Direct and Noninvasive Measurement of Cerebral Metabolic Rate of ATP in Cat brain and its Physiological Implications

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INTRODUCTION Adenosine triphosphate (ATP) production and utilization are fundamental biochemical processes for supporting the energy needs of all living species. In the brain, majority of ATP is formed in mitochondria through the oxidative phosphorylation of ADP and then utilized in cytosol for sustaining brain activity and normal function. *In vivo* ^{31}P MRS in combine with the magnetization transfer (MT) approach had been applied to directly and non-invasively measure the cerebral metabolic rate of ATP (CMR_{ATP}) in human and rat brains at high field [1-3]. In principle, the cerebral ATP production (Pi → ATP) and utilization (ATP → Pi), with reaction rate constants of k_f and k_r, respectively, are regulated rigorously for maintaining a stable ATP level in the brain. The cerebral metabolic rate of ATP is defined as $CMR_{ATP} = k_f \times [Pi]$; and $k_r \times [ATP] = k_f \times [Pi]$ in normal resting brain [2]. In this study, we apply the same approach to measure CMR_{ATP} in the isoflurane anesthetized cat brain at 9.4T. The results of the CMR_{ATP} measurement have been evaluated by comparing the CMR_{ATP} values obtained with different magnetization transfer techniques. At normal physiological condition, there should be a tight coupling between the cerebral oxidative phosphorylation and oxygen utilization. If the CMR_{ATP} value measured by the *in vivo* ^{31}P MT experiment can truly reflect the oxidative phosphorylation rate, then it has to satisfy the simple relation of $CMR_{ATP} \approx 2 \times CMRO_2 \times$P:O ratio. Thus, we have compared the measured CMR_{ATP} with the estimated CMR_{ATP} derived from the $CMRO_2$ previously obtained from the same cat model [4] and the P:O ratio of 2.5 taken from the literature [5]. In addition, the experimentally determined CMR_{ATP} in cat, as well as that in rat and human brain [1-3], are correlated with their corresponding $CMRO_2$ values [4,6-8], respectively. This allows us for the first time to directly assess the P:O ratios in these three species *in vivo,* which would have profound physiological implications for understanding the dynamic relation of cerebral ATP metabolism, oxidative metabolism and brain function.

METHODS All ^{1}H images and ^{31}P spectra were acquired with a home-built ^{1}H-^{31}P dual RF surface coil probe on a 9.4T/30cm bore Magnex magnet equipped with Varian INOVA consoles. Female adolescent cats under gaseous anesthesia (0.9-1.2 % isoflurane in a mixture of 70% nitrous oxide and 30% oxygen) and artificial ventilation were used for this study. Progressive saturation of γ-ATP with varied saturation time (t_{sat} ranging from 0.5 to 9.8s) under fully relaxed condition using an adequate pre-saturation delay (=12s) was performed for determining the intrinsic longitudinal relaxation time and the reaction rate constant k_f in cat brain (n=5). A 90° adiabatic pulse of 300 µs was used for spin excitation, and the BISTRO pulse train was used for frequency-selective saturation of γ-ATP; and for collecting *in vivo* ^{31}P MT spectra (spectral width=5,000 Hz and 64 averages). Steady-state MT experiments with multiple single-site saturation (MSS technique [2]) of γ-ATP, Pi and PCr, respectively, and a control (i.e. without saturation) were carried out on a separate group of cats (n=9) with TR of 16s and t_{sat} of 9.8s to explicitly determine the reaction rate constants of k_f and k_r. The ^{31}P spectra were processed with LB=10 Hz before Fourier transformation and analyzed using AMARES time domain spectra fitting algorithm in the JMRUI software package. All results were presented by mean±SD.

Table 1. Summarized results of reaction rate constant for Pi ↔ ATP and PCr ↔ ATP reactions determined in isoflurane anesthetized cat brain at 9.4T.

Pi ↔ ATP	*Progressive Saturation [a]*	*Steady-State Saturation [b]*	*Prediction from Unity [c]*
k_f (s^{-1})	0.11 ± 0.02	0.10 ± 0.02	---
k_r (s^{-1})	---	0.04 ± 0.01	0.04

PCr ↔ ATP	*Progressive Saturation [a]*	*Steady-State Saturation [b]*	*Prediction from Unity [c]*
k_f (s^{-1})	0.32 ± 0.02	0.27 ± 0.03	---
k_r (s^{-1})	---	0.37 ± 0.04	0.37

a. *T_1^{int} of 5.5s for Pi and 3.6s for PCr were obtained (n=5).*
b. *k_f and k_r were measured independently using the MSS technique (n=9).*
c. *k_r was determined using $k_r = k_f \cdot [Pi] / [ATP]$ and known k_f value.*

RESULTS AND DISCUSSION Table 1 summarizes the results of the Pi ↔ ATP reaction rate constant measurements using progressive and steady-state saturation techniques, respectively, as well as the predicted k_r value based on the unity relation of the forward and reverse reaction fluxes using the measured k_f and the absolute concentrations of Pi, ATP and PCr that were experimentally determined in a separate study ([Pi]=1.14±0.14mM, [PCr]=3.81±0.24mM and [γ-ATP]=2.81±0.11mM in 1% isoflurane anesthetized cat brain). The results in Table 1 indicate that the progressive saturation and the steady-state saturation techniques provide almost identical k_f values for the Pi → ATP reaction. These measures were used to determine the CMR_{ATP} value (= 6.5±1.2 µmol/g/min) in the cat brain. In addition, the rate constant for ATP utilization (k_r), i.e. the ATP → Pi reaction, determined by the direct measurement and prediction from the unity relation are also equivalent. Similar results were also obtained for the PCr ↔ ATP reaction and are included in Table 1. Therefore, the overall results demonstrate not only the validity of the *in vivo* ^{31}P MT techniques for studying ATP metabolism in cat brain, but more importantly, they also provide a reliable measurement of CMR_{ATP} *in vivo*.

There is a general consensus that a tight coupling between the cerebral oxidative phosphorylation and oxygen utilization exists under normal physiological condition, and the theoretical P:O ratio should be 3 although the value of P:O ratio as reported in the literature was close to 2.5 [5]. With the *in vivo* ^{31}P MT approaches established in our lab, we are able to directly measure CMR_{ATP} in cat brain in this study and in rat and human brains in previous studies [1-3]. We have also established an *in vivo* ^{17}O MRS/MRSI approach at high field for directly imaging $CMRO_2$ in anesthetized rat and cat brains [4,6]. Accordingly, we are able to experimentally assess the P:O ratio in a living brain using the *in vivo* ^{31}P and ^{17}O MRS methods. The correlations between the paired CMR_{ATP} and $CMRO_2$ values determined in resting rat, cat and human brains, respectively, are displayed in Fig. 1. The human brain $CMRO_2$ value is taken from the PET studies [7-8]. The green and yellow lines in Fig. 1define the P:O ratio zone between 2.5 (green line) and 3 (yellow line). It is clear that the paired $CMRO_2$-CMR_{ATP} values experimentally determined in rat, cat and human brain are closely resided within the P:O ratio range of ~2.5-3. This comparison result provides vital evidence supporting our hypothesis that CMR_{ATP} measured by *in vivo* ^{31}P MT techniques truly represents the net rate of oxidative phosphorylation, which dominates the ATP production in the brains.

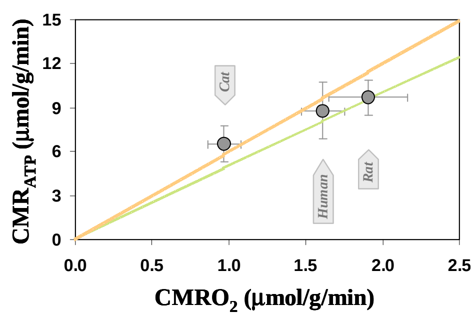

Fig. 1 Correlation of CMR_{ATP} with $CMRO_2$ in rat, cat and human brains. The yellow and green lines depict the P:O ratio value of 3 and 2.5, respectively.

CONCLUSION The *in vivo* ^{31}P MT techniques at high field allow us to directly and non-invasively measure the cerebral metabolic rates of ATP in anesthetized animal and awaked human brain. The CMR_{ATP} of the cat brain and the reliability of the measurement have been determined and evaluated in this study. The comparison between CMR_{ATP} and $CMRO_2$ obtained in the same brain region under the similar physiological condition offers a new way for directly assessing the P:O ratio *in vivo*. The results of such comparison in anesthetized rat and cat, and awaked human brains indicate that oxidative phosphorylation indeed dominates the ATP production in these resting brains. The findings from this study would have profound impact for understanding the physiological meanings of the CMR_{ATP} measurement and the relation of the cerebral ATP metabolism with brain function in healthy and diseased brains.

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