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Metabolic alterations in focally activated primary somatosensory cortex of α-chloralose-anesthetized rats measured by ^{1}H MRS at 11.7 T

Su Xu, Jehoon Yang, Charles Q. Li, Wenjun Zhu, and Jun Shen*

Molecular Imaging Branch, National Institute of Mental Health, Building 10, Room 2D51A, 9000 Rockville Pike, Bethesda, MD 20892, USA

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Previously, magnetic resonance spectroscopy studies of alterations in cerebral metabolite concentration during functional activation have been focused on phosphocreatine using ^{31}P MRS and lactate using ^{1}H MRS with controversial results. Recently, significant improvements on the spectral resolution and sensitivity of in vivo spectroscopy have been made at ultrahigh magnetic field strength. Using highly resolved localized short-TE ^{1}H MRS at 11.7 T, we report metabolic responses of rat somatosensory cortex to forepaw stimulation in α-chloralose-anesthetized rats. The phosphocreatine/creatine ratio was found to be significantly decreased by 15.1 ± 4.6% (mean ± SEM, $P < 0.01$). Lactate remained very low (∼<0.3 μmol/g w/w) with no statistically significant changes observed during forepaw stimulation at a temporal resolution of 10.7 min. An increase in glutamine and a decrease in glutamate and *myo*-inositol were also detected in the stimulated state. Our results suggest that, under the experimental conditions used in this study, increased energy consumption due to focal activation causes a shift in the creatine kinase reaction towards the direction of adenosine triphosphate production. At the same time, metabolic matching prevails during increased energy consumption with no significant increase in the glycolytic product lactate in the focally activated primary somatosensory cortex of α-chloralose-anesthetized rats.
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Introduction

The metabolic events accompanying neuronal activities can be measured in vivo using functional magnetic resonance spectroscopy (MRS). Previously, MRS studies of alterations in cerebral metabolite concentration due to functional activation have been focused on phosphocreatine (PCr) using ^{31}P MRS and lactate (Lac) using ^{1}H MRS. Many studies using ^{31}P MRS techniques to investigate the response of PCr to photic stimulation of human subjects have shown that focal brain activation causes a decrease in PCr, consistent with the role of PCr as a reservoir of high energy phosphates. For example, Sappey-Marinier et al. (1992) reported that PCr/P_i (inorganic phosphate) ratio in the visual cortex decreased from 100 ± 9% to 59 ± 9% during a 12.8-min photic stimulation of healthy subjects at 1.5 T. The reduction in PCr during photic stimulation of human subjects has since been qualitatively confirmed by others (Kato et al., 1996; Rango et al., 1997; Murashita et al., 2000). In a study of monkey brain subjected to visual stimulation using ^{31}P spectroscopic imaging, PCr in visual cortex was reported to be decreased in two out of the four experiments performed (Mora et al., 1991). Rango et al. (2001) reported no changes in high energy phosphates in the visual cortex of human brain during continuous photic stimulation at 1.5 T, in contrast to their earlier work which found a decreased PCr using a short stimulation paradigm (Rango et al., 1997). A 4-T photic stimulation study (Chen et al., 1997) demonstrated that the pseudo first-order rate constant of the creatine kinase reaction in the direction of adenosine triphosphate (ATP) synthesis from PCr (k_f) increased by 34% in the human visual cortex without a statistically significant change in the concentration of PCr. With improved sensitivity at the high magnetic field strength of 7 T and using a three-dimensional ^{31}P chemical shift imaging technique, the same group found a 4% statistically significant decrease in PCr signal intensity in the visual cortex of human subjects during photic stimulation (Lei et al., 2004).

Several groups have reported that visual (Prichard et al., 1991; Sappey-Marinier et al., 1992; Frahm et al., 1996), somatosensory (Ueki et al., 1988; Madsen et al., 1999), motor (Kuwabara et al., 1995), and cognitive stimuli (Urrila et al., 2003) cause a significant increase in the brain Lac level in the activated region, which has been interpreted as the evidence for uncoupling between glucose (Glc) and oxygen consumption (Fox et al., 1988). However, no increase in Lac concentration (Merboldt et al., 1992; Etta et al., 1994; Boucard et al., 2005) or a very brief decrease in Lac concentration (Mangia et al.,

* Corresponding author. Fax: +1 301 480 2397.
E-mail address: shenj@intra.nimh.nih.gov (J. Shen).
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2003) has also been reported using the photic stimulation paradigm. A previous visual stimulation study of α-chloralose-anesthetized cats (Kauppinen et al., 1997) found no accumulation of Lac using short-TE ^{1}H MRS at 4.7 T even when tissue oxygenation was reduced by approximately 50%. In contrast, study of α-chloralose-anesthetized rats using bioluminescence (Ueki et al., 1988) has found a significant increase in Lac-induced bioluminescence as a result of forepaw stimulation. A 30% increase in Lac-induced bioluminescence was measured in the center of the activated region with highest increase of cerebral blood flow and Glc utilization.

Recently, significant improvements on the spectral resolution and sensitivity of in vivo spectroscopy have been made at very high magnetic field (e.g., Shen et al., 2004a,b). In the short-TE ^{1}H spectra of rat neocortex acquired at 11.7 T, although the PCr and Cr methyl protons overlap with each other at 3.02 ppm, the PCr methylene peak at 3.93 ppm and the Cr methylene peak at 3.91 ppm are spectrally resolved, allowing detection of alterations in PCr to Cr ratio (PCr/Cr) with the sensitivity of ^{1}H MRS. Using highly effective outer volume suppression, the Lac methyl signal at 1.32 ppm, which is spectrally resolved from macromolecule signals resonating in the aliphatic region, can also be unequivocally measured without contamination from subcutaneous lipid signals. Excellent water suppression was also achieved (Shen et al., 2004a,b). Using the highly resolved ^{1}H MRS at 11.7 T, we report metabolic responses of rat somatosensory cortex to forepaw stimulation in α-chloralose-anesthetized rats. α-Chloralose was chosen for anesthesia because it affects metabolic responses to somatosensory stimulation much less than halothane or barbiturates. More importantly, it maintains the coupling between cerebral blood flow and metabolism (Ueki et al., 1988), allowing detection of blood oxygenation level-dependent (BOLD) signals in the primary somatosensory cortex due to electrical stimulation of forepaws (e.g., Hyder et al., 1994; Gyngell et al., 1996; Silva et al., 1999). In addition to PCr/Cr and Lac, the resolution achieved at 11.7 T allows spectral separation of many other metabolites including the separation of glutamate (Glu) H-4 methylene protons at 2.35 ppm from the glutamine (Gln) H-4 methylene protons at 2.46 ppm. In this study using ^{1}H MRS at 11.7 T and α-chloralose-anesthetized rats, the decrease in PCr/Cr caused by functional stimulation was unambiguously demonstrated. Despite significant variations in Lac concentration between individual rats, it remained below ~0.3 μmol/g w/w with no statistically significant accumulation observed during forepaw stimulation. An increase in Gln and decreases in Glu and *myo*-inositol (mI) were also detected in the stimulated rat primary somatosensory cortex.

Materials and methods

Animal preparation

In vivo experiments were performed according to procedures approved by the Institutional Review Board's Animal Care and Use Committee of the National Institute of Mental Health. Male adult Sprague–Dawley rats (170–210 g, n = 12) were used for the forepaw stimulation experiments. The rats were anesthetized by a gas mixture of O_2, N_2O, and isoflurane (O_2:N_2O = 3:7, isoflurane: 1.5%). The animals were orally intubated. A femoral artery was cannulated for monitoring arterial blood gases (pO_2, pCO_2), pH, and arterial blood pressure. A femoral vein was cannulated for administration of anesthetics. Cannulation was performed while the rat was under isoflurane anesthesia. After surgery, the rat was placed supine in a homemade head holder and fixed by means of a bite bar and two ear pins. Then, anesthesia was switched to and maintained with intravenous infusion of α-chloralose (an initial dose of 80 mg/kg followed by supplementary of doses 26.7 mg/kg/h throughout the experiment). Pancuronium bromide was administrated (2 mg/kg iv) for muscle relaxation. Arterial blood pressure, rectal temperature, end-tidal CO_2, tidal pressure of ventilation, and heart rate were monitored continuously. Oxygen saturation, pH, and pCO_2 were kept between 130 and 230, 7.25 and 7.35, and 26 and 38 mm Hg, respectively. The body temperature was maintained at 37 ± 0.5°C using an external pump for water circulation (BayVoltex, Modesto, CA). Both forepaws were stimulated through needles inserted in between digits 1, 2, and 3, 4 under the skin, respectively. The needles were connected to an external electrical stimulator (GRASS Astro-Med, Inc., West Warwick, RI) that generated square pulses (2 mA, 0.3 ms, 3 Hz per forepaw) (Hyder et al., 1994; Marota et al., 1999; Silva et al., 1999) for electrical stimulation of the forepaws. This stimulation paradigm has been shown to sustain an elevated BOLD response over ~1 h continuous stimulation (e.g., Hyder et al., 1996).

Functional magnetic resonance imaging (fMRI) and MRS methods

All NMR experiments were performed on a Bruker 11.7 T AVANCE microimaging spectrometer (Bruker, Billerica, MA) equipped with an 89-mm inner diameter vertical bore magnet (Magnex Scientific, Abingdon, UK) and a 57-mm inner diameter gradient insert with a maximum gradient strength of 3 G/mm at a rise time of 100 μs. A ^{1}H surface RF coil of 15-mm diameter was used for transmitting and receiving radiofrequency signals at 500.14 MHz. It was positioned ~0–1 mm posterior to bregma. The probe was rotated inside the magnet from superior to left by ~30° to allow positioning of a non-oblique spectroscopy voxel at the location of the primary somatosensory cortex. Three-slice (coronal, horizontal, and mid-sagittal) scout rapid acquisition with relaxation enhancement (RARE) images (field of view (FOV) = 2.5 cm, slice thickness = 1 mm, repetition time (TR)/echo time (TE) = 200/15 ms, rare factor = 8, 128 × 128 data matrix) were used to localize the volume of interest (VOI). Adjustment of all first- and second-order shims was accomplished with a fully automatic procedure described previously (Chen et al., 2004). Typically, the in vivo shimming procedure resulted in a ~10-Hz half-height line width from the PCr/Cr methyl group at 3.02 ppm acquired from a 3.5 × 2.0 × 4.5 mm^3 (31.5 μl) spectroscopy voxel placed at the location of the primary somatosensory cortex in the right hemisphere. The timing diagram of the pulse sequence was shown in Fig. 1. Outer-volume suppression (OVS) was achieved using 90° hyperbolic secant pulses (nominal 90° flip angle, 2 ms, μ = 5, 1% truncation) along the x (10-mm slab), $-x$ (10-mm slab), y (3-mm slab), $-y$ (5-mm slab), z (10-mm slab), and $-z$ (10-mm slab) directions. Water suppression was accomplished using interleaved nominally 90° sinc and nominally 90° Gaussian pulses based on the chemical shift-selective (CHESS) scheme. The CHESS and OVS pulses were interleaved and