Effects of a glutamate transport inhibitor on brain metabolism

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Introduction

Central nervous tissue takes up L-glutamate by a high-affinity, sodium-dependent transport system (GluT), which recently has attracted considerable attention due to implication in neurodegeneration [1]. In this study we used a powerful inhibitors of GluT, (2*S*,1'*S*,2'*R*)-2-(carboxycyclopropyl)glycine (L-CCG III) [2] to study the effect of GluT inhibitors on glutamate metabolism in the guinea pig cortex.

Methods

Guinea pigs were killed by cervical dislocation and the cortex dissected and sliced (350 um). Slices were prepared as described [3,4], washed and incubated with 2 mM [3-^{13}C]pyruvate (99%) (control) or 2 mM [3-^{13}C]pyruvate with 5 or 50 uM L-CCG III. After 1h, slices were extracted in 6% (w/v) PCA, the supernatant neutralised, lyophilised and reconstituted in $^{2}H_2O$ containing 2 mM ^{13}Cformate as reference. ^{1}H-Decoupled (WALTZ-16) ^{13}C NMR spectra were obtained at 100.13 MHz, and ^{13}C-decoupled (WURST)^{1}H NMR spectra were obtained at 600.13 MHz. Peak areas were integrated and compared to those from ^{13}Cformate after compensating for natural abundance, nOe and relaxation effects.

Results

L-CCG III (50 uM) significantly decreased incorporation of ^{13}C from [3-^{13}C]-pyruvate into Glu C4, Gln C4, Lac C3 and Ala C3 (Fig. 1).

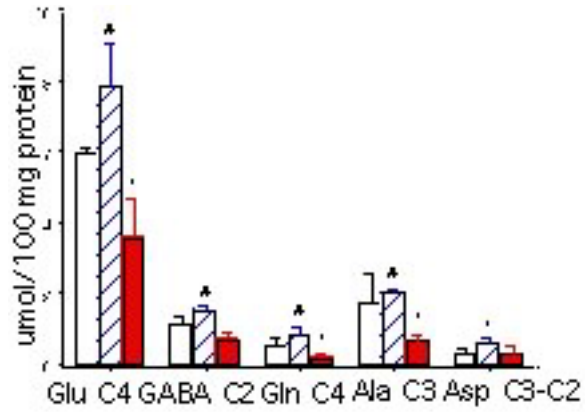

Fig. 1 Flux into individual isotopomers. Clear boxes, control; shaded boxes, 5 uM L-CCG III; dark boxes, 50 uM L-CCG III. * indicates significantly different to controls, # indicates significantly different to 50 uM L-CCG III.

The metabolite pool sizes of Lac, Glu, GABA, Asp and Gln were also decreased (Fig. 2) but there was no change in the fractional enrichment of any isotopomer. The presence of 5 uM L-CCG III resulted in a significant increase in flux of label through pyruvate carboxylase (Asp C3-C2) and an increase in the fractional enrichment of Glu, GABA, Gln and Lac, but had no effect on metabolite pool sizes.

L-CCG III was accumulated by the tissue slices at 50 uM concentrations, most likely by uptake mediated by the Na^+ dependent transporters (Fig. 3). Mean concentrations of L-CCG III in slices incubated with a 50 uM solution were estimated from the cyclic CH_2 resonances to be 8.54 uM. Resonances from L-CCG III were not detected in spectra of extracts of slices incubated with 5 uM L-CCG III.

Discussion

Results from incubation of slices with 5 uM L-CCG III are indicative of a mild stimulatory effect on metabolism, consistent with blockade of the transporter resulting in increased clearance times for glutamate and hence increased stimulation. The measured increase in anaplerotic pathway activity (Asp C3-C2) is indicative of increased glial cell metabolism.

At a concentration of 50 uM, L-CCG III had a mild inhibitory effect but maintenance of normal intracellular K^+ concentrations in these slices suggests that the effect is not neurotoxic. As L-CCG III is transported into cells at this concentration, it may well be exerting other effects on metabolism, such as inhibition of enzyme activities.

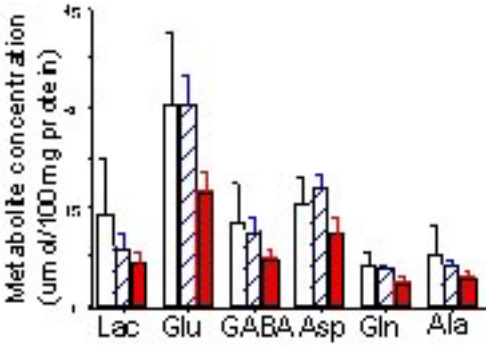

Fig. 2. Metabolite pool sizes. Clear boxes, control; hatched boxes, 5 uM L-CCG III; dark boxes, 50 uM L-CCG III.

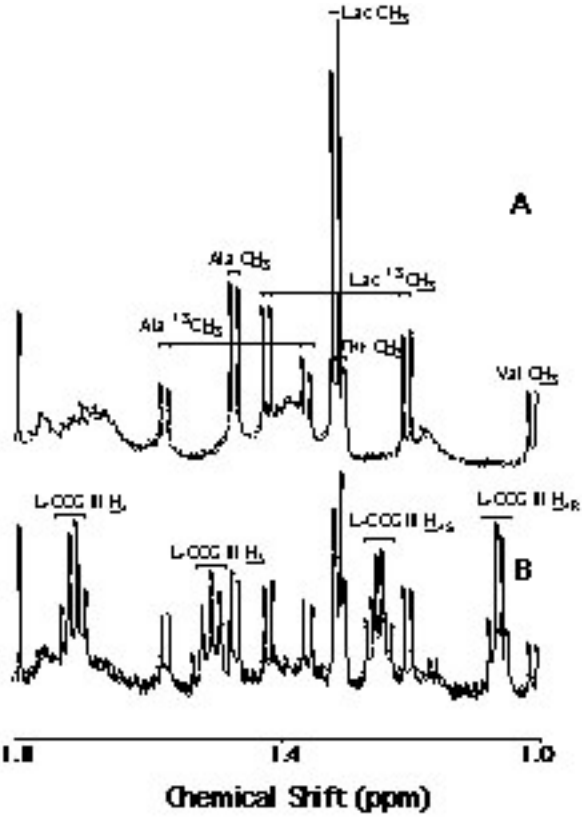

Fig. 3. Section of 600.13 MHz ^{13}C-decoupled^{1}H NMR spectra of brain tissue slice extracts. A Control, B, 50 uM LCCG III.

References

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