Neuronal and Glial Tricarboxylic Acid Cycles And The Glutamine Cycle As Precursors Of Cerebral Glutamine And GABA In The Adult Rat Brain.

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Abstract. The relative contributions of the neuronal and glial tricarboxylic acid cycles (TCAn and TCAg) and the glutamine cycle (GC) to the production of cerebral glutamine or GABA have been evaluated by high resolution ^{13}C NMR during (1-^{13}C) glucose infusions using trifluoroacetic acid (TFA) or methionine sulfoximine (MSO) as inhibitors of TCAg and GGC, respectively. TCAg contributed ca. 70% of the energy (or carbon skeletons) required for glutamine synthesis while glutamine was the precursor of approximately 40% of cerebral GABA.

Introduction. The glutamine cycle plays a central role in cerebral physiology by coupling neuronal activation to glial metabolism (1). GC is currently thought to be a major cerebral pathway (2). In this pathway, synaptic glutamate derived from glutamatergic transmission is recaptured by the astrocyte (Glu_s) to produce glutamine (Gln) from astrocytic glutamate (Glu_g). Gln is then secreted to the extracellular medium and metabolized back to neurotransmitter glutamate in glutamatergic neurons or to neurotransmitter GABA in gabaergic neurons. However, Glu_g may be produced alternatively from endogenous α-ketoglutarate in the glial tricarboxylic acid cycle. Similarly, neuronal GABA may be produced from endogenous glutamate derived from the metabolism of glucose or lactate in the neuronal tricarboxylic acid cycle. Therefore two possible sources of Gln and GABA exist in brain, but their relative contributions remain insufficiently characterized. This study reports the results obtained using a combination of high resolution ^{13}C NMR and pharmacological inhibition methods to address this question.

Materials and Methods. Male Wistar rats (n=10, 200-250g) received (1-^{13}C) glucose infusions in the right jugular vein (8 μmols. min^{-1}. 100g^{-1}, 60 min). Glutamine synthase activity was inhibited in some animals using an i.p. injection of methionine sulfoximine (MSO, 150 mg. Kg^{-1}, n=3, t = -6h). In other animals, the glial tricarboxylic acid cycle was inhibited by co-infusing sodium trifluoroacetate (TFA, 24 μmols. min^{-1}. 100g^{-1}, pH: 7.2, n=3). The remaining animals received only the (1-^{13}C) glucose infusion and were used as controls. At the end of the infusion, perchloric acid extracts from both brain hemispheres, obtained by funnel-freezing, were prepared and analyzed by high resolution ^{13}C NMR (90.55 MHz, pH 7.2, 22 °C). Acquisition conditions were: 600 pulses, 18.5 KHz spectral width, 1.77 s acquisition time, 64 kwords data table and 5.8 s recycling time. The total content of glutamate, glutamine and GABA was determined by automatic ion exchange chromatography after ninhidrin derivatization.

Results. ^{13}C NMR analysis of extracts allowed the determination of the amount of ^{13}C label from (1-^{13}C) glucose incorporated in cerebral glutamate C4, glutamine C4 and GABA C2 carbons under the different experimental conditions used (Fig. 1).

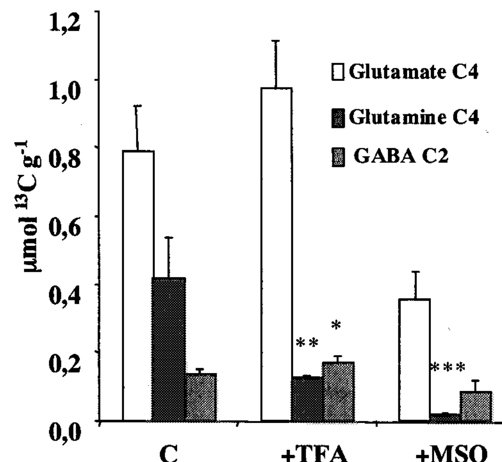

Fig. 1. ^{13}C incorporation (μmol .g^{-1})in glutamate C4, glutamine C4 and GABA C2 in the absence and presence of TFA or MSO. (* $p< 0.05$, ** $p<0.01$, *** $p< 0.001$)

The cerebral content (Control, TFA, MSO, μmol. g^{-1}) of these amino acids were; glutamate (8.3±1.0, 7.8±1.6 , 5.5±0.2), glutamine (6.6±0.8, 3.7±2.2 , 2.4± 0.4) and GABA (1,5± 0.2, 1.2±0.1, 0.6±0.2), respectively. MSO reduced ^{13}C incorporation in glutamate C4 (-55%), glutamine C4 (-96%) and GABA C2 (- 40 %), while TFA increased incorporation in glutamate C4 (23%) and GABA C2 (25%) and decreased in glutamine C4 (-69%).

Discussion. The pharmacological inhibition approach used in this study was aimed to provide a model independent assessment of the relative contributions of neuronal and glial oxidative metabolisms and the GC to cerebral glutamine and GABA production. Present results indicate that glutamine synthesis and therefore the GC is sensitive to TFA inhibition of TCA_g. Indeed the contribution of glial oxidative metabolism to glutamine synthesis appears to be dominant (ca. 70%) with glycolisis playing a smaller role. Similarly, almost complete inhibition of glutamine synthesis, resulted in a reduction of only 40% in GABA content, suggesting a dominant contribution of neuronal tricarboxylic acid cycle, rather than GC, to cerebral GABA. synthesis.

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