Simultaneous and Ultrafast Monitoring of $CMRO_2$, CBF and pO_2 Changes in Response to Acute Global Ischemia in Rat Brain

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INTRODUCTION Determination of the cerebral metabolic rate of oxygen utilization ($CMRO_2$) is essential for understanding the central role of oxidative metabolism in brain function at physiologic and/or pathologic states. Ischemia is an important pathologic condition encountered in neurology and stroke. Its effects on brain function and pathologic damage depend on the cellular oxidative metabolism change occurred during the ischemia, in which $CMRO_2$ can significantly decrease due to the lack of O_2 supply in the brain tissue (i.e., pO_2) after reducing the cerebral blood flow (CBF), and these parameters can be linked by the equation of $CMRO_2 = k\cdot[Cyt\ a_3^{2+}]\cdot[O_2]_t = k\cdot[Cyt\ a_3^{2+}]\cdot CBF\cdot[O_2]_a$, where k is a constant, Cyt a_3^{2+} is reduced cytochrome oxidase: one key enzyme for oxidative phosphorylation, $[O_2]_a$ and $[O_2]_t$ are the oxygen concentrations in artery and brain tissue, respectively [1]. It is known that before the $[O_2]_t$ approaches a critical low concentration, $CMRO_2$ normally remains constant via the compensation by increasing [Cyt a_3^{2+}]. However, when $[O_2]_t$ decreases to a critical point due to insufficient blood supply during an ischemia, $CMRO_2$ starts to decrease. Aiming to quantitatively understand the interplay between the oxygen supply and utilization during ischemia, we have conducted a study for determining CBF, pO_2 and $CMRO_2$ simultaneously in the rat brain before, during and after a 12-minute global ischemia using four-blood vessel occlusion (4BVO) model. *In vivo* ^{17}O MRS was applied to detect the labeled $H_2{}^{17}O$, which is metabolized from inhaled $^{17}O_2$, at 9.4T for determining $CMRO_2$ with ultrafast temporal resolution of a few seconds [2]. CBF and pO_2 were obtained in real time using laser Doppler flowmetry and optical fluorescence technology.

METHODS: Male SD rats were used in this study. An electrical occlusion of bilateral vertebral arteries was performed 24-48 hours before MR experiments. The common carotid arteries were exposed and surrounded with two plastic occluders (Harvard apparatus, MA). The anesthetization was maintained by continuous α-chloralose infusion (25mg/kg/hr) and 60/40 N_2O/O_2 mixtures. The forebrain ischemia was achieved by inflating the occluders for 12 minutes; the occluders were then deflated for reperfusion. All experiments were conducted on a 9.4 Tesla Magnex magnet interfaced to a Varian INOVA console. A multinuclear surface-coil probe consisting of an oval-shape ^{17}O coil (1 cm × 2 cm) and a butterfly-shape ^1H coil was used. The spatial localization of ^{17}O signal was achieved through the spatially limited B_1 profile of a ^{17}O surface coil, which covers most of the rat brain. The single-pulse acquisition sequence was used to collect ^{17}O spectra with the acquisition parameters of 10 ms TR, 50 μs pulse width for a nominal 90° pulse, spectral width=30 kHz and 100 averages (1 second temporal resolution). The measured time course of the metabolic $H_2{}^{17}O$ increase during the inhalation was fitted to a polynomial function and the first order derivation of the polynomial function provided the $CMRO_2$ value as a function of time [3, 4]. Dual-channel OxyLab LDF/OxyFlo instruments (Oxford Optronix, UK) were used to simultaneously monitor cerebral pO_2 and CBF via the placement of a combined pO_2/perfusion sensor in the rat cortex. The simultaneous $CMRO_2$/pO_2/CBF measurements were performed during the control, ischemia and reperfusion periods.

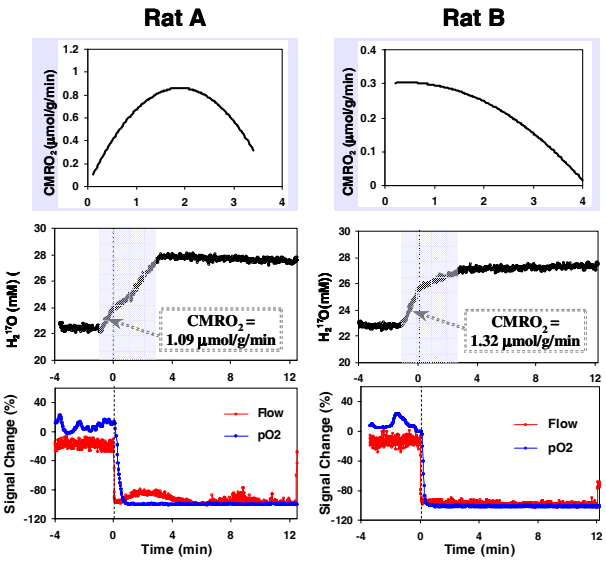

Fig. 1 Simultaneously measured CBF/pO_2 (bottom), $H_2{}^{17}O$ (middle) and $CMRO_2$ (top) values before and during global brain ischemia in Rat A and B. The dash line and the shading area indicate the start of ischemia and duration of the $^{17}O_2$ inhalation, respectively.

RESULTS and DISCUSSION: Figure 1 demonstrates time courses of changes in CBF, tissue pO_2, metabolic $H_2{}^{17}O$ content and $CMRO_2$ in two representative rat brains during a 3.5-minute $^{17}O_2$ inhalation experiment which started 1.5 minute before the ischemia. The accumulation rate of the metabolic $H_2{}^{17}O$ (i.e. the $CMRO_2$ value) during the period before ischemia reflect the basal $CMRO_2$ values of 1.09 (***Rat A***) and 1.32 (***Rat B***) μmol/g/min, respectively. During the ischemia, however, these two rats behaved differently in terms of CBF and $CMRO_2$ responses to the occlusion. In ***Rat B***, both CBF and pO_2 approached zero (≤ 5% of control value) due to the occlusion, and the accumulation rate of $H_2{}^{17}O$ decreased dramatically which reflected the decline of $CMRO_2$ during the ischemia (see Fig. 1). After the termination of $^{17}O_2$ inhalation, the brain $H_2{}^{17}O$ level remained constant because the residue CBF was extremely low during the ischemia for this rat. In contrast, although the pO_2 in ***Rat A*** reached the same minimal level as in Rat B, its CBF responses during ischemia were significantly different, which can be characterized by initial rapid drop to ≤ 5% followed by gradual recovery to ~20% of control CBF, then decreasing again. The significant residue level of CBF during ischemia in this rat would have different metabolic consequences and we were able to, for the first time, directly monitor the dynamic change of $CMRO_2$ during ischemia using the *in vivo* ^{17}O MRS technique. The striking finding is that the oxygen utilization rate decreased significantly at the beginning of ischemia which then recovered rapidly to approach pre-ischemic level even though the tissue pO_2 was remained near zero. Moreover, the $H_2{}^{17}O$ level after the termination of $^{17}O_2$ inhalation was decreasing substantially indicating a significant blood circulation during the ischemia. These results demonstrated that the dynamic change of $CMRO_2$ is very sensitive to the residue CBF rather than the tissue pO_2, and the $CMRO_2$ response during the ischemia can be linked to the behavior of CBF response. We found that in our study using 4BVO model, the tissue pO_2 approached zero during 12 min occlusion for most rats indicating that the tissue oxygen was consumed instantly in mitochondria when the blood oxygen supply was below a critical level, therefore, the residue CBF directly controls the oxygen metabolism rate under this circumstance. In addition, when the heart arrest occurred (induced by KCl injection) whereas CBF ≈ 0 and pO_2 ≈ 0 rapidly, the production of metabolic $H_2{}^{17}O$ during a $^{17}O_2$ inhalation was also stopped immediately (i.e., $CMRO_2$ ≈ 0) as shown in Figure 2. This experiment demonstrates that the oxygen availability in the brain is highly limited and it relies on the continuous supply via blood circulation, therefore, any vascular insult which interrupts the oxygen supply could lead to severe brain damage.

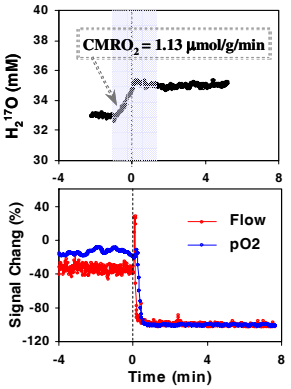

Fig. 2 The CBF / pO_2 and $H_2{}^{17}O$ values before and after KCl injection (dash line) in a rat brain. The shading area indicates the duration of the $^{17}O_2$ inhalation.

CONCLUSION: In this work, we demonstrate the feasibility for performing simultaneous $CMRO_2$/CBF/pO_2 measurements with ultrafast temporal resolution. These measurements have been successfully applied to study the rat brain ischemia *in vivo*. The results should provide better understanding and insights about the interplay of oxygen supply (CBF and pO_2) and oxygen metabolism ($CMRO_2$) during vascular insult and its effect on the outcomes of ischemia.

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