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Quantitative imaging of energy expenditure in human brain

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ABSTRACT

Despite the essential role of the brain energy generated from ATP hydrolysis in supporting cortical neuronal activity and brain function, it is challenging to noninvasively image and directly quantify the energy expenditure in the human brain. In this study, we applied an advanced *in vivo* ^{31}P MRS imaging approach to obtain regional cerebral metabolic rates of high-energy phosphate reactions catalyzed by ATPase (CMR_{ATPase}) and creatine kinase (CMR_{CK}), and to determine CMR_{ATPase} and CMR_{CK} in pure gray mater (GM) and white mater (WM), respectively. It was found that both ATPase and CK rates are three times higher in GM than WM; and CMR_{CK} is seven times higher than CMR_{ATPase} in GM and WM. Among the total brain ATP consumption in the human cortical GM and WM, 77% of them are used by GM in which approximately 96% is by neurons. A single cortical neuron utilizes approximately 4.7 billion ATPs per second in a resting human brain. This study demonstrates the unique utility of *in vivo* ^{31}P MRS imaging modality for direct imaging of brain energy generated from ATP hydrolysis, and provides new insights into the human brain energetics and its role in supporting neuronal activity and brain function.

Introduction

Cellular energy metabolism is a fundamental process that underlies and enables all the biological functions within a living organism. Adenosine triphosphate (ATP), which mainly formed in mitochondria through coupled electron transport chain and F_1F_0-ATPase (ATPase) enzyme activities (*i.e.*, the oxidative phosphorylation process), provides the chemical energy for virtually all energy-dependent cellular processes. In the brain, a majority of energy generated from ATP hydrolysis is utilized in the cytosol for supporting ongoing neuronal activity at resting state and elevated neuronal activity during a working state (Attwell and Laughlin, 2001; Erecinska and Silver, 1989; Raichle and Mintun, 2006; Shulman et al., 2004). Therefore, determining the rate of ATP hydrolysis is critical for understanding the bioenergetics of human brain at both normal and diseased conditions.

The energy demand in the human brain is not uniform and it is expected to be higher in the cortical gray matter (GM) than that of white matter (WM) based on the fundamental premise that GM tissue, characterized with high populations of synapses and mitochondria and intensive neuronal activity, needs more energy compared to the WM even though WM is intricately involved in signal transduction. Direct experimental verification of this premise, however, is limited by the availability of quantitative neuroimaging approaches capable of directly and noninvasively assessing the ATP metabolism and its rate with adequate spatial resolution for differentiating GM and WM in the human brain.

So far, most studies aiming for understanding the human brain energetics were commonly based on indirect neuroimaging measurements of cerebral metabolic rate of glucose (CMR_{glc}) or oxygen ($CMRO_2$) consumption for estimating the ATP utilization rate in the brains. These estimations rely on two hypothetical parameters: the molar ratio of oxygen consumption to glucose uptake (*i.e.*, the oxygen–glucose index OGI) and the cerebral ATP synthesis to oxygen consumption ratio (P/O ratio). However, large uncertainties in determining the actual values of the OGI and P/O ratios in the human brain could result in substantial errors in estimating the cerebral metabolic rate of ATP. Theoretically, one glucose molecule will react with six

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; PCr, phosphocreatine; Pi, inorganic phosphate; HEP, high-energy phosphate; ATPase, F_1F_0-ATPase; CK, creatine kinase; MRS, magnetic resonance spectroscopy; MT, magnetization transfer; CMR_{ATPase}, forward ATPase reaction flux, also define as cerebral metabolic rate of ATPase reaction; CMR_{CK}, forward CK reaction flux, also define as cerebral metabolic rate of CK reaction.

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oxygen molecules through oxidative metabolism and form 36 ATP molecules, leading to an OGI of 6 and P/O ratio of 3. Nevertheless, the OGI value measured by positron emission tomography (PET) in the healthy human brain at resting state was lower than 6 and also not uniform across the brain. For example, the OGI was reported to be ranging from 4.5 to 5.9 at different human brain regions (Vaishnavi et al., 2010), and it was also reported to be 4.1 in the human visual cortex at rest and dropped to 2.8 during visual stimulation (Fox et al., 1988). Moreover, the P/O ratio has been reported to be ≤2.5 (Du et al., 2008; Hinkle, 2005; Kingsley-Hickman et al., 1987; Zhu et al., 2002) apparently due to substantial proton leaking in the mitochondria (Rolfe and Brown, 1997). Therefore, the ability to directly measure the cerebral metabolic rate of ATP production is essential for determining the true brain energy status associated with brain function or dysfunction. One available approach that in principle is capable of providing such information is the *in vivo* ^{31}P magnetic resonance (MR) spectroscopy (MRS) imaging.

In vivo ^{31}P MRS is a powerful tool for non-invasive detection of the intracellular contents of high-energy phosphate (HEP) compounds (*e.g.*, ATP and phosphocreatine (PCr)) and inorganic phosphate (Pi) in the brain (*e.g.*, Kemp, 2000; Zhu et al., 2009, and references therein). The concentrations of HEP and Pi or their ratio can alter under pathological conditions; yet in normal brain, ATP production and utilization appear to be tightly regulated to maintain the HEP and Pi levels, in particular the ATP level, fairly constant across a wide range of physiological states where the brain activity level and associated ATP turnover rate can vary substantially (Du et al., 2008). Therefore, for assessing the cerebral energy metabolism and its effect on the brain function in healthy human brain, the measurement of HEP and Pi contents *per se* is not as informative as that of ATP metabolic rates (Du et al., 2008). The latter can be obtained using the *in vivo* ^{31}P MRS approach in combination with the magnetization transfer (MT) preparation (Forsen, 1963), which allows simultaneous measurement of two important *unidirectional* ATP production rates associated with the creatine kinase (CK) (*i.e.*, PCr→ATP) and ATPase (*i.e.*, Pi→ATP) reactions in the brain, that are defined as cerebral metabolic rate of CK reaction (CMR_{CK}) and ATPase reaction (CMR_{ATPase}), respectively (Du et al., 2007; Lei et al., 2003a and references therein). Early attempts of using *in vivo* ^{31}P MRS-MT in the brain largely focused on measuring CMR_{CK} (Bottomley and Hardy, 1992; Shoubridge et al., 1982) given the relatively high intracellular PCr content and better MRS signal in the brain. The CK reaction plays only a supportive role in facilitating ATP metabolism. A direct measure of net ATP production in the brain cell should be obtained through CMR_{ATPase}. However, *in vivo* measurement of CMR_{ATPase} faces a daunting challenge owing to a low intracellular Pi concentration (~1 mM) and limited Pi signal change induced by the MT and chemical exchange effects in the brain, a major signal source for measuring the value of CMR_{ATPase}.

Recently, with the advancement in high-field MRI/MRS technology and relevant methodology development, it has been shown that CMR_{ATPase} can be reliably measured in the human occipital lobe using the *in vivo* ^{31}P MRS-MT approach and a radio-frequency (RF) surface coil at 7 Tesla (T) (Du et al., 2007; Lei et al., 2003a). Moreover, the CMR_{ATPase} values obtained in both human and animal brains were found to reflect the mitochondrial oxidative phosphorylation rates (Chaumeil et al., 2009; Du et al., 2007; Lei et al., 2003a; Shoubridge et al., 1982), and are sensitive to the variation in the basal brain activity in animal models across a wide range of brain activity levels (Du et al., 2008). The previous human studies, however, measured CMR_{ATPase} from a small portion of the human brain located in the occipital lobe without spatial differentiation of GM and WM tissues (Du et al., 2007; Lei et al., 2003a).

In the present study, we have extended the utility of *in vivo* ^{31}P-MT approach by integrating three-dimensional (3D) chemical shift imaging (CSI), newly developed ^{1}H-^{31}P dual-frequency volume RF coil (Vaughan et al., 1994; Zhang et al., 2003) and a novel quantification strategy (Xiong et al., 2011) to quantitatively determine regional CMR_{ATPase} values of the human brain *in vivo* at 7 T, ultimately, to: i) quantify and differentiate the cerebral ATP production rates in the gray and white matters of the human brain; ii) determine the energy expenditure differences between cortical GM and WM, and between neuron and non-neuronal cells; and iii) estimate the number of ATP molecules utilized per second by a single cortical neuron in a resting human brain.

Materials and methods

Human subject

Seven healthy volunteers (ages 20 to 34 years) from the University of Minnesota and local communities were recruited for the study. The Institutional Review Board at the University of Minnesota approved all procedures, and written informed consents were obtained from all subjects.

MRI/MRS scanner and RF coil

All studies were performed on a 7 T/90 cm magnet (Magnex Scientific, Abingdon, U.K.) interfaced with a Varian (Varian Inc., Palo Alto, CA, USA) INOVA console. A circular-polarized ^{1}H-^{31}P double-tuned TEM volume RF coil was constructed (Vaughan et al., 1994; Zhang et al., 2003). For the ^{31}P and ^{1}H measurements, the averaged RF power delivered through the coils was monitored and controlled at well below the FDA specific absorption rate (SAR) limit.

^{1}H MRI and brain tissue segmentation

Anatomic images were obtained by T_1-weighted multi-slice Turbo-FLASH sequence (Haase et al., 1986) (inversion-recovery time: 1.2 s, repetition and echo times: TR/TE = 8.8/3.9 ms; matrix size: 128×128; slice thickness: 5 mm) with whole brain coverage, and they were processed and segmented using the tools from the FMRIB (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain) Software Library (www.fmrib.ox.ac.uk/fsl). Non-brain (*e.g.*, scalp) regions were first removed using an automated brain extraction tool (BET) (Smith, 2002); and then three tissue types of gray matter, white matter and cerebrospinal fluid (CSF) were segmented using an automatic segmentation tool (FAST), which is based on a hidden Markov random field model and an associated Expectation-Maximization (EM) algorithm (Zhang et al., 2001). The fractional tissue contribution of gray-matter (f_{GM}) and white-matter (f_{WM}) to a particular ^{31}P-CSI voxel was calculated based on the corresponding segmented ^{1}H MRI data and the coordinates/dimensions of a selected ^{31}P-CSI voxel.

3D ^{31}P-MT CSI measurement

3D ^{31}P-MT CSI data were acquired using the Fourier Series Window (FSW) CSI technique (Hendrich et al., 1994) in which the k-space sampling is weighted according to the Fourier coefficients of a predetermined voxel shape, thus, the data was oversampled in central k-space with partial truncation of higher k-space lines. The following acquisition parameters were used: 5000 Hz spectral bandwidth; nominal excitation pulse flip angle of $\beta = 36°$ with a 500-μs hard pulse; 20×20×22 cm^3 field of view (FOV); 15×15×13 phase encodes; 1269 k-space lines; 3888 total scan number; 0.73 s TR; cylindrical voxel (circular shape on the transverse orientation and 2.4 cm diameter) with 10.9 ml actual (or 2.3 ml nominal) voxel size; and total acquisition time of 47.3 min per 3D CSI dataset. A frequency-selected saturation pulse train, constructed with multiple hyperbolic Sech pulses (pulse duration of 50 ms; bandwidth of 150 Hz) with varying amplitude according to the B_1 insensitive selective train to obliterate signal (BISTRO) scheme (de Graaf et al., 1996;