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MRS studies of neuroenergetics and glutamate/glutamine exchange in rats: Extensions to hyperammonemic models

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ABSTRACT

In vivo Magnetic Resonance Spectroscopy is a useful tool to characterize brain biochemistry as well as its alteration in a large number of major central nervous system diseases. The present review will focus on the study of the glutamate-glutamine cycle, an important biochemical pathway in excitatory neurotransmission, analyzed using *in vivo* MRS of different accessible nuclei: ^{1}H, ^{13}C, ^{15}N and ^{31}P. The different methodological aspects of data acquisition, processing and absolute quantification of the MRS data for each nucleus will be presented, as well as the description of the mathematical modeling approach to interpret the MRS measurements in terms of biochemical kinetics. The unique advantages of MRS, especially its non-invasive nature enabling longitudinal monitoring of brain disease progression and/or effect of treatment is illustrated in the particular context of hyperammonemic disorders with a specific focus on animal models. We review the current possibilities given by *in vivo* MRS to investigate some of the molecular mechanisms involved in hyperammonemic disorders and to give a better understanding of the process of development of hepatic encephalopathy, a severe neuropsychiatric disorder that frequently accompanies liver disease.

1. Introduction

Compared to magnetic resonance imaging (MRI), where the output consists of an image in a grey scale, MR spectroscopy (MRS) provides a spectrum as readout. This spectrum consists of peaks at different resonant frequencies (or resonances) representing signal intensities. Resonant frequency is often expressed in parts per million (ppm), which is the magnetic field independent frequency scale. When performing single voxel MRS, spectra are acquired from a well-defined single volume, usually positioned in a specific brain region. In spectroscopic imaging (MRSI), also called chemical shift imaging (CSI), spectra are simultaneously acquired from different brain regions and thus the spatial distribution of metabolites in various regions of the brain can be efficiently studied. After quantification of the entire set of acquired spectra, each acquired from different spatial positions (volumes), the result is usually displayed as a metabolic map (images of individual metabolite concentrations using a grey or colored scale from the lowest to the highest concentration). Spectroscopic imaging can be performed in rodents with μL spatial resolution, which is comparable in resolution to animal positron emission tomography (PET) [1], or even better.

In vivo MRS is a powerful and reliable diagnostic tool with unique advantages due to its non-invasiveness and consequently its possibility to be used in a longitudinal manner. It thus enables the monitoring of disease progression and/or effect of treatment, and makes a bridge between the clinical diagnostics and basic research. *In vivo* studies of energy metabolism and/or glutamate-glutamine cycle can be performed using different nuclei (^{1}H, ^{31}P, ^{13}C, ^{15}N, ^{17}O). These studies can be more or less complex depending on the nuclei under investigation. Fig. 1 shows an example of representative ^{1}H, ^{31}P, ^{13}C and ^{15}N spectra acquired *in vivo* in the rat brain. As can be seen, all these spectra look quite different. However, since the majority of organic compounds contain hydrogen, carbon and also nitrogen or phosphorus, these spectra often offer complementary information on the same metabolites by detecting different atoms in the molecule (e.g. phosphocreatine in ^{1}H and ^{31}P

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Abbreviations

ADP	adenosine diphosphate
AHP	adiabatic half passage
Ala	alanine
ALF	acute liver failure
AMARES	Advanced Method for Accurate, Robust, and Efficient Spectral fitting
Asc	ascorbate
Asp	aspartate
ATP	adenosine triphosphate
BBB	blood-brain-barrier
BDL	bile duct ligation
CK	creatine kinase
CLD	chronic liver disease
Cr	creatine
CRLB	Cramér-Rao lower bounds
CSI	chemical shift imaging
GABA	γ-aminobutyrate
Glc	glucose
Gln	glutamine
Glu	glutamate
GLUTs	glucose transporters
Gly	glycine
GPC	glycerophosphocholine
GS	glutamine synthetase
GSH	glutathione
HA	hyperammonemia
HE	hepatic encephalopathy
Ins	myo-inositol
Lac	lactate
MCTs	monocarboxylate transporters
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRSI	magnetic resonance spectroscopic imaging
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
OVS	outer volume suppression
PAG	phosphate activated glutaminase
PC	Pyruvate carboxylase reaction
PCA	portocaval anastomosis
PCho	phosphocholine
PCr	phosphocreatine
PE	phosphoethanolamine
PET	positron emission tomography
PDH	pyruvate dehydrogenase complex reaction
Pi	inorganic phosphate
RF	radio-frequency
SNR	signal-to-noise ratio
Tau	taurine
TCA	tricarboxylic acid cycle
TE	echo time
TR	repetition time
VOI	volume of interest
WS	water suppression

spectra; glutamate and glutamine in ^{1}H and ^{13}C spectra). Moreover, all these spectra were acquired using different acquisition parameters and setups, and consequently their processing/quantification will be different as will be described below. ^{1}H MRS enables the direct detection of an important number of markers of energy metabolism (lactate, glucose, alanine, phosphocreatine, creatine) and neurotransmitters (glutamate, aspartate, glycine, γ-aminobutyrate, N-acetylaspartylglutamic acid). ^{31}P MRS provides information about energetically important metabolites as the three phosphates of adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi) and some chemical reaction rates between them. ^{13}C and ^{15}N MRS together with infusions of isotopically labeled substrates can be used to monitor the flow of the isotope of interest into different metabolic intermediates that will appear in the spectrum. ^{13}C MRS offers the possibility to study non-invasively the fluxes through the tricarboxylic acid cycle and glutamine-glutamate cycle *in vivo*. ^{15}N MRS is an alternative approach to ^{13}C MRS to study glutamate-glutamine metabolism. Finally, ^{17}O MRS is also used to measure energy metabolism and is presented in a separate review within this special issue.

A large number of central nervous system pathological states can be characterized using MRS, e.g. brain tumors, demyelinating and neurodegenerative diseases, epilepsy, acute stroke, brain ischemia, infectious brain lesions as well as neonatal and pediatric disorders (hypoxia-ischemia, inherited metabolic diseases, and traumatic brain injury) [2]. The focus of the present review will be on some acquired hyperammonemic disorders [3,4] and on the usefulness of MRS (^{1}H, ^{13}C, ^{15}N and ^{31}P) to investigate *in vivo* some of the molecular mechanisms involved in this type of disease in animal models.

Hyperammonemia (HA) can lead to hepatic encephalopathy (HE), a neuropsychiatric disorder that frequently accompanies severe liver disease (acute or chronic). Increasing evidence points to high blood and brain ammonia concentrations together with its metabolic product, glutamine, as key factors involved in the pathogenesis of HE [3,5–19]. Normally, ammonia is maintained at low levels by the liver (kidney and muscles can also contribute [20]), as excess ammonia is toxic to central nervous system [11,16]. Cerebral ammonia removal relies on formation of glutamine by glutamine synthetase (GS) in astrocytes [14] (unique astrocytic expression of GS [6,21]), leading to astrocytes swelling and brain edema in hyperammonemic cases. Despite important amount of research in the field, the precise mechanisms and their relative contributions to the chronological events leading to astrocytes swelling, brain edema and neurological alterations are very complex and not yet fully elucidated. These mechanisms are only beginning nowadays to be understood. Studies, performed mainly on animal models (*in vivo* or *ex vivo*) or in cell cultures, showed that the main pathogenic mechanisms involved in HE are: amino acids disturbances (i.e. glutamine increase); alterations in neurotransmission/neurotransmitters (i.e. glutamate, γ-aminobutyrate changes); cerebral energy disturbance (i.e. ATP, creatine, phosphocreatine, lactate modifications); alteration of nitric oxide synthesis and oxidative stress which leads to induction of the mitochondrial permeability transition; impairment of axonal and dendritic growth during brain development; signaling transduction pathways; alterations in channels and transporters activity [8,11,14,20,22–25].

To date, MRS (mainly ^{1}H MRS) was successfully used *in vivo* to investigate and monitor acquired hyperammonemic disorders in humans and animal models [3,7,22,26–39]. The main finding of