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Amide proton signals as pH indicator for *in vivo* MRS and MRI of the brain—Responses to hypercapnia and hypothermia

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ABSTRACT

Using proton MRS and MRI of mouse brain at 9.4 T, this work provides the first *in vivo* evidence of pH-dependent concurrent changes of three amide signals and related metabolic responses to hypercapnia and hypothermia. During hypercapnia, amide proton MRS signals of glutamine at 6.8–6.9 ppm and 7.6 ppm as well as of unspecific compounds at 8.1–8.3 ppm increase by at least 50% both at 37 °C and 22 °C. These changes reflect a reduced proton exchange with water. They are strongly correlated with intracellular pH which ranges from 6.75 ± 0.10 to 7.13 ± 0.06 as determined from a shift in creatine phosphokinase equilibrium. In MRI, saturation transfer from aliphatic as well as aromatic and/or amide protons alters slightly during hypercapnia and significantly during hypothermia. The asymmetry in magnetization transfer ratios decreased slightly during hypercapnia and hypothermia. Regardless of pH or temperature, saturation transfer from aliphatic protons between −2 and −4 ppm frequency offset to water protons is significantly greater than that from aromatic/amide protons at corresponding offsets between +2 and +4 ppm. Irradiation of aliphatic compounds at −3.5 ppm frequency offset from water predominantly saturates lipids and water associated with myelin. Taken together, the results indicate that, for the B_1 power used in this study, dipolar coupling between aliphatic and water protons rather than proton exchange is the dominant factor in Z-spectra and magnetization transfer ratio asymmetry of the brain *in vivo*.

Introduction

Magnetic resonance (MR) spectroscopy (MRS) and imaging (MRI) play important roles in translational biomedical research and in particular allow for a biochemical phenotyping of the brain. Complementing the neurochemical profiling by *in vivo* MRS, there have been manifold investigations of pH dependencies, mainly with the use of ^{31}P MRS. Exploiting the higher sensitivity of ^{1}H MRS at 4.7 T, amide signals in brain spectra were found to change with pH, because they exchange protons with water in base-catalyzed reactions (Kanamori and Ross, 1997; Mori et al., 1998; Zhou and van Zijl, 2006). While two amide resonances of glutamine (Gln) at 6.8–7.6 ppm were studied by nuclear Overhauser enhancement (NOE) through ^{15}N excitation after administration of exogenous $^{15}NH_4^+$ (Kanamori and Ross, 1997), amide signals of proteins and peptides around 8.3 ppm were identified by MRS sequences with minimized saturation transfer from water (Mori et al., 1998; Zhou and van Zijl, 2006).These base-catalyzed exchange reactions are also responsible for the contrast described in chemical exchange saturation transfer (CEST) MRI (Zhou and van Zijl, 2006).

Here, we report saturation transfer MRS and MRI studies of proton exchange in mouse brain *in vivo* at 9.4 T, which detect all aforementioned amide protons without the use of $^{15}NH_4^+$ or the need for special pulse sequences. At a high field of 9.4 T, the application of a common MRS technique with effective water pre-saturation yields a significant increase of amide signals, i.e., reduced amide–water proton exchange, in response to hypercapnia and hypothermia.

Materials and methods

Animals and anesthesia

A total of 11 female C57BL/6N mice (4–5 months, 23–29 g) were studied in accordance with German animal protection laws after approval by the responsible governmental authority. As shown in Fig. 1, 5 mice underwent MRS of the forebrain and acquisition of the Z-spectra before and during hypercapnia at 37 °C (protocol 1-1)

Abbreviations: ADP, adenosine diphosphate; Ala, alanine; ATP, adenosine triphosphate; CEST, chemical exchange saturation transfer; CHESS, chemical-shift selective; Cr, creatine; FLASH, fast low-angle shot; Glc, glucose; Gln, glutamine; Glu, glutamate; HC, homocarnosine; His, histidine; H_E and H_Z, amide protons of glutamine; Lac, lactate; MR, magnetic resonance; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MTR, magnetization transfer ratio; MTR_{asym}, magnetization transfer ratio asymmetry; NAA, *N*-acetylaspartate; -NH_X, unspecific amide protons; NOE, nuclear Overhauser enhancement; Phe, phenylalanine; PCr, phosphocreatine; R_1, longitudinal relaxation rate; RF, radiofrequency; STEAM, stimulated-echo acquisition mode; Tau, taurine; Trypt, tryptophan.

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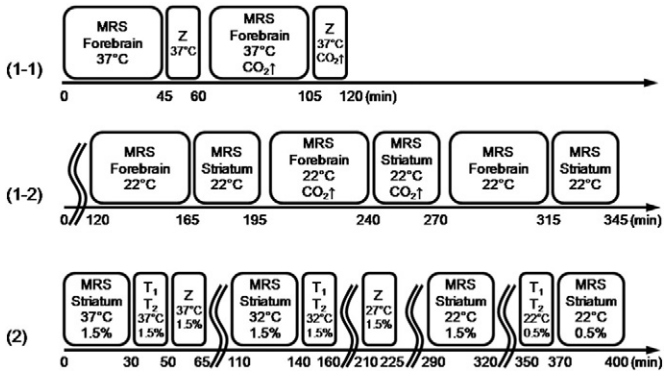

Fig. 1. Time course of three MRS and MRI protocols (Z-spectrum, T_1 and T_2 relaxation times) for two animal groups measured with or without hypercapnia ($CO_2\uparrow$) at different temperatures under 1.5% or 0.5% isoflurane anesthesia. Z = Z-spectrum. For details, see text.

followed by MRS of the forebrain and of the striatum before, during, and after hypercapnia at 22 °C one day later (protocols 1–2). Another 6 mice underwent MRS of the striatum, T_1, T_2, and/or Z-spectra measurements of the striatum at 37 °C ($n = 6$), 32 °C ($n = 4$), 27 °C ($n = 5$), and/or 22 °C ($n = 5$) under 1.5 or 0.5% isoflurane anesthesia (protocol 2). After induction of anesthesia with 5% isoflurane, animals were intubated with a purpose-built polyethylene endotracheal tube (0.58 mm inner diameter, 0.96 mm outer diameter) and artificially ventilated using an animal respirator (TSE, Bad Homberg, Germany) with a respiratory rate of 25 breaths per minute and an estimated tidal volume of 0.35 ml as previously described (Schulz et al., 2002; Watanabe et al., 2004; Boretius et al., 2013). The animals were then placed in a prone position on a purpose-built palate holder equipped with an adjustable nose cone. The Göttingen animal bed (Tammer et al., 2007) secured a reproducible and reliable fixation of the mouse head and receiver coil in the magnet isocenter. Respiratory movement of the abdomen as well as rectal temperature were monitored by a unit supplied by the manufacturer (Bruker Biospin MRI GmbH, Ettlingen, Germany). Thirty minutes after the rectal temperature reached 37 ± 1 °C, 32 ± 1 °C, 27 ± 1 °C, or 22 ± 1 °C with the use of a heating system (i.e., a water blanket and animal bed), the respective chemical shifts of the *N*-acetylaspartate (NAA) amide signal at 7.83–7.84, 7.87–7.88, 7.91–7.92, and 7.95–7.96 ppm confirmed the brain temperature to be within the target range (Arús et al., 1985). MRS and MRI data were acquired only after the equilibrium was established.

MRS

At 9.4 T (Bruker Biospin MRI GmbH, Ettlingen, Germany), localized proton MRS (STEAM, TR/TE/TM = 6000/10/10 ms) was performed with the use of a birdcage resonator (inner diameter 70 mm) and a saddle-shaped quadrature surface coil (both Bruker Biospin MRI GmbH, Ettlingen, Germany) on anesthetized mice as previously described (Boretius et al., 2013). A $(40\ mm)^3$ cubic volume-of-interest was centered on the forebrain or a $(20\ mm)^3$ volume-of-interest on the right striatum (Boretius et al., 2013). Water saturation was accomplished by means of three Gaussian-shaped CHESS radiofrequency (RF) pulses (90°–90°–180°), each of which with a duration of 7.83 ms and a bandwidth of 350 Hz. The overall duration of the CHESS module was 147 ms. Each CHESS pulse was followed by an associated spoiler gradient and a 37 ms outer volume saturation module covering a range of 3 mm around the volume-of-interest without gap.

Metabolite quantification involved spectral evaluation by LCModel (Provencher, 1993) and calibration with brain water concentration of 79% (Duarte et al., 2014), for which the unsuppressed water proton signal served as internal reference. Amide signal intensities of different compounds were normalized to and expressed as those of NAA in each spectrum. Intraindividual variations in NAA amide signal intensities during hypercapnia were corrected for variations in concentration by normalization with NAA as determined by LCModel. With TE = 10 ms, the unsuppressed water signal was assumed to be attenuated by 22% between the temperatures at 37 °C and 22 °C, because T_2 relaxation times of water protons in the $(20\ mm)^3$ volume-of-interest were determined by a multi-echo spin-echo MRI (TR/TE = 2500/10–123 ms) to be 41.0 ± 0.6 ms at 37 °C and 40.0 ± 1.5 ms at 22 °C ($n = 5$). T_1 relaxation times were determined with the use of a spin-echo saturation recovery sequence and 7 TR values from 0.15 to 6 s. Metabolites with Cramer–Rao lower bounds above 20% were excluded from further analysis. Significant differences between two groups of data were determined by a Student's two-sided *t*-test as well as by the Wilcoxon's signed rank (for paired comparisons) or rank sum (for unpaired comparisons) test. *p* values for the non-parametric tests are given as *p′*. The standard general linear model was fitted to examine a linear relationship between two variables because the animal effects did not account for a meaningful amount of variance in the random effects when the linear mixed-effects model was fitted (SPSS® version 21.0, IBM®).

In addition, for validating the source of observed MRS signals at 6.81–6.9 ppm and 7.6 ppm in brain *in vivo* as Gln as well as their dependence on pH, 10–100 mM Gln, Cr, or PCr (SERVA Feinbiochemica, Heidelberg, or Sigma-Aldrich Chemie, Taufkirchen, Germany) in 0.2 M sodium phosphate buffer with pH 6.2–7.4 was measured using localized MRS (STEAM, TR/TE/TM = 10,000–15,000/10/10 ms) at 16–37 °C.

pH estimation

Intracellular pH in brain tissue was estimated from the creatine phosphokinase equilibrium: $ATP + Cr \leftrightarrow ADP + PCr + H^+$ yielding $[H^+] = ([ATP] \times [Cr] \times K')/([ADP] \times [PCr])$. [ATP]/[ADP] is calculated to 11.47 with the equilibrium constant $K' = 7.09 \times 10^{-9}$ at 37 °C taken from the literature (Siesjö et al., 1972; Kass and Lipton, 1982; Whittingham et al., 1984). For the exothermic reaction, K' is corrected with $\Delta H = -1550$ cal/mol (Noda et al., 1954; Ohlmeyer, 1946) according to the van't Hoff equation yielding $K' = 7.39 \times 10^{-9}$ at 32 °C and 8.06×10^{-9} at 22 °C. The tissue concentrations of ATP and ADP are known to be unaffected by the type of anesthesia, hypercapnia, or hypothermia (Folbergrová et al., 1972; Hägerdal et al., 1975; Nilsson and Siesjö, 1974; Siesjö et al., 1972).

MRI

For both MRI and MRS, shimming of the B_0 field was carried out by FASTMAP (Gruetter, 1993). For measurements of S_{sat}/S_0, i.e., Z-spectrum (Zhou and van Zijl, 2006), an off-resonance Gaussian-shaped RF pulse with a duration of 12 ms and a flip angle of 180° (2.3 μT) was incorporated into a spin density-weighted gradient-echo MRI sequence (RF-spoiled 3D FLASH, TR/TE = 24/4.5 ms, flip angle 5°, field-of-view $(16\ mm)^3$, matrix 64 × 64 × 32, measuring time 48 s, duty cycle 50%) at a resolution of $250 \times 250 \times 500\ \mu m^3$ (Supplementary Fig. 1). The duration and power of the off-resonance pulse was optimized to observe the transfer of saturation from non-water protons to water protons in brain of mice *in vivo* (Natt et al., 2003; Watanabe et al., 2012). A stronger off-resonance irradiation, in particular at small frequency offset, would unfavorably increase a direct saturation of water protons and therefore was not used. High-resolution MTR maps were obtained from acquisitions with and without off-resonance irradiation at a resolution of $120^2 \times 600\ \mu m^3$ (TR/TE = 30/7.6 ms, flip angle 5°, field-of-view $15.4 \times 15.4 \times 19.2\ mm^3$, matrix 128 × 128 × 32, measuring time 8 min, $n = 3$). For the evaluation of MRI signal intensities, square-shaped regions of interest with 36 pixels were selected in the striatum (Supplementary Fig. 1) in a standardized manner. The analysis followed a strategy previously developed for intraindividual comparisons of MR images obtained after manganese administration (Watanabe et al., 2004).