^{13}C Magnetic Resonance Spectroscopy Studies of Alterations in Glutamate Neurotransmission

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Over the past a few years, significant progress has been made in refining the in vivo ^{13}C magnetic resonance spectroscopy technique and in applying it to studying the alterations in the glutamate-glutamine cycling flux. Meanwhile, the details of the metabolic modeling are being rigorously debated. Recent evidence against fast α-ketoglutarate-glutamate exchange across the mitochondrial membrane is examined. Previous reports have indicated that glutamate release or ^{13}C label incorporation into glutamine is attenuated at elevated concentrations of endogenous γ-aminobutyric acid (GABA). A recent study has shown that phenelzine administration reduces the glutamate-glutamine cycling flux while raising endogenous GABA levels in vivo. Effects of several metabotropic glutamate receptor agonists and antagonists and brain disorders on the glutamate-glutamine cycle are also summarized.

Key Words: Glutamate-glutamine cycle, in vivo ^{13}C magnetic resonance spectroscopy, glucose metabolism, acetate metabolism, GABA

Coordination of neuronal and glial metabolisms is essential for central nervous system (CNS) activities (Shepherd 1994). Important metabolic couplings exist between various cells through the use of common substrates and the exchange of several metabolic intermediates such as glutamate, glutamine, and γ-aminobutyric acid (GABA) (Erecinska and Silver 1990; Rothman et al 1999). Because glutamate also acts as the major excitatory neurotransmitter and GABA as the major inhibitory neurotransmitter in the CNS, the neurotransmission of glutamate and GABA is intimately related to the metabolism of glutamate, glutamine, and GABA. In vivo and in vitro studies (Behar and Rothman 2001; Bluml et al 2002; Cruz and Cerdan 1999; Erecinska and Silver 1990; Gruetter et al 2001; Hertz 1979, 2004; Künnecke et al 1993; Lebon et al 2002; Rothman et al 1999; Shank et al 1993; Shen and Rothman 2002; Shen et al 1998, 1999; Sibson et al 1997, 1998a, 1998b, 2001) have convincingly demonstrated that glutamate released by glutamatergic neurons into the synaptic cleft is taken up by surrounding glia and converted into its inactive form glutamine by the glia-specific glutamine synthetase. The expression of high capacity glutamate transporters on glial cell membrane ensures that the extracellular glutamate concentration is kept very low in normal brain to avoid excitotoxicity. To replenish the neuronal carbon lost to glia, resulting from synaptic glutamate release, glutamine is released by glia and recycled back to neurons where it is hydrolyzed into glutamate by phosphate-activated glutaminase. This so-called glutamate-glutamine cycle was conceptualized many decades ago (see reviews byErecinska and Silver 1990; Hertz 1979, 2004). Only recently, because of the rapid advances of in vivo ^{13}C and ^{15}N magnetic resonance spectroscopy (MRS) techniques, the glutamate-glutamine cycling flux was quantified in vivo in anesthetized rat brain (Shen et al 1998; Sibson et al 1997, 2001) and in human brain (Chhina et al 2001; Gruetter et al 2001; Lebon et al 2002; Shen et al 1999). It has been established that the glutamate-glutamine cycle between glutamatergic neurons and glia is a major metabolic flux, reflecting synaptic glutamate release (reviewed by Shen and Rothman 2002). This glutamate-glutamine cycle, together with the glial and neuronal tricarboxylic acid (TCA) cycles (Shen et al 1999), is schematically illustrated in Figure 1.

Because the large brain glutamate pool is predominantly from glutamatergic neurons, it acts as an effective trapping pool for ^{13}C labels of the neuronal TCA cycle through exchange with mitochondrial α-ketoglutarate. The kinetics of the ^{13}C label incorporation into glutamine, which is predominantly localized in glial cells (Erecinska and Silver 1990), contains the quantitative information of the glutamate-glutamine cycling flux. Therefore, the in vivo ^{13}C MRS technique allows noninvasive assessment of synaptic glutamatergic activities. Figure 2 shows an in vivo ^{13}C MRS spectrum obtained at 4.7 Tesla from an approximately 30-mL voxel located in a rhesus monkey brain. The spectrum was acquired during intravenous infusion of [1-^{13}C]glucose with a newly developed mostly adiabatic single-shot INEPT-based ^{1}H–^{13}C polarization transfer method (Li et al 2005). As shown in Figure 2, owing to the large ^{13}C chemical shift dispersion, signals from glutamate, glutamine, aspartate, lactate, and GABA, which are involved in brain energetics and neurotransmission, are spectrally resolved.

Despite the long history of ^{13}C MRS (Morris and Bachelard 2003), application of in vivo ^{13}C MRS is still at its infancy, largely owing to technical difficulties in its implementation on commercial spectrometers. Nevertheless, tremendous interests have been generated in the neuroscience community after the initial quantification of the glutamate-glutamine cycling flux in the human brain, because it provides a novel parameter for noninvasive characterization of glutamate neurotransmission, which plays a fundamental role in normal brain function as well as in a variety of psychiatric and neurological brain disorders (e.g., Hertz 2004; Morris 2002; Pellerin et al 2001; Sanacora et al 2003; Shulman and Rothman 2001). Over the past a few years, exciting progress has been made in probing the alterations of the glutamate-glutamine cycling flux as a result of drug effects and brain disorders. Meanwhile, although it is agreed by different authors that the glutamate-glutamine cycling is a major metabolic flux, the details of the metabolic models are still rigorously debated. In this article, the current debate on modeling the exchange between α-ketoglutarate and glutamate and the latest experimental results regarding the effects of endogenous GABA concentration, metabotropic glutamate receptor modulators, and several brain disorders on the glutamate-glutamine cycle were reviewed.

Modeling of the Exchange Between α-Ketoglutarate and Glutamate

Recently, evidence (Berkich et al 2005; Gruetter et al 2001; Henry et al 2002) has been presented to criticize the traditional

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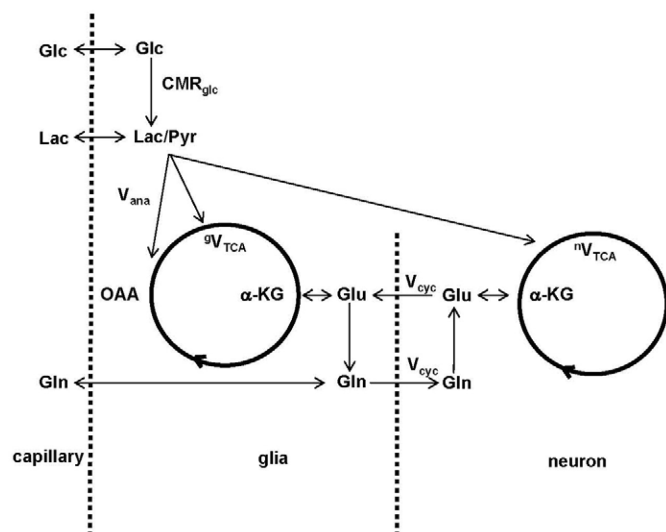

Figure 1. Schematic illustration of the glutamate-glutamine cycle between neurons and glia. Released neurotransmitter glutamate is transported from the synaptic cleft by surrounding glial end processes (adapted from Shen et al 1999). In glia, glutamate is converted into glutamine by glutamine synthetase. Glutamine is then released by the glia, transported into the neurons, and converted back into glutamate by phosphate-activated glutaminase, which completes the cycle. Glc, glucose; Pyr/Lac, pyruvate/lactate; OAA, oxaloacetate; α-KG, α-ketoglutarate; Glu, glutamate; Gln, glutamine; CMR_{glc}, cerebral metabolic rate of glucose utilization; V_{ana}, anaplerotic flux for de novo synthesis of oxaloacetate; $^gV_{TCA}$, glial tricarboxylic acid cycle flux; V_{cyc}, glutamate-glutamine cycling flux; $^nV_{TCA}$, neuronal tricarboxylic acid cycle flux.

metabolic modeling results (Mason et al 1992, 1995) that concluded that the exchange rate between α-ketoglutarate and glutamate (V_x) across the mitochondrial inner membrane in brain is much faster than the rate of the TCA cycle (V_{TCA}). With a metabolic modeling approach incorporating the turnover kinetics of both glutamate C4 and C3 measured from human brain, Gruetter et al (2001) concluded that $V_x = V_{TCA}$. With the same modeling approach, Henry et al (2002) modeled both glutamate C4 and C3 turnover, measured from α-chloralose anesthetized rat brain, and concluded that V_x is approximately equal to V_{TCA}. Although incorporating glutamate C3 turnover into metabolic models might seem to be appealing, it is difficult to incorporate the more significant isotopic dilution associated with glutamate C3. Because TCA cycles are in constant exchange with non-TCA cycle fluxes (Stryer 1995), the isotopic labels are progressively more diluted as they travel through the TCA cycles. Because glutamate C3 is formed during the second turn of the TCA cycle, the isotopic dilution at C3 is significantly larger than that at glutamate C4. In fact, in all published in vivo and in vitro brain ^{13}C MRS spectra including those acquired at isotopic steady state, the total glutamate C3 intensity is always significantly lower than that of glutamate C4 (e.g., Henry et al 2003; Sibson et al 1997). None of the dilution effects were taken into account by authors who modeled the turnover kinetics of C3 (Gruetter et al 2001; Henry et al 2002; Mason et al 1992, 1995). The lower C3 enrichment and slower than expected ^{13}C incorporation into glutamate C3 could be compensated by a significantly slower V_x during metabolic modeling when the dilution fluxes are not taken into account. Because both modeling approaches (Mason et al 1992, 1995 vs. Gruetter et al 2001; Henry et al 2002) included the turnover kinetics of glutamate C3, it appears that V_x can not be properly determined or estimated unless other factors (e.g., the additional isotope dilution at glutamate C3, the possibly incomplete label randomization in the symmetric citrate acid cycle intermediates succinate and fumarate) could be accounted for independently.

The α-ketoglutarate-glutamate exchange across the mitochondrial inner membrane has recently been characterized directly, with isolated rat brain mitochondria (Berkich et al 2005). It was concluded that intramitochondrial α-ketoglutarate was not in isotopic equilibrium with cytosolic glutamate. It is, however, debatable whether results obtained from isolated mitochondria could be extrapolated to the physiologically relevant in vivo situations. Brain endogenous oxaloacetate and α-ketoglutarate are quickly depleted during ischemia (Siesjo 1978) and, therefore, during the preparation of isolated mitochondria. Oxaloacetate is also known to be chemically unstable. It readily decomposes into pyruvate and bicarbonate at pH approximately equal to 7, especially at the presence of metal cations (Kurz et al 1985). In vivo, most of oxaloacetate molecules are probably bound to enzymes (Price and Stevens 2001); a condition might not be mimicked with isolated mitochondria. Because both extramitochondrial and intramitochondrial α-ketoglutarate and oxaloacetate are crucial components of the malate-aspartate shuttle, the destruction of the intimate network of metabolic pathways involved in the malate-aspartate pathways by the use of isolated mitochondria might significantly hamper the α-ketoglutarate-glutamate exchange process across the mitochondrial inner membrane.

Recently, the carbon-13 magnetization transfer (CMT) effect due to the exchange process between α-ketoglutarate and glutamate catalyzed by aspartate aminotransferase has been detected in vivo (Shen 2005). It is conceivable, although difficult, to use this new approach to follow the α-ketoglutarate and glutamate exchange process across the mitochondrial membrane. For example, a shift reagent could be used to separate the chemical shift of the extramitochondrial α-ketoglutarate from that of the intramitochondrial α-ketoglutarate in a properly constructed in vitro system. Then, V_x can be measured directly by selectively saturating mitochondrial α-ketoglutarate. Regard-

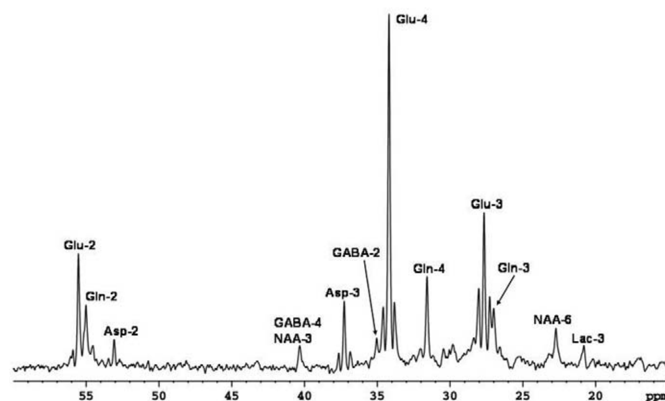

Figure 2. Three-dimensionally localized ^{13}C NMR spectrum of the rhesus monkey brain. The spectrum was accumulated for 1 hour from 80 to 140 min after the start of [1-^{13}C]glucose infusion. Twelve signals are clearly observed: glutamate C2 (55.5 ppm), glutamine C2 (55.0 ppm), aspartate C2 (53.0 ppm), GABA C4 and N-acetylaspartate C3 (40.2 ppm), aspartate C3 (37.4 ppm), GABA C2 (35.2 ppm), glutamate C4 (34.2 ppm), glutamine C4 (31.7 ppm), glutamate C3 (27.8 ppm), glutamine C3 (27.1 ppm), N-acetylaspartate C6 (22.8 ppm) and Lactate C3 (21.0 ppm). Additional splittings due to homonuclear ^{13}C-^{13}C coupling from the resonances of glutamate C3-C4, glutamine C3-C4, and aspartate C2-C3 were clearly resolved.

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