

Review

In vivo ^{13}C NMR studies of compartmentalized cerebral carbohydrate metabolism

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Abstract

Localized ^{13}C nuclear magnetic resonance (NMR) spectroscopy provides a unique window for studying cerebral carbohydrate metabolism through, e.g. the completely non-invasive measurement of cerebral glucose and glycogen metabolism. In addition, label incorporation into amino acid neurotransmitters such as glutamate (Glu), GABA and aspartate can be measured providing information on Krebs cycle flux and oxidative metabolism. Given the compartmentation of key enzymes such as pyruvate carboxylase and glutamine synthetase, the detection of label incorporation into glutamine indicated that neuronal and glial metabolism can be measured in vivo. The purpose of this paper is to provide a critical overview of these recent advances into measuring compartmentation of brain energy metabolism using localized in vivo ^{13}C NMR spectroscopy. The studies reviewed herein showed that anaplerosis is significant in brain, as is oxidative ATP generation in glia and the rate of glial glutamine synthesis attributed to the replenishment of the neuronal Glu pool and that brain glycogen metabolism is slow under resting conditions. This new modality promises to provide a new investigative tool to study aspects of normal and diseased brain hitherto inaccessible, such as the interplay between glutamatergic action, glucose and glycogen metabolism during brain activation, and the derangements thereof in patients with hepatic encephalopathy, neurodegenerative diseases and diabetes. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the traditional context of neuroscience the brain's tasks are mainly accomplished by the neurons, with the surrounding glial cells having a rather passive function in neurotransmission. However, the glial cells are more than just passive components in neuronal function in that they are intimately involved in the process of neurotransmission through, e.g. glial uptake of glutamate (Glu) from the synaptic cleft (Yudkoff et al., 1993; Zigmond, 1999). Glu is the major excitatory neurotransmitter (Shank and Aprison, 1979) which is present in the mammalian brain in high concentrations. Despite the high intracellular concentration of Glu, the extracellular concentration must be maintained very low (~ 0.004 mM) to avoid excitotoxicity. Presynaptic release of Glu into the synaptic cleft therefore requires efficient uptake mechanisms, which are achieved by Glu transporters (Arriza et al., 1994). Most of the metabolic evidence suggests that uptake by glia is an important process which has led to the concept of the Glu–glutamine

cycle (Brand et al., 1997; Westergaard et al., 1995; Yudkoff et al., 1988). Recent studies have again supported the participation of glia in neurotransmission through rapid clearance of Glu from the synaptic cleft into neighboring glia, based on conductance currents associated with Glu uptake (Bergles et al., 1997) and consequent metabolism to glutamine. The uptake of Glu into the astrocytes is associated with increased glucose metabolism (Eriksson et al., 1995; Magistretti et al., 1993), thereby linking stimulated energy metabolism between glial and neuronal cells during neurotransmission. This paper reviews recent contributions of in vivo nuclear magnetic resonance (NMR) spectroscopy to the assessment of neurotransmitter metabolism.

2. Features of ^{13}C NMR spectroscopy in vivo and relationship to other modalities

The administration of a tracer, whether stable or radioactive, and the ability to follow its metabolism provides neurochemists and neuroscientists alike tools to study in vivo metabolism non-invasively. When using radiotracers, label in different metabolic pools cannot be distinguished,

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which has led to the use of non-metabolizable analogs, such as deoxy-glucose. On the other hand, tracer studies can be performed using stable isotopes. In conjunction with the non-invasive detection using NMR the metabolism of stable isotopes can be followed, although administration of the “tracer” requires a higher enrichment. Because NMR spectroscopy can be used to detect label in different molecules and different chemical positions, it offers the attractive possibility to follow metabolism of the native compound, labeled at one or more specific positions. For example, glucose can be labeled at the 1 position and flow of the label into metabolic pools further downstream can be followed non-invasively. Applications are predominantly focused on areas where a stable isotope is present at low natural abundance, examples include ^2H , ^{15}N , ^{19}F and ^{13}C , the latter of which shall be dealt with in this review.

Ideally, an investigative method applied to biomedical problems is fully developed and “mature”. Unfortunately, the development of ^{13}C NMR spectroscopy in vivo has been limited to a handful sites worldwide (Bluml, 1999; Chen et al., 2001; Gruetter et al., 1998a; Rothman et al., 1999) and largely requires further development, as biomedical problems in general and neurochemical questions in particular develop. When considering these constraints in the application of this method, it is clear that methodological advances are likely required and expected when developing the biomedical questions. This shall in the following be illustrated with a historical perspective on the development of the application to the intact in vivo brain: The first application of ^{13}C MRS to the head (Behar et al., 1986), was admittedly struggling with achieving the necessary spectral resolution in vivo to separate the glutamine from the Glu resonances in vivo. In retrospect, it is clear from the detection of intense lipid signals in this and other early in vivo papers on the brain, that signals from outside the brain were dominant, such as the intense resonances from natural abundance lipids (Beckmann et al., 1991; Behar et al., 1986; Bluml, 1999; Sibson et al., 1997). In brain, the concentration of mobile lipids is generally too low to be detected in vivo (although this can be done in extracts) and therefore, the lipid resonances seen in ^{13}C NMR spectra of the head must be attributed to extra-cerebral fat tissue, such as subcutaneous fat. In 1991, it was shown for the first time that glucose can be administered to humans, and resonances from Glu (Beckmann et al., 1991; Rothman et al., 1985; Rothman et al., 1992) and glucose (Gruetter et al., 1992a; Mason et al., 1992a) were detected following ^{13}C -labeled glucose administration. Most of these early studies continued to be hampered by the lack of adequate spectral resolution and/or localization. In 1992, two important advances in MR methodology were introduced to in vivo ^{13}C NMR spectroscopy: First, the use of automated shimming (i.e. in vivo optimization of the main static magnetic field, B_0 , such that it becomes largely independent of the spatial coordinates) dramatically improved sensitivity by narrowing linewidths by almost an order of magnitude (Gruetter, 1993; Gruetter and Boesch,

1992). Second, the introduction of three-dimensional localization allowed for well-defined volumes to be measured (Gruetter et al., 1992a; Gruetter et al., 1992b). Two immediate advances were immediately realizable, namely the complete elimination of the intense scalp lipid signals from the extra-cerebral tissue, which overlap with numerous signals from amino acids, and the collection of signals from a well-defined volume together with excellent shimming improved the spectral resolution. The improvements in sensitivity were demonstrated with the rather surprising observation that natural abundance signals from brain metabolites can be observed in vivo, such as those from *myo*-inositol (Gruetter et al., 1992b). Also in 1992, these methodological advances lead to the landmark discovery that labeling of glutamine can be detected in the in vivo brain (Gruetter, 1993; Gruetter et al., 1994), which is now recognized as a window to study cerebral metabolic compartmentation (Bachelard, 1998; Cruz and Cerdan, 1999; Magistretti et al., 1999; Rothman et al., 1999). These advances have placed the ^{13}C NMR method into the neuroscience theater to provide a unique in vivo window on the brain. NMR spectroscopy itself holds promise to studying a number of neurochemical events that are otherwise inaccessible by non-invasive means, and thus opens the potential to study neurochemistry in patients.

A discussion of methodological issues surrounding the use of ^{13}C NMR in vivo would be incomplete without the side-by-side comparison of the two principal detection methods available, namely direct and indirect detection. Direct detection of the ^{13}C signals takes advantage of the large chemical shift dispersion of the ^{13}C nucleus. In addition, the spin physics of the ^{13}C nucleus are rather simple, provided ^1H decoupling is applied (Gruetter et al., 1996). However, the increased specificity of direct detection comes at the price of a substantial loss of sensitivity (Chen et al., 1998). For example, the signal-to-noise ratio from an equimolar solution at a given static field B_0 can be as much as 12-fold weaker for ^{13}C than the corresponding ^1H signal in vivo. It is, therefore, attractive to exploit the fact that ^1H nuclei in close chemical proximity to a ^{13}C nucleus are coupled through the heteronuclear J coupling, which with appropriate “spin gymnastics” can be exploited to filter the magnetization of those protons that are attached to a carbon nucleus (Hyder et al., 1997; Pan et al., 1997; Pfeuffer et al., 1999; Rothman et al., 1985; Schupp et al., 1993; Van Zijl et al., 1993). The ensuing “ ^{13}C -edited” ^1H spectrum stands to benefit from the improved sensitivity of ^1H NMR. It also stands to suffer from the same shortcoming, namely the limited spectral dispersion, as the corresponding chemical shifts in biomedical applications are crammed into a 10-fold reduced bandwidth compared to carbon and additional efforts are required to achieve adequate spectral resolution (Watanabe et al., 2000).

The spectral resolution is especially limiting at low magnetic fields, such as the widely deployed magnetic field of 1.5 T, which does not allow the accurate separation of resonances of Glu and glutamine. When increasing the magnetic

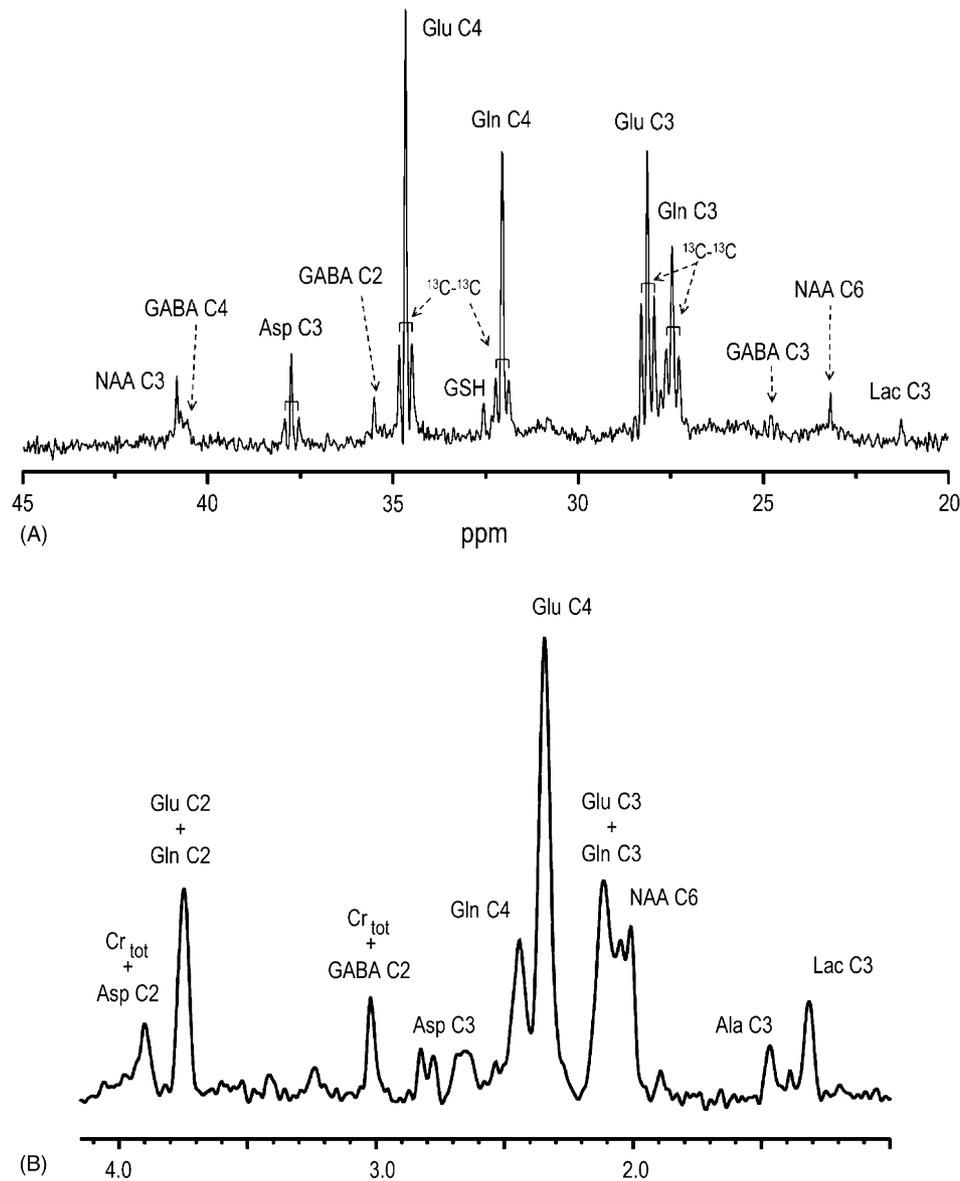


Fig. 1. Illustration of the information content achievable in vivo by ^{13}C and ^1H -detected ^{13}C NMR at 9.4 T. (A) is a direct-detected ^{13}C NMR spectrum from a 500 μl volume in approximately 1 h and is from Choi et al., 2000. In addition to the resonances from the amino acids, label incorporation into the Glu C4 resonance of glutathione (GSH) was detected. For resonance assignments, see Fig. 2. (B) is a ^1H NMR spectrum obtained from a 130 μl volume in the rat brain in the first 1 h of glucose infusion showing resonances coupled to ^{13}C only and is taken from Pfeuffer et al., 1999. The improved sensitivity allowed the detection of label incorporation into alanine C3 (Ala). Natural abundance signal is detected for creatine (Cr_{tot}) and NAA.

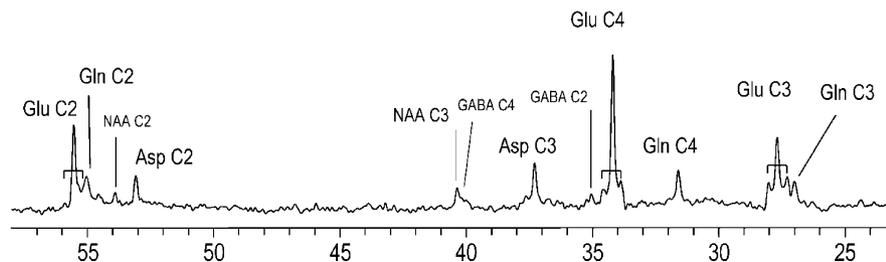


Fig. 2. ^{13}C NMR detection of label incorporation into cytosolic amino acids in a 45 ml volume in the human occipital lobe at 4 T, from Gruetter et al., 2001. Shown is a representative spectrum obtained from a 3 cm \times 3 cm \times 5 cm volume in the human visual cortex in 45 min. Resonance assignments are as follows: Glu C2 at 55.6 ppm, Gln C2 at 55.0 ppm, NAA C2 at 54.0 ppm, Asp C2 at 53.7 ppm, NAA C3 at 40.5 ppm, GABA C4 at 40.45 ppm, Asp C3 at 37.6 ppm, GABA C2 at 35.3 ppm, Glu C4 at 34.2 ppm, Gln C4 at 31.7 ppm, Glu C3 at 28.0 ppm, Gln C3 at 27.7 ppm. In addition to these resonances, resonances ascribed to the homonuclear ^{13}C - ^{13}C coupling were readily detected at the positions of all Glu resonances (brackets).

field some of these limitations can be overcome due to improved spectral dispersion (Gruetter et al., 1998c), allowing the detection of resolved signals from ^{13}C labeled Glu and glutamine by ^1H NMR spectroscopy (Pfeuffer et al., 1999). Fig. 1A illustrates that the resonances of most amino acids in ^{13}C NMR spectra are easily resolved, whereas in ^1H NMR spectra the resolution of a number of resonances is limited when using only direct editing for the ^1H attached to ^{13}C . For example, the C2 resonances of Glu and glutamine are not easily separated in the ^1H spectra (Fig. 1B), whereas in ^{13}C NMR spectra even at 4 T as shown in Fig. 2 (Gruetter et al., 1998a; Gruetter et al., 2001), and to some extent at 1.5 and 2.1 T, the C2 of Glu and glutamine are readily separated (Bluml, 1999; Gruetter, 1993; Shen et al., 1999).

3. The ^{13}C glucose labeling studies of the brain

Most ^{13}C NMR studies of the brain are designed along the principles of tracer studies, and as a matter of fact the mathematics involved are not different in substance. However, because of the low sensitivity of MR and the non-toxic nature of using the natural substrate, such as glucose, the label is administered in quantities that increase the isotopic enrichment well above what can be considered tracer level and isotopic enrichments ranging between 50 and 100% are not uncommon.

A majority of studies have focused on measuring Glu turnover (Hyder et al., 1997; Lukkarinen et al., 1997; Pan et al., 2000; Rothman et al., 1992), which was motivated by the fact that cerebral Glu is linked to the metabolism of the Krebs cycle (Chatham et al., 1995; Cruz and Cerdan, 1999; Gruetter et al., 1998a; Gruetter et al., 2001; Mason et al., 1995; Mason et al., 1992b; Yu et al., 1997), and that the Glu C4 resonance, which is labeled in the first turn of the Krebs cycle, presents a readily detectable NMR signal due to the high concentration of Glu in the CNS.

4. Glucose transport

Unless glucose is injected directly into the brain, the label must cross the blood–brain barrier before it is metabolized by the brain cells. Aside from lactate and possibly glycogen (Choi et al., 1999), glucose is the only sizable kinetic pool in the Embden–Meyerhof pathway that is capable of influencing the labeling kinetics of pyruvate and thus ultimately of acetyl-CoA. Therefore, precise knowledge of the size of the brain glucose pool and its physical distribution space is critical for the derivation of absolute fluxes from, e.g. Glu labeling curves. Many studies have measured the kinetics of glucose transport (Knudsen et al., 1990; Pardridge, 1994; Van Zijl et al., 1997) given that the dominant method to measure cerebral glucose consumption is by measuring the activity accumulated in the phosphorylation product of a glucose analog, such as the widely used autoradiography of deoxyglucose, or the non-invasive fluoro-deoxy-glucose

positron emission tomography (Tyler et al., 1988). In vivo NMR spectroscopy stands out among these methods in that it can provide a direct, localized measurement of brain glucose content (Choi et al., 2001; Gruetter et al., 1992a; Gruetter et al., 1998b) as well as the diffusion behavior of glucose (Pfeuffer et al., 2000). The diffusion behavior of glucose and lactate was found to be different from that of known intracellular metabolites such as NAA, creatine and Glu. The differential diffusion behavior was ascribed to the signal component in extracellular space and comparison with intracellular signals was used to estimate the physical distribution space of glucose (Pfeuffer et al., 2000). From the relationship between brain and plasma glucose, steady-state glucose transport kinetics can be derived (Choi et al., 2001; Gruetter et al., 1992a; Gruetter et al., 1998b; Mason et al., 1992a). Based on previous modeling it has been argued that the turnover of brain glucose is too rapid to be able to significantly influence labeling kinetics of the large Glu pool (Mason et al., 1992a). However, based on numerical simulations we have found this to be only true when the changes in Glu labeling kinetics are large. When observing rather small changes in brain Glu turnover, it is quite possible that these changes can be due to changes in flux through the Embden–Meyerhoff pathway, which can influence the rate of labeling of pyruvate, which ultimately is reflected in a changed rate of fractional labeling of acetyl-CoA. In other words, it is in principle possible to observe a change in the labeling of Glu, even though the flux through pyruvate dehydrogenase has not changed. The question arises as to whether such a difference in the labeling rate can affect the Glu turnover curves and this question is addressed in Fig. 3, which illustrates the simulated labeling curves of Glu C4 assuming a 50% increase in flux through the Embden–Meyerhof pathway (solid line) compared to resting metabolism (dashed line), while all other metabolic rates

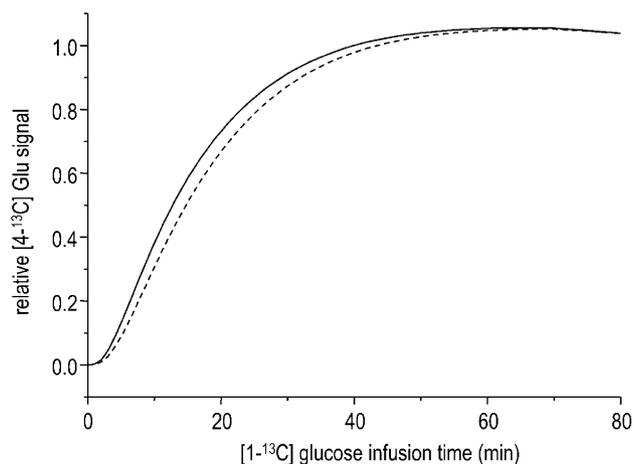


Fig. 3. Effect of increased rate of labeling of pyruvate due to increased flux through glycolysis. The dashed line represents the rate of label incorporation into Glu C4 at rest and the solid line the rate of label incorporation, when assuming that glycolysis increased by 50% (Fox et al., 1988) and all other fluxes remained unchanged.

were held constant. In other words, if the observed changes in Glu labeling are small, it is not clear whether they are attributed to a large change in the Embden–Meyerhoff pathway and small changes in oxidative metabolism, as they have been reported (Fox et al., 1988) or whether the changes are due to smaller fractional changes in oxidative metabolism tightly coupled to glucose consumption. In mathematical terms, this ambiguity results in a negative numerical covariance of CMR_{glc} and flux through pyruvate dehydrogenase, V_{PDH} . However, when large changes in oxidative metabolism are observed, this covariance can be expected to be much smaller to the point that it may even be irrelevant. Several-fold changes in Glu turnover have been reported during forepaw stimulation (Hyder et al., 1996; Hyder et al., 1997), and when such large changes occur, the increased turnover rate of the cerebral glucose pool is expected to have a lower relative influence.

Glucose is the single most important substrate for normal function, and the brain relies on a continuous import of glucose from the blood, which must occur across the blood–brain-barrier. Glucose transport rates into the brain

thus are indicative of the maximal sustainable rate of glucose consumption, CMR_{glc} . We have recently shown in the conscious human and the α -chloralose anesthetized rat, that the maximal sustainable rate of glucose consumption is approximately 60–90% above the basal rate of glucose metabolism (Choi et al., 2001; Gruetter et al., 1998b). Recent studies by Hyder et al. have reported that over a 45 min period of forepaw stimulation, oxidative glucose metabolism was increased by more than three-fold (Hyder et al., 1996; Hyder et al., 1997). Such an increase in cerebral glucose metabolism is clearly beyond what transport across the blood–brain barrier can sustain alone and the implication is that other sources of fuel must have been increasingly utilized.

5. Brain glycogen, the forgotten energy store

As pointed out in the previous section, some of the reported increases in brain glucose metabolism observed during focal activation imply increased usage of

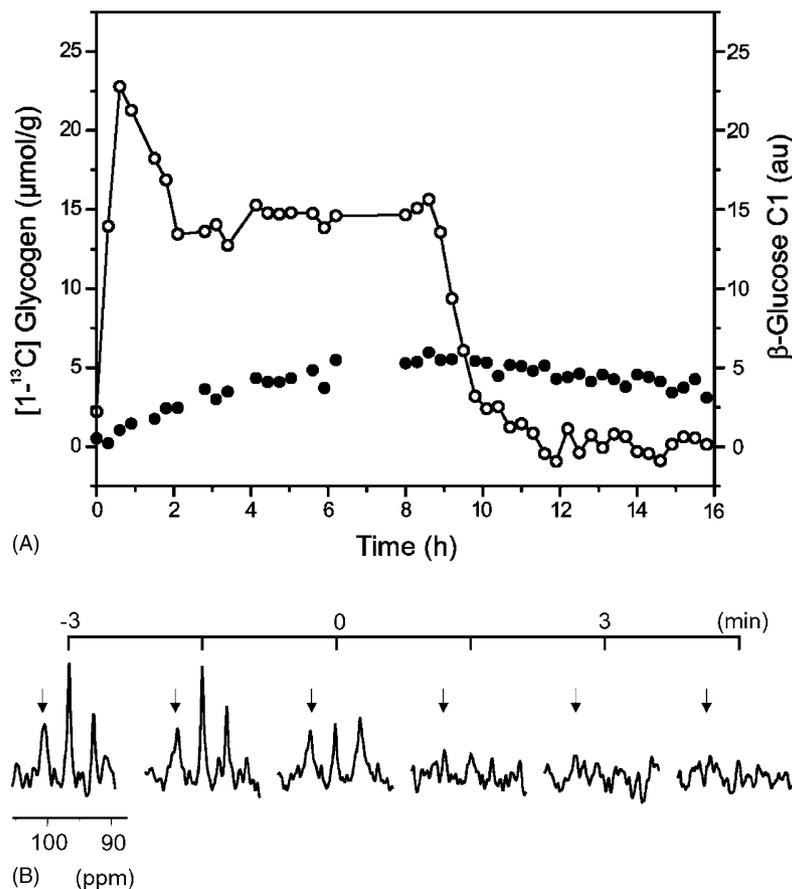


Fig. 4. Direct detection of brain glycogen metabolism, adapted from Choi et al., 1999. (A) Rapid mobilization of glycogen and glucose is illustrated with a horizontal stack plot of ^{13}C NMR spectra before and after inducing hypoxic ischemia by intravenous KCl injection at $t = 0$ min. The spectra are from a $500 \mu l$ volume and were collected at 9.4T with a 90s temporal resolution. The arrows indicate the position of the glycogen resonance. (B) During ($1-^{13}C$) glucose infusion, the ^{13}C glucose signal rises rapidly (right scale), leading to label transfer into glycogen (left scale). Following the infusion with unlabeled (cold) glucose (pulse-chase experiment), the glucose label is rapidly washed out leading to a much slower reduction in brain glycogen signal implying a slow turnover of glycogen under resting conditions.

carbohydrates other than blood glucose. One such source of glucose equivalents is brain glycogen, which is present in sizable amounts in brain (Choi et al., 1999). It remains to be determined whether the changes reported by Hyder et al. are accounted for by brain glycogen alone. Studies have reported that brain glycogen metabolism appears to be activated during focal stimulation (Swanson, 1992). Brain glycogen metabolism and concentrations obviously can be measured by NMR (Choi et al., 2000; Choi et al., 1999). Based on the high metabolic activity of the brain, label wash-in and wash-out was expected to be fast. However, such pulse-chase experiments clearly demonstrated that glycogen phosphorylase in the α -chloralose anesthetized rat was very slow, on the order of $0.5 \mu\text{mol}/(\text{g h})$ during glucose infusions, as illustrated by the slow label washout during the chase in Fig. 4A. Clearly, as illustrated in Fig. 4B, the brain glycogen reservoir was rapidly mobilized during global ischemia combined with hypoxia and the question arises as to what is the mechanism by which brain glycogen concentrations are controlled. One possibility is that glycogen, like in other tissues, is a reservoir of glucosyl units that is mobilized when there is demand in excess of glucose supply. During global ischemia, we indeed observed that depletion of brain glycogen occurred on a time scale comparable to that of brain glucose (Fig. 4B), which supported the view that glycogen appears to be a glucosyl reservoir, of probably limited storage capability. To further test this hypothesis, we studied the effect of profound hypoglycemia on brain glycogen. Textbook literature implies that brain glycogen is a limited storage form for glucose and based on that reasoning its role as a glucose reservoir has been dismissed. However, during hypoglycemia, glycogen need only account for part of the deficient glucose supply and hence can survive longer periods of sustained hypoglycemia. Our results showed that brain glycogen indeed was only slowly degraded during hypoglycemia and that this degradation started when brain glucose approached zero, which is the point at which glucose transport becomes rate-limiting for metabolism (Gruetter et al., 2000). Interestingly, at this point cerebral blood flow was increased abruptly, indicating an attempt by the brain to increase supply, by decreasing the arterio-venous gradient for glucose (Choi et al., 2001). We reported rates of label incorporation into glycogen using the native glucose that were very similar to those observed using deoxyglucose (Nelson et al., 1984), implying that the rate of incorporation of deoxyglucose into glycogen is as efficient as it is for glucose. These considerations raise the interesting possibility that deoxyglucose measurements of brain glucose metabolism can be affected by brain glycogen metabolism in focal neural activation.

6. Glu turnover: oxygen metabolism

Because of the ever increasing importance of functional MRI, which depends on an activation-dependent change in

the venous concentration of deoxyhemoglobin, the question whether there is tight coupling between glucose and oxygen consumption in the brain has become of paramount importance. The landmark study by Fox and Raichle in the late 1980s suggested that there is indeed a large increase in glucose metabolism that exceeds the changes in oxygen metabolism (Fox et al., 1988). The concept of uncoupled oxygen metabolism has been supported by reports of small increases in brain lactate during focal activation (Prichard et al., 1991), that initially were very controversial (Merboldt et al., 1992) and that are very difficult to perform. The relatively small magnitude of change in brain lactate is difficult to reconcile with the reported large uncoupling between oxygen and glucose consumption (Madsen et al., 1999). To address this question, we performed a study to measure the effect of focal activation on cerebral Glu turnover. This was achieved using the following study design: Using hemi-field activation, we verified that only one side of the human visual cortex was activated. Measurement of Glu turnover was then performed in this area and simultaneously in a control area placed symmetrically to the midline, which was verified to be devoid of activated pixels. Comparison of the rate of label incorporation indicated a significant difference between the activated and the resting voxel. Modeling of the data (Fig. 5) indicated that oxygen consumption increased at most by 30%, which is approximately half of the blood flow increase measured using this stimulation paradigm (Chen et al., 2001). Unfortunately, the change in Glu turnover was small enough to be influenced by changes in brain glucose turnover as illustrated by the discussion above and by the simulation presented in Fig. 3, and thus an unambiguous answer was not possible. Nevertheless, this study supports

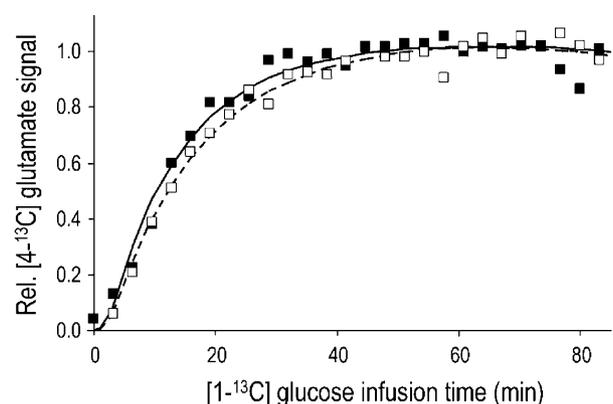


Fig. 5. Focal brain activation of the human brain. Shown is the average label incorporation curve into brain Glu from five studies. The solid squares are the measurements from the activated side, and the open squares are the resting half of the human visual cortex during hemifield stimulation. Adapted from Chen et al., 2001. The solid line shows the fit of a model of brain metabolism similar to what is shown in Fig. 6A and the dashed line is the fit to the data obtained from the resting visual cortex. The small changes in Glu labeling indicate at most a 30% increase in Glu labeling.

Table 1
Relative fluxes in human visual cortex as measured by ^{13}C NMR following glucose infusion (from Gruetter et al., 2001)

	Relative flux rate (%)
$V_{\text{NT}}/\text{CMR}_{\text{glc}}^{\text{a}}$	41 ± 14
Oxidative ATP/total ATP production in glia	80 ± 5
Glial/neuronal ATP synthesis	16 ± 9
$V_{\text{x}}/V_{\text{PDH}}$	100 ± 35

^a For a definition of fluxes see Fig. 6B and (Gruetter et al., 2001).

the idea that oxygen consumption increases are less than the associated blood flow increases, leading to a net decrease in deoxy-hemoglobin content during focal activation, which forms the basis of blood-oxygen-level-dependent functional MRI (Ogawa et al., 1998).

Measurements of Krebs cycle flux from the flow of label from glucose C1 to Glu C4 are inherently affected by the assumptions made in the modeling. Of critical importance in that regard is the fact that most amino acids are located in the cytosol, whereas the reactions leading to the scrambling of the label take place in the mitochondrion. Therefore, transport has to occur across the highly charged inner mitochondrial membrane. It is likely that the transport of Glu (a charged amino acid, whose transport is generally associated with concomitant ion transport) or oxoglutarate (an acid as well) is controlled, since rapid transport of these acids is likely to interfere with chemiosmosis. In brain and other tissues, exchange does occur via the malate–aspartate shuttle (illustrated by the scheme in Fig. 6A) and our observation that V_{x} is comparable to the flux through pyruvate dehydrogenase (Table 1) implies that the malate–aspartate shuttle may be a major mechanism mediating the exchange of label across the mitochondrial membrane (LaNoue and Tischler, 1974). It is in this context also important to recognize that the pool size that is in exchange with the Krebs cycle intermediate can also affect the accuracy by which the labeling of the amino acid represents that in the Krebs cycle. Table 2 illustrates that the calculation of V_{PDH} from aspartate labeling is much less prone to the specific assumption made for V_{x} , which in part reflects the fact that aspartate is approximately four–six times less concentrated in brain than is Glu.

Table 2
Krebs cycle flux (V_{PDH})^a as a function of the rate of exchange, V_{x} , measured from Asp (Asp₂₃) or Glu C4 (Glu₄)

V_{x} ($\mu\text{mol}/(\text{g min})$)	Asp ₂₃	Glu ₄
57	1.04	0.53
10	1.04	0.55
2	1.03	0.65
1	1.01	0.83
0.75	1.00	1.00

^a Obtained by fitting V_{out} and V_{PDH} keeping the remainder of the fluxes fixed and by dividing the values by the V_{PDH} obtained at $V_{\text{x}} = 0.75 \mu\text{mol}/(\text{g min})$, reproduced from Gruetter et al., 2001.

7. Glutamine turnover: The hallmark of cerebral compartmentation

Brain metabolism is exquisitely compartmentalized, with at least two major compartments (attributed to the “neuronal” and “glial” compartment) that are differentiated by the size of the respective Glu pools associated with the Krebs cycle and that are metabolically linked by the Glu–glutamine cycle (Yudkoff et al., 1993). It has now become accepted that the large Glu pool is in the neuronal compartment and the small Glu pool in the glial compartment. The compartmentation extends further to a number of enzymes, such as glutamine synthetase (Martinez-Hernandez et al., 1976) and pyruvate carboxylase (Shank et al., 1985) being almost exclusively in the glial compartment. Furthermore, glycogen appears to be mainly in the glial compartment in normal adult brain (Dringen et al., 1993). The compartmentation of brain metabolism and associated enzymes is summarized in more detail elsewhere (Bachelard, 1998; Cruz and Cerdan, 1999; Gruetter et al., 2001). The scheme in Fig. 6B summarizes some of the salient features that can be exploited in modeling brain metabolism. It is of interest to note that the exclusively glial localization of glutamine synthetase implies that the observation of glutamine synthesis *in vivo*, first achieved in human brain (Gruetter, 1993; Gruetter et al., 1994), is a direct manifestation of glial metabolism, whereas the observation of label incorporation into Glu implies a mainly neuronal event. Clearly, glutamine synthesis can be measured non-invasively by NMR, as shown in Figs. 1 and 2, and therefore implicates the ability of NMR to study cerebral compartmentation non-invasively.

A key feature to cerebral compartmentation is the inactivation of neurotransmitter Glu by uptake into perisynaptic astrocytes and conversion into electrophysiologically inactive glutamine (Daikhin and Yudkoff, 2000; McKenna et al., 2000; Nicklas et al., 1987; Schousboe et al., 1993). Clearly, this mechanism implies a much more active role for astrocytes than is conventionally assumed, since the conversion of neurotransmitter Glu to glutamine invokes glial energy metabolism (Eriksson et al., 1995; Magistretti and Pellerin, 1996; Silver and Erecinska, 1997). The neuron–astrocyte pair thus has to be considered the functional unit intimately involved in achieving chemical transmission which has been proposed in the last decade by Magistretti and others (Magistretti et al., 1999; Magistretti et al., 1993; Tsacopoulos and Magistretti, 1996). The link between astrocytes and neurons is generally accepted from a metabolic and from a neurophysiological standpoint (Bergles et al., 1997), yet differences exist as to the precise coupling and the specific energetics involved.

For example, a recent proposal suggested that the rate of Glu–glutamine inter-conversion (also termed the “Glu–glutamine cycle”), identified in the scheme in Fig. 6A by $V_{\text{NT}}^{\text{app}}$, is equal to the glucose consumption rate (Sibson et al., 1998). The proposed near 1:1 stoichiometry

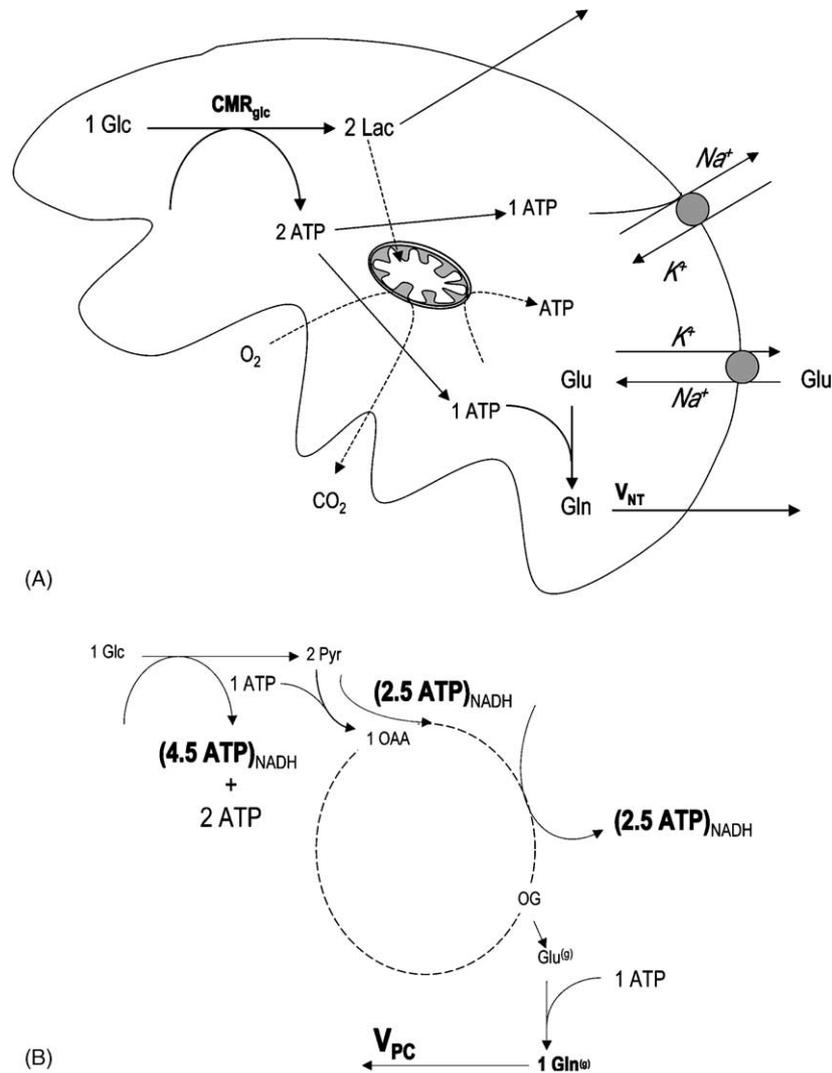


Fig. 7. Stoichiometry of glutamatergic action. The implications of the model proposed by Shulman et al. (A) The 1:1 stoichiometry between CMR_{glc} and V_{NT}^{app} proposed recently. In this model it is assumed that the two ATP required for the Na/K ATPase to restore the ion balance after glutamate uptake and for glutamine synthesis are produced exclusively by the Embden–Meyerhof pathway as proposed (Magistretti et al., 1999; Rothman et al., 1999; Sibson et al., 1998), which neglects oxidative generation of ATP (dashed arrows). (B) Energetics of anaplerotic glutamine synthesis from glucose in the glial compartment. Reactions yielding ATP through oxidative phosphorylation are indicated by the subscript NADH. These include glyceraldehyde-3-phosphate dehydrogenase, pyruvate dehydrogenase and isocitrate dehydrogenase. One glucose forms two pyruvate and in that process two ATP; one ATP is consumed by the consumption of one pyruvate to form oxaloacetate. Net synthesis of citrate is followed in this scheme by net removal of 2-oxoglutarate to form glutamine, which requires an additional ATP per glutamine molecule formed, thereby maintaining a constant concentration of glial Krebs cycle intermediates. For abbreviations, see also caption of Fig. 6.

at $V_{NT} = 0$ (“isoelectricity”) is approximately half of that at normal resting conditions, which is in good agreement with reported decreases in oxygen consumption during isoelectric pentobarbital anesthesia in humans. The proposal put forth by Shulman et al. (Rothman et al., 1999; Sibson et al., 1998), that the glial ATP production needed to maintain neuronal Glu is solely provided by the Embden–Meyerhoff pathway is intriguing as it emphasizes the coupling between neurons and glia at the level of energy metabolism. However, only a few percent of pyruvate need to be diverted to the Krebs cycle to generate as many ATP as are formed in the absence of oxidative metabolism of glucose in the astrocytes (aerobically generation of ATP is illustrated by the

dashed arrows in Fig. 7A). Astrocytes clearly have oxygen metabolism (Eriksson et al., 1995), and this shall be further exemplified in the case of anaplerosis, a mitochondrial reaction confined to the glial compartment. The energetics of glutamine synthesis from glucose via anaplerosis imply substantial oxidative ATP generation: As illustrated in the scheme in Fig. 7B, almost 10 ATP are generated per glutamine molecule synthesized due to the reducing equivalents produced by glyceral-3-phosphate dehydrogenase, pyruvate dehydrogenase and isocitrate dehydrogenase. Pyruvate carboxylase activity is significant in vivo (Gruetter et al., 1998a; Gruetter et al., 2001; Lapidot and Gopher, 1994; Shen et al., 1999). This calculation assumed a P:O ratio of 2.5:1, when

assuming a P:O ratio of 3:1 as in previous studies (Sibson et al., 1998), almost 12 ATP are generated per glutamine molecule, which is approximately a third as efficient as complete glucose oxidation, yet five–six times as efficient as anaerobic glycolysis alone. Although differences exist as to the magnitude of the flux through pyruvate carboxylase, the labeling pattern is different between glutamine and Glu (Gruetter et al., 1998a; Gruetter et al., 2001; Lapidot and Gopher, 1994; Martin et al., 1993), which implies substantial contribution of pyruvate carboxylase to the flux through glutamine synthetase. Even the lowest reported value of $0.04 \mu\text{mol}/(\text{g min})$ (Shen et al., 1999) results in an ATP generation of $0.4 \mu\text{mol}/(\text{g min})$, which corresponds to 50–75% of the ATP needed for Glu uptake and conversion to glutamine (Gruetter et al., 1998a; Gruetter et al., 2001). Adding the small glial Krebs cycle flux of $\sim 0.04 \mu\text{mol}/(\text{g min})$ results in a rate of oxidative ATP production well in excess of what is required for the Glu–glutamine interconversion, which is in agreement with the observation that under basal conditions the energy requirements of the Na/K ATPase are 20% of the ATP synthesized in astrocytes (Silver and Erecinska, 1997). Given that autoradiographs are acquired typically following extended periods of tracer administration, it is unlikely that in such periods of activation, glial cells do not increase their oxygen consumption. In fact, oxygen consumption has been reported to increase in cultured astrocytes when exposed to extracellular Glu (Eriksson et al., 1995) and large increases in oxygen consumption have been reported (Hyder et al., 1996; Hyder et al., 1997), which support the idea that oxygen metabolism in astrocytes is stimulated during focal activation. It should be stressed that the concept of oxidative metabolism still is compatible with the idea that lactate produced in astrocytes is the fuel for oxidative metabolism in neurons. Our results suggest that in the human brain approximately one sixth of the ATP production from glucose measured by NMR is in the glial compartment, leaving five-sixths of the lactate for export to neurons, when assuming the extreme case that phosphorylation of glucose is an exclusively glial process.

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