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Brain Tissue PO₂ Measurement During Normoxia and Hypoxia Using Two-Photon Phosphorescence Lifetime Microscopy

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Abstract

Key to the understanding of the principles of physiological and structural acclimatization to changes in the balance between energy supply (represented by substrate and oxygen delivery, and mitochondrial oxidative phosphorylation) and energy demand (initiated by neuronal activity) is to determine the controlling variables, how they are sensed and the mechanisms initiated to maintain the balance. The mammalian brain depends completely on continuous delivery of oxygen to maintain its function. We hypothesized that tissue oxygen is the primary sensed variable. In this study two-photon phosphorescence lifetime microscopy (2PLM) was used to determine and define the tissue oxygen tension field within the cerebral cortex of mice to a cortical depth of between 200–250 μm under normoxia and acute hypoxia ($\text{FiO}_2 = 0.10$). High-resolution images can provide quantitative distributions of oxygen and intercapillary oxygen gradients. The data are best appreciated by quantifying the distribution histogram that can then be used for analysis. For example, in the brain cortex of a mouse, at a depth of 200 μm , tissue oxygen tension was mapped and the distribution histogram was compared under normoxic and mild hypoxic conditions. This powerful method can provide for the first time a description of the delivery and availability of brain oxygen *in vivo*.

Keywords

Oxygen partial pressure; 2PLM; Tissue oxygen tension; Distribution histogram; Mouse

1 Introduction

The mammalian brain depends completely on continuous delivery of oxygen to maintain its function. There are numerous intrinsic and extrinsic mechanisms that maintain a suitable level of oxygen availability to the neurons and other cells of the central nervous system. The brain tissue parenchyma exists in a low oxygen environment. For example, the oxygen partial pressure (PO_2) distribution histogram and cumulative occurrence function suggests that 90% of the oxygen levels are below 30 mmHg in rat brain [1]. Hypoxia is any decrease in the availability of oxygen. Under hypoxic condition, decreases in inspired oxygen produce decreases in tissue PO_2 . Mammals adapt to prolonged moderate hypoxia by increased ventilation, increased blood hemoglobin and failure to gain weight. Prolonged hypoxia also results in an increase in capillary density, resulting in decreased intercapillary distance achieved through angiogenesis that allows brain tissue oxygen diffusion flux to remain adequate [2]. We have previously demonstrated a right shift and broadening in the distribution of capillary segment lengths in rat brain after 3 weeks of hypoxia [3].

Determining the range of optimal oxygen concentrations in the mammalian brain is important, however, the tools and techniques for quantitative oxygen measurements in the awake, normally functioning brain have been limited, mostly by the need for noninvasiveness. In addition, for any method used, it requires adequate response time and signal localization in both temporal and spatial heterogeneities at the microregional level. This includes the methods such as polarography, optical, electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), positron emission tomography (PET), and mass spectrometric techniques [4]. Two-photon phosphorescence lifetime microscopy (2PLM) of PO_2 can provide detailed distributions of the absolute oxygen concentration in tissue [5–7]. This method enables us to measure PO_2 with high temporal and spatial resolution in three dimensions, and features a measurement depth of up to 250 μm , sub-second temporal resolution. High-resolution images can document the intercapillary oxygen concentration gradients [5]. This method can also be used with intravascular dye administration to provide detailed descriptions of the blood oxygen concentration in arterioles, veinules and capillaries [5, 8].

In this study, 2PLM of PO_2 was used to map the tissue oxygen tension in the mice cerebral cortex down to a cortical depth of 200–250 μm . These data enabled us to determine quantitative distributions of oxygen concentration in the brain tissue.

2 Methods

2.1 Animal Preparation

Animals were prepared under a protocol approved by the Subcommittee on Research and Animal Care at Massachusetts General Hospital. As described previously [5], for imaging of PO_2 in the microvasculature, C57BL/6 mice (male, 25–30 g, 3 months old) were anesthetized by isoflurane (1–2% in a mixture of O_2 and air) under constant temperature. Mice were tracheotomized and a catheter was inserted in the femoral artery to monitor the blood pressure and blood gases and to administer the dyes. A cranial window was opened in the parietal bone with intact dura and sealed with a 150- μm thick microscope coverslip. The

concentration of the oxygen-sensitive phosphorescent dye PtP-C343 in the blood immediately after administration was $\sim 16 \mu\text{M}$.

2.2 Two-Photon Laser Scanning Microscopy

Partial O_2 pressure in tissue (PtO_2) was measured using *in vivo* two-photon high-resolution PO_2 measurements in mouse cortical microvasculature and tissue, by combining an optimized imaging system with a two-photon-enhanced phosphorescent nanoprobe [5–8]. In brief, the imaging was performed on the brain of anesthetized animals through the sealed cranial windows. The two-photon excitation made it possible to confine the phosphorescence quenching by oxygen to the immediate vicinity of the focal plane, thus minimizing oxygen consumption and/or phototoxicity. During the measurement, isoflurane was discontinued and anesthesia was maintained by first injecting a 50-mg/kg intravenous bolus of α -chloralose followed by continuous intravenous infusion at 40 mg/(kg/h). To reduce possible animal motion during experiments, we administered an intravenous bolus of pancuronium bromide (2 mg/kg) followed by continuous intravenous infusion at 2 mg/(kg/h). Tissue PO_2 values were measured during normoxia with the fraction of inspired oxygen (FiO_2) of 21% and hypoxia ($\text{FiO}_2 = 10\%$) [9].

3 Results

Two-photon laser scanning microscopy was used to map the tissue oxygen tension within the mice cerebral cortex of down to a cortical depth of 200–250 μm . Our preliminary data showed that the tissue PO_2 was in the range of 20–60 mmHg and 2–18 mmHg for normoxia and hypoxia, respectively. Figure 20.1 shows an example image from the mouse cortex at a depth of 200 μm , tissue oxygen tension was mapped under normoxic and hypoxic conditions and the tissue PO_2 distribution histograms were compared. The tissue PO_2 during hypoxia was in the range of 8–18 mmHg, lower than the tissue PO_2 during normoxia (25–50 mmHg). There was no overlap between the PO_2 distributions of the two conditions.

4 Discussion

Key to the understanding of the principles of physiological and structural acclimatization to changes in the balance between energy supply (represented by substrate and oxygen delivery, and mitochondrial oxidative phosphorylation) and energy demand (initiated by neuronal activity), is to determine the controlling variables, how they are sensed, and the mechanisms initiated to maintain the balance. We hypothesized that tissue oxygen is the primary sensed variable during hypoxic acclimatization. In this study, 2PLM of PO_2 allowed us to map the tissue oxygen tension within the mouse cerebral cortex down to a cortical depth of 20–250 μm . The data are best appreciated by quantifying the tissue PO_2 distribution histogram that can then be used for analysis. For example, the images from the cortex of a mouse demonstrated significantly different tissue PO_2 distribution histograms under normoxic and mild hypoxic conditions. We have previously modelled the expected oxygen field changes for the “unit capillary” in rat cerebral cortex and showed the predicted oxygen tension along the length of a “unit capillary” and with respect to tissue depth (distance from the capillary). The model predicts a significant flattening of the distribution curve and tissue oxygen gradients after 3 weeks of mild hypoxia [10]. Global measurements of brain tissue

oxygenation generally corroborate these predictions [11]. Two-photon phosphorescence lifetime microscopy of PO_2 will enable us to directly test this model in the mouse studies.

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References

1. LaManna JC (2007) Hypoxia in the central nervous system. *Essays Biochem* 43:139–151 [PubMed: 17705798]
2. LaManna JC, Vendel LM, Farrell RM (1992) Brain adaptation to chronic hypobaric hypoxia in rats. *J Appl Physiol* 72:2238–2243 [PubMed: 1629078]
3. LaManna JC, Cordisco BR, Knuese DE et al. (1994) Increased capillary segment length in cerebral cortical microvessels of rats exposed to 3 weeks of hypobaric hypoxia. *Adv Exp Med Biol* 345:627–632 [PubMed: 7521566]
4. Ndubuizu O, LaManna JC (2007) Brain tissue oxygen concentration measurements. *Antioxid Redox Signal* 9(8):1207–1219 [PubMed: 17536959]
5. Sakadžić S, Roussakis E, Yaseen MA et al. (2010) Two-photon high-resolution measurement of partial pressure of oxygen in cerebral vasculature and tissue. *Nat Methods* 7(9):755–759 [PubMed: 20693997]
6. Finikova OS, Lebedev AY, Aprelev A et al. (2008) Oxygen microscopy by two-photon-excited phosphorescence. *ChemPhysChem* 9(12):1673–1679 [PubMed: 18663708]
7. Lecoq J, Parpaleix A, Roussakis E et al. (2011) Simultaneous two-photon imaging of oxygen and blood flow in deep cerebral vessels. *Nat Med* 17(7):893–898 [PubMed: 21642977]
8. Sakadžić S, Mandeville ET, Gagnon L et al. (2014) Large arteriolar component of oxygen delivery implies a safe margin of oxygen supply to cerebral tissue. *Nat Commun* 5:5734 [PubMed: 25483924]
9. Baraghis E, Devor A, Fang Q et al. (2011) Two-photon microscopy of cortical NADH fluorescence intensity changes: correcting contamination from the hemodynamic response. *J Biomed Opt* 16(10):106003 [PubMed: 22029350]
10. Lauro KL, LaManna JC (1997) Adequacy of cerebral vascular remodeling following three weeks of hypobaric hypoxia. Examined by an integrated composite analytical model. *Adv Exp Med Biol* 411:369–376 [PubMed: 9269451]
11. Dunn JF, Grinberg O, Roche M et al. (2000) Noninvasive assessment of cerebral oxygenation during acclimation to hypobaric hypoxia. *J Cereb Blood Flow Metab* 20(12):1632–1635 [PubMed: 11129779]

Fig. 20.1.

Tissue PO_2 in a mouse brain measured by two-photon phosphorescence lifetime microscopy (2PLM) of PO_2 , 200 μm below the cortical surface under normoxic and hypoxic ($FiO_2 = 0.10$) conditions, stack $725 \times 725 \times 360 \mu\text{m}^3$. Tissue oxygen tension was mapped and the tissue PO_2 distribution histograms were compared between normoxia (range 25–50 mmHg) and hypoxia (range 8–18 mmHg). There was no overlap between the PO_2 distributions of the two conditions