Study of Correlation between Brain Activity and ATP Metabolic Rates by means of ^{31}P Magnetization Transfer and Electroencephalograph Measurement

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Introduction

Increases in neuronal activity are accompanied by elevated rates of cerebral glucose and oxygen consumption (CMR_{glc}, $CMRO_2$, respectively), as well as increased cerebral blood flow (CBF) at the activated sites. The end result of glucose and oxygen consumption is the production of Adenosine-5'-triphosphate (ATP) by mitochondrial proton ATP_{ase}. ATP is the fundamental cellular energy currency, and its hydrolysis to Pi and ADP is coupled to all energy requiring process in the cell. ATP in muscle and brain tissue is also buffered by the phosphocreatine (PCr) pool through the creatine kinase (CK) reaction. In the brain, the majority of the ATP energy budget is spent for the restore the transmembrane sodium and potassium gradients that are diminished by neuronal firing employed in cell signaling. In view of the central role played by ATP metabolism in brain function, it is important to have methods that can be employed to measure rates of enzymes involved in ATP synthesis and hydrolysis *in vivo,* and utilize these methods to investigate the coupling between ATP turnover and brain activity. In this study, this goal was achieved using *in vivo* ^{31}P magnetization saturation transfer (ST) approach which was applied to determine the forward chemical exchange fluxes of CK and ATP_{ase} simultaneously and non-invasively at different states of brain activity attained by using different anesthesia chemicals and doses. The magnetic resonance (MR) measured fluxes were correlated to brain activity measured by EEG.

Methods

Animal Preparation: Male Sprague-Dawley rats (260-350g) were divided to two groups (each group has 9-12 rats). They were first anaesthetized by inhalation of 2% (vol-vol) isoflurane (*Iso*) in nitrous oxide/oxygen (3:2), then switch to α chloralose (*αch*: 40mg/kg bolus followed by constant infusion at 25mg/kg/h) or pentobarbital with two different doses (*Pen_30*: 30mg/kg bolus with 30mg/kg/h infusion rate; and *Pen_70*: after Pen_30 infusion rate increased to 70mg/kg/h) to control brain basal activity. *In vivo* ^{31}P ST experiments and EEG recordings were performed when the animal physiologic conditions approached steady states.

EEG Measurements: Two electrodes recorded EEG signal. One was put on the nose of rat serving as a reference and the tip of the another electrode was inserted into the cortex through a small hole on the skull (3mm deep, 3mm from bregma, 3mm lateral midline). A Shannon entropy method [1] analyzed EEG data and quantified the brain activity at varied brain states.

MRS Experiments: *In vivo* ^{31}P ST experiments were performed at the 9.4T horizontal animal magnet (Magnex Scientific, Abingdon, U.K.) interfaced with Unity INOVA console (Varian Inc., Palo Alto, CA). ^{31}P ST approaches were carried by means of frequency-selective saturation of γ-ATP [2-4] to determine simultaneously the unidirectional ATP synthesis flux from PCr to ATP and from inorganic phosphate (P_i) to ATP, referred to as the "forward direction" in each case.

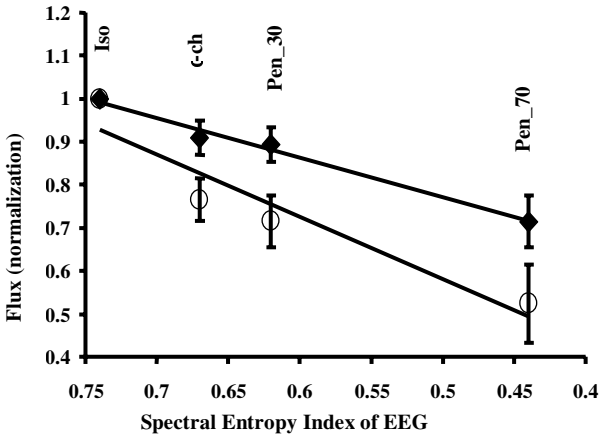

Fig.1 Correlations between averaged ATP fluxes of CK (upper line) and ATP_{ase} (lower line) and averaged spectral entropy index of EEG at different basal states in the rat brain. The EEG became silence (i.e., iso-electric state) with a spectral entropy index of EEG of 0.44±0.08 when the infusion rate of pentobarbital increased to 70mg/kg/h. The linear regression fitting gave a regression coefficient of R ≥0.95.

Results and Discussion

Figure 1 summarizes the correlations between the averaged rat brain activity (reported as EEG spectral Entropy) and the unidirectional ATP synthesis fluxes from PCr (upper line) and from P_i (lower line). While the PCr-to-ATP synthesis can be unequivocally assigned to the CK reaction, the P_i-to-ATP synthesis can have multiple contributions. However, the P_i-to-ATP flux determined by *in vivo* ^{31}P ST approach in the rat brain anesthetized with αchloralose was in good agreement with the net ATP synthesis rate estimated from the literature value of $CMRO_2$ using $P:O_2$=5~6 [6]; thus, the ^{31}P ST measurement of unidirectional flux from P_i-to-ATP is assigned to the net ATP synthesis by the mitochondrial proton ATP_{ase} through the oxidative phosphorylation process. The apparent unidirectional rate constants ($\mathbf{k_f}$), and unidirectional fluxes ($\mathbf{F_f}$, i.e., the multiplier of $\mathbf{k_f}$ and the related substance concentration) for both CK and ATP_{ase} reactions decreased when the brain activity was suppressed (reflected by the decreased spectral entropy index of EEG) by varying the anesthesia depth. Relative to the initial physiological state with light anesthesia (isoflurane, spectral entropy index of EEG = 0.74±0.06), $\mathbf{k_f}$ and $\mathbf{F_f}$ of CK decreased 20% and 28%, respectively, at iso-electric state (spectral entropy index of EEG = 0.44±0.08). In contrast, $\mathbf{k_f}$ and $\mathbf{F_f}$ of ATP_{ase} decreased 59% and 48%, respectively; the latter reduction is in good agreement with the 50% decrease in CMR_{glc} previously reported for iso-electric condition compared to an awaken state [5]. The findings from this study indicate that (i) coupling between brain activity and oxidative phosphorylation processes (and hence oxidative glucose consumption) can be measured *in vivo* in the brain; (ii) the ATP synthesis rates from PCr [7] and from P_i are tightly correlated to brain activity; (iii) the ATP synthesis rate via ATP_{ase} reaction decreased more than that via CK reaction with decreasing neuronal activity; (iv) at isoelectric point, ~50% oxidative ATP synthesis capacity remains; (v) "house keeping" processes and energy consumption related to neuronal signaling must each account for ~50% of ATP utilization in the brain and (vi) *in vivo* ^{31}P approach for noninvasively determining ATP metabolic rate would provide a useful modality for studying brain function and bioenergetics.

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