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In vivo N-15 MRS study of glutamate metabolism in the rat brain

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ABSTRACT

In vivo ^{15}N MRS has made a unique contribution to kinetic studies of the individual pathways that control glutamate flux in the rat brain. This review covers the following topics: (1) the advantages and limitations of in vivo ^{15}N MRS and its indirect detection through coupled ^{1}H; (2) kinetic methods; (3) major findings from our and other laboratories in the areas: (a) the uptake of the neurotransmitter glutamate from the extracellular fluid into glia; (b) the metabolism of glutamate to glutamine; (c) glutamine transport to the extracellular fluid; (d) hydrolysis of neuronal glutamine to glutamate; and (e) contribution of transamination from leucine to replenish the glutamate nitrogen.

In vivo glutamine synthetase activities measured at several levels of hyperammonemia showed that this enzyme becomes saturated at blood ammonia concentration >0.9 μmol/g, and causes the elevation of brain ammonia. Implications of the results for the cause of hyperammonemic encephalopathy are discussed. Leucine provides >25% of glutamate nitrogen. An intriguing possibility that supplementing leucine may restore cognitive function after brain injury is discussed. Finally, some characteristics of ^{15}N MRS that may facilitate the future application of this technique to the study of the human brain at 4 or 7 T are described.

1. Introduction

In vivo ^{15}N magnetic resonance spectroscopy (MRS) is a useful technique that has made a unique contribution to our understanding of metabolic flux. The in vivo studies were first performed in microorganisms (reviewed by Ref. [1]) and since the 1990's in the mammalian brain (reviewed by Refs. [2,3]).

Brain metabolites of neuropathological importance include glutamate (GLU), which mediates most of the excitatory neurotransmission, and glutamine (GLN) which is closely linked to glutamate in the glutamine/glutamate "cycle". Fig. 1 shows schematically the major metabolic and transport pathways of GLU and GLN in the neuron, the glia, and the extracellular compartment of the brain. The neurotransmitter GLU (GLU_{NT}) is released from presynaptic vesicles in axon terminals to the extracellular fluid (ECF) and binds to the receptor of the postsynaptic neuron for neurotransmission. It is then mainly taken up into the glia by the excitatory amino acid transporter subtype 2 (EAAT2) and metabolized to GLN by glia-specific glutamine synthetase (GS). The rapid clearance of the glutamate in the extracellular fluid (GLU_{ECF}) from the synaptic space prevents the excessive stimulation of the GLU receptor (GLU excitotoxicity) which is implicated in a wide range of neurological disorders [4–6]. GLN is then transported from glia to the extracellular fluid by the sodium-coupled neutral amino acid transporter subtype 3 (SNAT3) [7,8] and possibly by the subtype 5 (SNAT5) (Section 3.1.1). Glutamine in the extracellular fluid (GLN_{ECF}) is taken up into neurons by the sodium-coupled neutral amino acid transporter subtypes 1 and 2 (SNAT1 and 2) [9,10]. (Corresponding earlier nomenclatures for the transporters are given in Fig. 1 caption). Neuronal GLN is then hydrolyzed, by phosphate-activated glutaminase (GLNase), to GLU. According to the concept of the glutamine/glutamate cycle [11,12], this completes the recycling of GLU from GLN. An aminotransferase, viz. branched-chain aminotransferase (BCAT), also contributes to GLU

Abbreviations: BCAA, branched-chain amino acid; BCAT, branched-chain aminotransferase; EAAT2, excitatory amino acid transporter subtype 2; ECF, extracellular fluid; GDH, glutamate dehydrogenase; GLN, glutamine; GLNase, glutaminase; GLN_{ECF}, glutamine in the extracellular fluid; GLU, glutamate; GLU_{ECF}, glutamate in the extracellular fluid; GLU_{NT}, neurotransmitter glutamate; GS, glutamine synthetase; HE, hepatic encephalopathy; HMQC, heteronuclear multiple-quantum coherence; HSQC, heteronuclear single-quantum correlation; ISIS, image-selected in vivo spectroscopy; LEU, leucine; MRS, magnetic resonance spectroscopy; MSO, L-methionine-DL-sulfoximine; NOE, nuclear Overhauser effect; NOEF, nuclear Overhauser enhancement factor; SNAT1,2,3,5, sodium-coupled neutral amino acid transporter subtypes 1,2,3,5; T_1, spin lattice relaxation time.

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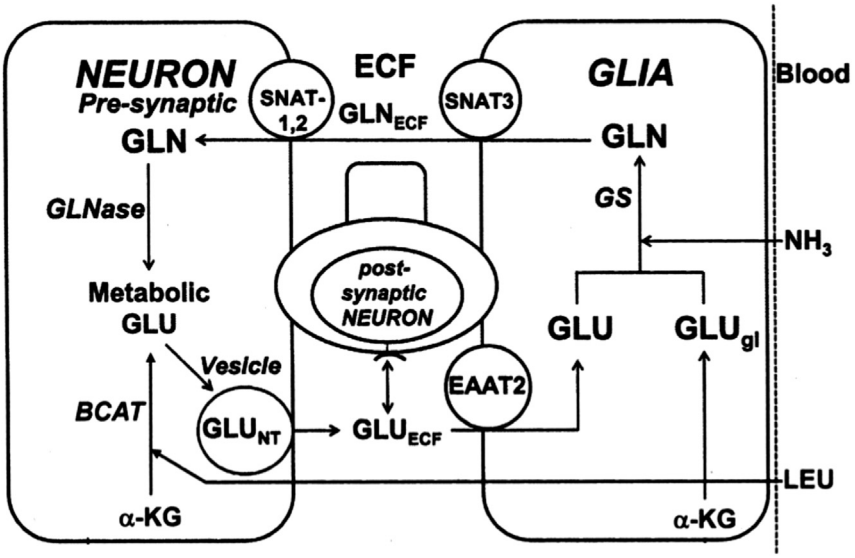

Fig. 1. Major metabolic and transport pathways that contribute to glutamate flux in the brain. α-KG, α-ketoglutarate; BCAT, branched-chain aminotransferase; EAAT2, excitatory amino acid transporter subtype 2; ECF, extracellular fluid; GLU, glutamate; GLU_{ECF}, GLU in the extracellular fluid; GLU_{gl}, glial GLU derived from α-KG; GLU_{NT}, neurotransmitter GLU; GLN, glutamine; GLN_{ECF}, GLN in the extracellular fluid; GLNase, glutaminase; GS, glutamine synthetase; LEU, leucine; SNAT1,2, sodium-coupled neutral amino acid transporter subtypes 1 and 2 (also called SAT1,2); SNAT3, subtype 3 (also called SN1).

synthesis from leucine [13–15]. These major pathways control the flux of the metabolic and neurotransmitter pools of GLU and involve the formation or transfer of amide and amine groups. Hence, non-invasive ^{15}N MRS makes a unique contribution to the kinetic studies of these individual pathways in vivo.

The classical study by Berl et al. [16] which examined ^{15}N labeling of brain glutamate and glutamine in $^{15}NH_4^+$-infused cat led to the important concept of compartmentation of glutamate metabolism in the brain: there is a small pool that rapidly turns over that is kinetically distinct from a larger pool that slowly turns over. Using ^{13}N, the positron-emitting isotope with a half-life of 10 min that can be easily quantified, Cooper et al. measured the brain uptake index of ammonia [17] and its incorporation into glutamine. While the ^{13}N studies clearly demonstrated the importance of glutamine synthetase in the metabolism of brain ammonia, these short-term (10–25 min) experiments did not provide a numerical estimate of the rate of glutamine synthesis in the brain (reviewed by Ref. [18]). Measurement of the in vivo glutamine synthetase activity in the rat brain using ^{15}N MRS was initiated in our laboratory at 4.7 T in the 1990s and then in other laboratories at higher field strengths (7 and 9.4 T) (Section 3.1).

2. Methodology of ^{15}N MRS

2.1. Characteristics of in vivo ^{15}N MRS

The most abundant isotope of nitrogen, ^{14}N (natural abundance 99.6%), has seldom been used for in vivo MRS because it has a nuclear spin quantum number I = 1 and hence a quadrupole moment, leading to rapid relaxation and broad signals in most molecules of biomedical importance. We will focus on ^{15}N which has a nuclear spin I = ½ and provides a MRS signal with a narrow linewidth. First, the characteristics of ^{15}N MRS (by direct ^{15}N detection) that are important for in vivo studies are described.

2.2. Direct ^{15}N detection

^{15}N has a high spectral resolution because of its broad chemical shift range (500–900 ppm) [19]. Its low natural-abundance (0.365%) is an advantage for in vivo MRS because only those metabolites that have been ^{15}N enriched by the intravenous infusion of a labelled precursor are normally observed, without interference from naturally occurring ^{15}N. This permits the measurement of the fluxes of ^{15}N-metabolites upon a clean background. The sensitivity of ^{15}N is 1/1000th of that of ^{1}H and 1/15th of that of ^{13}C for equal numbers of nuclei at a constant field. However, proton-decoupling not only removes ^{15}N-^{1}H splitting, but can also lead to the enhancement of the ^{15}N signal by the nuclear Overhauser effect (NOE) for ^{15}N nuclei that relax via dipolar interaction with proton(s). Due to the negative gyromagnetic ratio of ^{15}N, the nuclear Overhauser enhancement factor (NOEF) is negative, with a theoretical maximum value of −4.93. This NOEF, when added to the original signal intensity (+1.0), results in an *inverted* and enhanced signal with a maximum NOE enhancement of −3.93 [19]. Near-maximum NOE is observed in many molecules of biomedical importance, such as GLU and GLN. The spin-lattice relaxation times, T_1, of these nitrogens are relatively short, < 5 s, permitting fairly rapid signal acquisition. Consequently, ^{15}N enriched brain metabolites in the 1–10 mM range can be observed with a time resolution of 5–30 min by proton-decoupled NOE-enhanced ^{15}N MRS in vivo at a magnetic field strength of 4.7 T (20 MHz for ^{15}N) [20], 7 T [21] and 9.4 T [22]. Demonstrated improvement in the sensitivity at 9.4 T is discussed in Section 3.1.2. In Fig. 2, the top spectrum shows an in vivo proton-decoupled NOE-enhanced ^{15}N spectrum of [5-^{15}N]GLN and [2-^{15}N]GLU/GLN in the rat brain acquired in 19 min during intravenous infusion of $^{15}NH_4Cl$ [20]. The time resolution permits the measurement of the reaction rates on the order of 0.1–10 μmol/g/h, which comprise the in vivo rates of many key reactions that control the flux of GLU.

2.3. Indirect ^{15}N detection through coupled ^{1}H

The sensitivity of ^{15}N detection is significantly enhanced by indirect detection through coupled ^{1}H by ^{1}H-^{15}N heteronuclear multiple-quantum coherence (HMQC) transfer MRS [23] and heteronuclear single-quantum correlation (HSQC) MRS [24]. These methods permit the selective detection of ^{1}H spin-coupled to ^{15}N if the proton is non-labile at physiological pH and temperature.