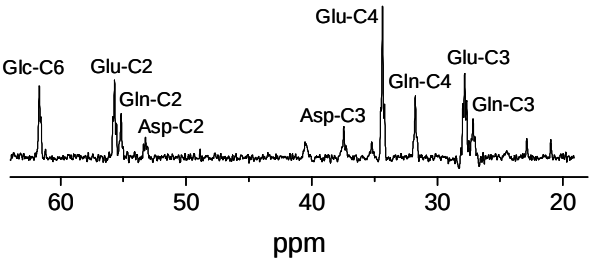

Fig.1. Example of ^{13}C spectrum obtained with ^{1}H-localized polarization transfer (27 minutes acquisition) under morphine anesthesia. Note the resolved signals of glutamate and glutamine at the C3 and C2 positions.

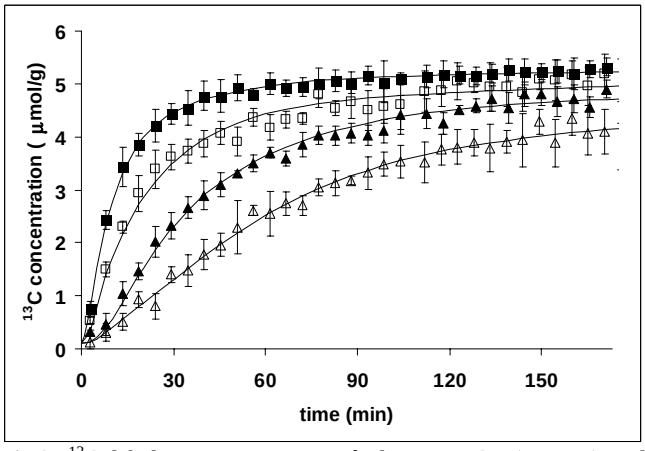

Fig.2. ^{13}C labeling time courses of glutamate C4 (squares) and glutamate C3 (triangles) under morphine (filled symbols) and α–chloralose (empty symbols) anesthesia. Each curve represents the average from 5 different animals with SD indicated by error bars. Label incorporation into glutamate was faster with morphine than with α-chloralose, reflecting higher metabolic rates.

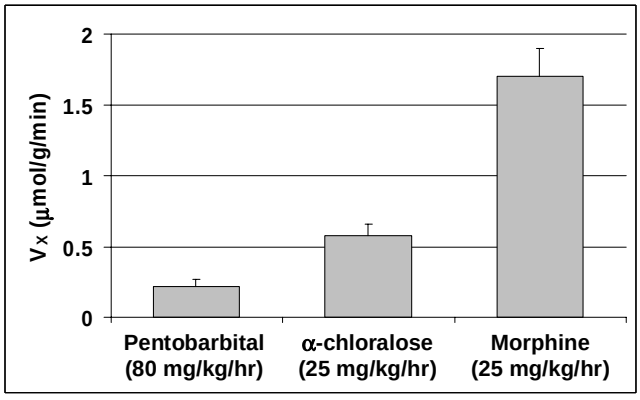

Fig.3. Changes in the exchange rate V_x under different anesthetic conditions. Pentobarbital is taken from Ref. 4.

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