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Simultaneous measurement of brain tissue oxygen partial pressure, temperature, and global oxygen consumption during hibernation, arousal, and euthermia in non-sedated and non-anesthetized Arctic ground squirrels

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Abstract

This study reports an online temperature correction method for determining tissue oxygen partial pressure (P_{tO_2}) in the striatum and a novel simultaneous measurement of brain P_{tO_2} and temperature (T_{brain}) in conjunction with global oxygen consumption (VO_2) in non-sedated and non-anesthetized freely moving Arctic ground squirrels (AGS, *Spermophilus parryii*). This method fills an important research gap—the lack of a suitable method for physiologic studies of tissue P_{O_2} in hibernating or other cool-blooded species.

P_{tO_2} in AGS brain during euthermia (21.22 ± 2.06 mm Hg) is significantly higher ($P=0.016$) than during hibernation (13.21 ± 0.46 mm Hg) suggests brain oxygenation in the striatum is normoxic during euthermia and hypoxic during hibernation. These results in P_{tO_2} are different from blood oxygen partial pressure (PaO_2) in AGS, which are significantly lower during euthermia than during hibernation and are actually hypoxic during euthermia and normoxic during hibernation in our previous study. This intriguing difference between the P_{O_2} of brain tissue and blood during these two physiological states suggests that regional mechanisms in the brain play a role in maintaining tissue oxygenation and protect against hypoxia during hibernation.

Keywords

Temperature correction; Correction factor; Online calculation; Tissue O_2 partial pressure; Simultaneous multi-measurements; Real-time; In-vivo

Introduction

Many different methods have been used to monitor cerebral oxygenation, including electron paramagnetic resonance oximetry (EPR) (Liu et al., 1995; Dunn et al 2000), positron emission tomography (Coles et al, 2004a, b), magnetic resonance spectroscopy (Garnett et al, 2000), jugular venous oximetry (Coles et al, 2004b), near-infrared spectroscopy (NIRS) (Hadolt &

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Litscher, 2003; Imray et al, 2000, 2005), and 2-nitroimidazole-binding techniques (Koch, 2002). Although each of these methods has its advantages, none can determine the spatial differences of cerebral oxygenation, and most provide only indirect measurement of brain oxygenation. Measurement of tissue oxygen partial pressure (P_{tO_2}) has been explored as an alternative way to monitor cerebral oxygenation (Mass et al, 1993; Dings et al, 1996a & 1996b; Kiening et al, 1996; Van Santbrink et al, 1996; Hoffman et al, 1996; Zauner et al, 1997; Gopinath et al, 1999). P_{tO_2} measured by a platinum wire electrode coated with a gas permeable membrane, usually referred to in the biomedical literature as a “Clark-type” O_2 electrode, is now recognized as the most precise and accurate measurement of brain oxygenation in both clinical and animal research applications (Nortji & Gupta, 2006). To date, all reported P_{tO_2} data both from patients with brain trauma and from animal models have been measured under an anesthetized or restrained condition at a body temperature maintained near 37 °C. A big challenge has been to obtain the real-time value of P_{tO_2} without the effects of anesthesia because anesthetics have significant influence on P_{tO_2} and result in very different patterns of response of the P_{tO_2} in the brain (Liu et al., 1995; Swart et al, 2003; Hou et al 2005).

Besides the effects of anesthesia on P_{tO_2} , temperature affects both the P_{O_2} electrode and physiologic metabolism. Care must be taken to distinguish between the effects of temperature on metabolic processes and the physiologic handling of oxygen and the effects of temperature on the measurement of oxygen levels by a polarographic current effect induced by a Clark-type P_{O_2} electrode.

For hibernation studies, correction of the effects of temperature on the P_{O_2} electrode presents an even greater challenge for measuring real-time dynamic changes of P_{tO_2} because of the huge, rapid, and continuous changes in body temperature (from -2 °C to 40 °C) during transition periods in the three physiological states of hibernation, arousal, and euthermy. Moreover, the temperature changes during arousal from or entrance into the hibernation are heterogeneous in different body tissues. For example, change in mouth temperature occurs before changes in abdominal temperature and rectal core body temperature (Tøien et al, 2001; Ma et al 2005). Therefore, we must measure real-time dynamic brain temperature simultaneously with P_{tO_2} in order to correct for temperature's effects on P_{tO_2} . Previously, there has been no temperature correction method to achieve such a purpose in hibernation studies. Therefore, this study addressed the need for a real-time temperature correction method to eliminate temperature effects induced by the O_2 electrode for in vivo measurement of P_{tO_2} in hibernation studies.

The aims of this study were twofold: (i) to provide an on line temperature correction method for real-time in vivo measurement of P_{tO_2} from non-sedated and non-anesthetized AGS and (ii) to obtain pioneering data in P_{tO_2} during different physiological states in active AGS.

Material and Methods

Animals

All procedures were performed in accordance with NIH guidelines for the care and use of laboratory animals and approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee. Adequate measures were taken to minimize pain or discomfort. Arctic ground squirrels (AGS; *Spermophilus parryii*) were used for these experiments. Adult AGS of both sexes were trapped during mid-July in the northern foothills of the Brooks Range, Alaska, approximately 40 miles south of the Toolik Field Station of the University of Alaska Fairbanks (68°38'N, 149°38'W; elevation 809 m) and transported to Fairbanks (permit obtained from Alaska Department of Fish & Game). The animals were housed individually at 16–18 °C and fed rodent chow, sunflower seeds, and fresh carrots and apples ad libitum until

mid-September when they were moved to a cold chamber set to an ambient temperature (T_a) of 2 °C and a 4:20 h light:dark cycle.

Stereotaxic surgery for intracerebral guide cannula implantation

The animals used in this study were male and female AGS weighing 650–840 g at the time of the surgery. The surgeries were conducted after the beginning of the hibernation season, between September and October. Animals fasted at least 12 hours before surgery. Surgery was performed at room temperature under general anesthesia with isoflurane (Halocarbon Laboratories, River Edge, NJ), induced at 5%, and maintained at 1.5% to 3% mixed with 100% medical grade O₂ at a flow rate of 1.5 L/min.

During surgery, the animals' heads were put in a stereotaxic apparatus and their body temperature was maintained at 35–37 °C with a servo-controlled fluid-filled heating pad (Omni Medical Equipment Inc., Cincinnati, OH). Under strictly aseptic conditions, skin incision was made midline on the head and the working area was exposed with a retractor. The soft tissues were scraped with surgery blade and cleaned with sterilized cotton-tipped applicators. After tissue cleaning on the skull, two holes (1.8 mm diameter) to accommodate the guide cannulae were made with a sterilized trephine operated by a battery-driven drill at the coordinates (AP, 13.5 or 14 mm; L, ± 3.25 mm D, -4.0 mm) and another four holes were made with a steel burr (0.5 mm diameter) near the cannulae holes (two in front of and two in back of the guide cannula holes) on the skull for the foundation of dental cement. After completing the drilling process, four anchor stainless steel bone screws (BAS, West Lafayette, IN) were driven into the skull through the screw holes. Two guide cannulae (CMA11, Acton, MA) were stereotaxically positioned above the right and left striatum (AP, 13.5 or 14 mm; L, ± 3.25 mm D, -4.0 mm), as described in detail by Osborne et al (Osborne et al, 1999), and were slowly lowered 4 mm from the cortical surface. Cannulae were secured to the screws with dental cement. Antibiotics (Baytril; Bayer Corp., Shawnee Mission, KS, Dose: 5 mg/kg) were given twice a day, 1 day before surgery and 2 days postoperatively, by subcutaneous injection in the back of the neck. After surgery, daily cleanings were performed with 3% betadine for 10 to 15 days, and animals were allowed to recover completely at room temperature. The animals, housed individually under a light regime of 12:12 hours light:dark at 20 to 22 °C, were next transferred to the cold chamber with an ambient temperature of 2 °C on a 4:20 hours light:dark cycle for the hibernation bouts.

Five AGS were used to measure P_{tO_2} , T_{brain} , and VO_2 simultaneously and continuously during hibernation, arousal, and euthermia for data shown in Fig 6 and 7. Three winter euthermic AGS were used to conduct the stability tests of P_{tO_2} measurement in the striatum with P_{O_2} electrodes for data shown in Fig. 9. All brains of eight AGS were used to identify the tip locations of P_{O_2} electrodes in the striatum as showing in Fig. 8.

Calibration of P_{O_2} electrode and thermocouple

For the measurement of P_{tO_2} , a P_{O_2} microelectrode (Model: IPS-020; 200 μm ; Inter Medical Co. Ltd, Japan) was calibrated in artificial cerebral spinal fluid (ACSF in mM: NaCl:124; Cl: 2.7; CaCl₂:1.2; MgCl₂: 0.85; D-Glucose:1.4; NaHCO₃:24, pH 7.4) at 37 °C equilibrated with air (21% O₂) and N₂ (0% O₂) respectively before and after each experiment to insure no obvious change (less than 10%). ACSF solution in a water-circulating double layer glass beaker (15 mL) was thermostatically maintained at a temperature of 37 °C by circulating anti-freezing solution through plastic tubing connected to a circulating bath, which could change the temperature of the circulating fluids to any given temperature from -2 to 40 °C. Compressed air containing 21% O₂ was used to set up a standard P_{O_2} value at a reading of 145–150 mm Hg, depending on the value of atmospheric pressure during calibration, and pure compressed nitrogen gas was used to set up a standard P_{O_2} value as 0 mm Hg on the P_{O_2} monitor (IMP-201,

Inter Medical Co. Ltd, Japan). The needle thermocouples were calibrated at 0 and 40 °C with an accuracy of ± 0.1 °C in a water circulating bath before gas-sterilization.

Temperature correction for P_{O_2} measurement

Under natural living conditions, the oxygen partial pressure (P_{O_2}) in tissue fluids is influenced by atmospheric pressure (P_{atm}), water vapor pressure (P_{H_2O}) at measured tissue temperature, and the inspired oxygen fraction (F_iO_2),

$$P_{O_2} = (P_{atm} - P_{H_2O}) F_iO_2 \quad (1)$$

Thus, measured P_{O_2} varies with changes of P_{atm} and P_{H_2O} when F_iO_2 is constant. The first factor, P_{atm} , varies daily and can be easily measured with a barometer. The second factor, P_{H_2O} , depends on temperature. Therefore, temperature's effects on P_{O_2} are decided by P_{H_2O} under natural living conditions.

To find the function of temperature's effect on P_{H_2O} , we used the values of water vapor pressure (P_{H_2O}) at different temperatures as cited in the *Handbook of Chemistry and Physics* (Weast RC, 1968) and plotted those values of P_{H_2O} against temperatures from 0 to 40 °C with a scale of 5 °C (Figure 1). Then we found the function of P_{H_2O} versus temperature by means of an Excel curve fitting program. We found that the water vapor pressure varies with temperature (t) following a power function:

$$P_{H_2O} = 4.871e^{0.0619t}$$

When P_{tO_2} is measured with a polarographic P_{O_2} electrode, temperature affects the polarographic current of the P_{O_2} electrode during measurement and the current is proportional to the values of measured PO_2 . Thus, all P_{O_2} values recorded at different temperatures ($P_{O_2, meas.}$) in AGS brain must be corrected empirically to get the actual values corresponding to the physiological effect without the influence of temperature ($P_{O_2, cal.}$). To perform such a temperature correction, the following steps were taken.

1. The P_{O_2} meter was initially calibrated to a P_{O_2} value of 144–150 mm Hg (the value depended on the local atmospheric pressure during calibration) when the electrode was exposed to ACSF equilibrated with compressed air (21% O_2) at 37 °C, and to a P_{O_2} value of zero when ACSF was equilibrated with compressed nitrogen gas (0% O_2) at 37 °C.
2. Under the condition of bubbling with 21% O_2 in ACSF, the signal outputs of the P_{O_2} electrode ($P_{O_2, meas.}$) were determined in ACSF solutions ranging in temperature from 0 to 40 °C (Figure 2).
3. The actual values of P_{O_2} ($P_{O_2, cal.}$), independent of temperature effects, were calculated at each given temperature according to the equation:

$$P_{O_2, cal.} \text{ (mm Hg)} = (P_{atm} - P_{H_2O})^* F_iO_2,$$

where the values of water vapor pressure (P_{H_2O}), which were obtained from the literature (Weast, 1968), were appropriate for a given temperature (Figure 3).

4. These data— $P_{O_2, meas.}$, $P_{O_2, cal.}$, and temperature in Celsius (t)—were used to derive a temperature correction factor ($f = A e^{-kt}$, where the values of A and k are determined by each electrode calibration) to calculate the actual P_{O_2} of tissues measured at various temperatures:

$$f = P_{O_2, cal.} / P_{O_2, meas.} = A e^{-kt}$$

We plotted " $P_{O_2, cal.} / P_{O_2, meas.}$ " against t at temperatures of 0 to 40 °C and fit the curve function with an Excel program to find the values of A and k (Figure 4). After

A and k values were defined, the function, $P_{O_2, cal.} = (A e^{-k t})(P_{O_2, meas.})$, was programmed into the data acquisition program (PowerLab) for online temperature correction of P_{O_2} measurements.

For each electrode and experiment, the calibration process was repeated as above.

Real-time measurement of P_tO_2 in the striatum of AGS

After animals recovered from stereotaxic surgery for guide cannulae implantation and a hibernation bout was begun approximately three to four weeks later at 2 °C in a cold chamber, the insertions of the reference electrode, the P_{O_2} electrode, and the micro thermocouples were conducted under deep torpor without anesthesia during the second or third hibernation bout for the hibernating AGS. For the euthermic AGS, the insertions of the reference electrode, the P_{O_2} electrode, and the micro thermocouples were conducted under anesthesia with 2–3% isoflurane at room temperature. A sterilized silver-silver chloride electrode (RC1, WPI, Sarasota, FL) was implanted to serve as a reference electrode under dorsal skin on the back through a soft silicone tube using general anesthesia with isoflurane gas before insertion of the P_{O_2} electrode and micro-thermocouple. After implantation of the reference electrode, the calibrated and sterilized P_{O_2} electrode and needle micro-thermocouple for T_{brain} were inserted through the guide cannulae into the left and right striatum, respectively, and secured by medical tape. The tips of the P_{O_2} electrode and needle micro-thermocouple extended about 2 mm beyond the guide cannulae tubes. Buprenorphine (0.03 mg/kg), a pain reliever, was given by subcutaneous injection on the back, with one single dose after insertions of the P_{O_2} electrode and thermocouple for both euthermic and hibernating AGS. Then for real-time in vivo recording of measurements, the animals were transferred into an experimental chamber with a normal swivel set-up for active animals. The wires connected from P_{O_2} electrode, reference electrode, and thermocouple were went through a sprint tube and came out the experimental chamber in the middle of the top, and connected to P_{O_2} meter and thermocouple meter respectively.

VO_2 of AGS was recorded by indirect calorimetry in a vertical cylindrical-shaped Plexiglas metabolic chamber (I.D. 29 cm, height 32 cm) shown in Figure 5. Air was drawn into a distribution tube through a vertical cylindrical-shaped Plexiglas metabolic chamber at a flow rate of 3 L/min by a membrane pump and measured by a mass flow meter (model AFSC-10 K, Teldyne Hastings-Raydist, Hampton, VA). A separate pump sampled gas from the distribution tube through a canister, which was filled by molecular sieves and connected in a series with a Nafion drying column. O_2 extraction was measured with an O_2 analyzer (Model: FoxBox 2.0, Sable System International, Inc, Las Vegas, NV), which was calibrated with air. VO_2 was calculated with an Excel program on the basis of the O_2 fraction differences between fresh air (before being used by an animal) and expired air (after being used by an animal), flow rate of sample air, animal body weight, and a known respiratory quotient (RQ) value from previous experiments with AGS (Tøien et al, 2001; Ma et al, 2005), according to the principles of equation 3a of Withers, $VO_2 = V_E * (F_{IO_2} - F_{EO_2}) / ((1 - (1 - RQ) * F_{IO_2}))$, (Withers, 1977).

The P_tO_2 , T_{brain} , and VO_2 were recorded simultaneously 20 minutes later after buprenorphine injection by a data acquisition program matched with the interface (Power Lab/8 sp, AD Instruments, Inc., Colorado Springs, CO) at a sample rate of 1/min and without further signal filtering. A P_{O_2} monitor and a thermocouple meter (Sable Systems, TC-1000 thermometer, Henderson, NV) were used as pre-amplifiers and the outputs of those two meters were connected to the interface to the data acquisition program. A Sable interface (model:U12, Henderson, NV) was used as A/D converter for the VO_2 measurement system, and the signals of the outputs were then input into the interface to the data acquisition program. The experiments for P_tO_2 , T_{brain} , and VO_2 measurement were conducted in the cold chamber at 2 °C. Data in $P_{O_2, meas.}$ recorded originally by the P_{O_2} electrode in one channel were online

calibrated into corresponding values of $P_{O_2, cal.}$ without the effects of temperature in another channel.

Histologic analysis of electrode tip location

After the experiment was completed for P_tO_2 , T_{brain} , and VO_2 recordings, the AGS were euthanized under deep anesthetic condition with isoflurane. The brain tissues were immediately frozen in 2-methylbutane at $-40^\circ C$ and kept in a freezer at $-80^\circ C$. The brain slide sections ($30\text{ }\mu m$) were cut with Cryostat (Cryocut 1800, Leica Instruments, Nussloch, Germany), and tissues were collected on precleaned microscope slides (Fisherbrand, Fisher Scientific, US) at $-18^\circ C$. The tissue slides were dried overnight at $-25^\circ C$ in a freezer and stained with Cresyl Violet the next day at room temperature.

Before staining, tissue slides were fixed in 10% formalin for 10 minutes and rinsed with PBS washing solution (NaH_2PO_4 : 1.9 mM; Na_2HPO_4 : 8.1 mM; $NaCl$: 154 mM; pH 7.4) three times. The tissues slides were then stained for 8 minutes in Cresyl Violet solution mixed with sodium acetate solution (0.1 M, 150 mL), glacial acetic acid (0.1 M, 130 mL), and Cresyl Violet water solution (1%, 110 mL), followed by rinsing with PBS washing solution two times. Next, the tissue slides were treated in de-ionized water for 2 min, and then in a series of alcohol-water solutions (50%, 70%, 90%, and 100% alcohol) for 1 min for each solution. After being treated with alcohol solutions, the slides were treated with a series of histoclear solutions in alcohol for 2 min for each treatment (50%, 70%, 90%, 100% histoclear solution in alcohol). Finally, the tissue slides were covered with Permount glue (SP 15–100, FairLawn, New Jersey) and cover glass ($24\times 60\text{ mm}$, Corning Labware & Equipment, US) from 100% histoclear. The finished slides were cleaned with 100% histoclear and kept at room temperature for 2–3 days for drying. After the slides dried, they were analyzed with a dissection microscope and pictures were taken with a digital camera attached to the microscope.

Data analysis

The data in P_tO_2 , T_{brain} , VO_2 are presented as means $\pm$ S.E.M. Statistical analysis was performed using *student t-test* (Sigma Stat, Systat Software, Inc., Richmond, CA), and results were considered significant when $P<0.05$ (Figure 7).

Results

P_tO_2 during hibernation, arousal, and euthermia

Figure 6 shows representative graphs of simultaneous measurement of $P_{O_2, meas.}$, $P_{O_2, cal.}$, T_{brain} , and VO_2 during hibernation, arousal, and euthermia in the striatum of AGS. Panel A shows the change of T_{brain} during arousal. Panel B shows the change of P_{O_2} without temperature correction ($P_{O_2, meas.}$). Panel C shows the change of P_{O_2} with temperature correction ($P_{O_2, cal.}$). Panel D shows the change in O_2 consumption of AGS during arousal. There is an obvious difference between the changes in $P_{O_2, meas.}$ and $P_{O_2, cal.}$ when P_tO_2 is measured without and with correction for the effect of temperature.

P_tO_2 in hibernating and euthermic AGS

The P_tO_2 , T_{brain} in the striatum, and global O_2 consumption of AGS during hibernation and euthermia recorded from five hibernating to aroused AGS is shown in Figure 7. Average values in P_tO_2 , T_{brain} , and VO_2 during hibernating and euthermic states in each individual animal (as shown in Figure 6) were used to calculate the mean value in each group and to perform statistical analysis. P_tO_2 during euthermia ($21.22 \pm 2.06\text{ mm Hg}$, $n=5$) is significantly higher ($P=0.016$) than during hibernation ($13.21 \pm 0.46\text{ mm Hg}$, $n=5$). VO_2 during euthermia ($1.22 \pm 0.07\text{ mL/g.hr}$, $n=5$) is significantly higher ($P=0.016$) than during hibernation ($0.10 \pm 0.01\text{ mL/}$

g.hr, n=5). Brain tissue temperature in the striatum during euthermia (35.88 ± 0.33 °C, n=5) is also significantly different ($P < 0.001$) from hibernation (5.11 ± 0.53 °C, n=5).

Location of the P_{O₂} electrode in the striatum

Five hibernating AGS and three winter euthermic AGS were used to identify the P_{O₂} electrode tip locations. All tips of the P_{O₂} electrodes with the coordinates (AP, 13.5 or 14 mm; L, ± 3.25 mm D, -4.0 mm) are evidenced in the left striatum of AGS brains. The typical locations of the P_{O₂} electrode and guide cannula tube are shown in Figure 8. The lower arrow points to a tip of the P_{O₂} electrode and the upper arrow points to the guide cannula tube. Some of the histologic pictures show only the guide cannula tube without clearly showing the location of the electrode tip. Whether both guide cannulae and P_{O₂} electrode locations are shown depends on the amount of time between when the electrode is pulled out of the brain and when the animal is euthanized to collect brain tissue. If the animal is euthanized for brain tissue collection immediately after the P_{O₂} electrode is pulled out from the guide cannula, then the space occupied by the P_{O₂} electrode tip is identified morphologically in the brain slide. If the P_{O₂} electrode is pulled out from the guide cannula at least 2–3 days before the animal is euthanized, then the tissue slide might show only the guide cannula tube (diameter 0.6 mm) without showing the P_{O₂} electrode tip location. This interesting result may be due to the fact that the striatum tissue of AGS brain is reported to be resistant to the damage induced by implantation of guide cannula, with an absence of tissue damage and cell neuropathology, especially in the hibernating AGS (Zhou et al, 2001; Zhou et al, 2002).

Discussion

Stability of P_{O₂} electrode during measurement

Compared with other methods of brain oxygenation measurement, the platinum wire membrane-coated P_{O₂} electrode has unique benefits despite the disadvantage of invasiveness. It can determine the spatial differences of cerebral oxygenation. This is a remarkable advance because the distribution of O₂ delivery and the P_tO₂ are both temporally and spatially heterogeneous in the different brain tissues (Chi et al, 1996; Ndubuizu & LaManna, 2007). P_tO₂ is a direct indicator of brain oxygenation, which represents a net balance between oxygen delivery (CBF) and O₂ consumption (brain regional metabolic rate for O₂, CMRO₂), and reflects the availability of O₂ in extracellular fluid for oxidative energy (ATP) production. Measurement of P_tO₂ by O₂ microelectrodes is the most localized, sampling only approximately 15 mm² of tissue around the tip (Nortji & Gupta, 2006). The rapid response of the O₂ microelectrode to altering tissue oxygen levels allows more accurate identification of adverse cerebral conditions. P_tO₂ measurement, therefore, has been emerging as a useful clinical tool to monitor brain oxygenation in anesthetized patients (Nortji & Gupta, 2006). However, the stability of the P_{O₂} electrode in biomedical applications has been a concern with regard to obtaining precise and accurate P_{O₂} data. The current drifting of this polarographic electrode during measurement is a major contributor to this stability problem. To address that, we conducted stability tests of the P_{O₂} electrode in both ACSF calibration solutions and in the brain tissues. Panel A of Figure 9 shows the stability of five P_{O₂} electrodes with and without bubbling of 21% O₂. Mean values in P_{O₂,meas. in ACSF solution at 37 °C were plotted against the time. The P_{O₂,meas. was measured in ACSF (15 mL) bubbled with 21% O₂ at flow rate of approximately 1.5 liter/min for 1 hour, which resulted in a reading drift of 5.41 mm Hg/hr in P_{O₂}. Then the bubbling 21% O₂ was turned off, and P_{O₂} was continuously measured in ACSF for 14 hours. The reading drift in P_{O₂} declined to a value of 0.50 mm Hg/hr. This result demonstrates that the stability of the P_{O₂} electrode in the calibration solution is heavily dependent on the bubbling condition and vaporization rate of the water during calibration. It is easy to think that the vaporization is faster with than without a bubbling condition, especially at a higher temperature such as 37 °C. Vaporization induces an increased salinity of the}}

calibration solution, a change known to affect dissolved O_2 . Therefore, the vaporization is the major cause of the drift in reading of the P_{O_2} electrode in the calibration solution. When the P_{O_2} electrode was used in the ACSF without 21% O_2 bubbling, the drift in the P_{O_2} reading was dramatically decreased because, without bubbling, the vaporization was reduced. This result suggests that the calibration of the P_{O_2} electrode in ACSF at 37 °C should not last for too long (no more than 1–2 hours). Vaporization does not exist under the living condition of brain tissue (Figure 9, panel B), and so the drift of the P_{O_2} electrode was significantly lower, -0.027 mm Hg/hr, indicating variation in P_tO_2 is mainly induced by physiologic factors after correction for temperature effects. Therefore, the drift of the P_{O_2} electrode reading in measurements of living tissues should not be a major concern, according to the results in panel A.

Real-time temperature correction for P_tO_2 measurement

The temperature correction for oxygen partial pressure in blood gases (PaO_2) has been well documented and empirical calculations recommended (Severinghaus JW, 1966; Porter, 1979; Andritsch et al, 1981; Van Stekelenburg, 1984; Fedde MR, 1991; Shapiro BA, 1995; Degen et al 1996; Merino, 1999). These methods use off-line correction between two measured values in P_{O_2} , which are obtained at the temperature of 37 °C for in vitro analysis of P_{O_2} with a blood gas analyzer and at the actual body temperature for in vivo sampling. Thus, the temperature correction of P_{O_2} of blood gases was performed between those two temperatures. However, for the real time in vivo measurement of P_tO_2 , temperature correction becomes a more challenging issue because the body temperature varies with time and with physiological conditions. For example, it is well known that conditions under anesthesia (Jiang et al, 1991; Hellyer et al, 1991) or hypoxia (Barnas & Rautenberg, 1990) induce changes in body temperature. In addition, the tissue temperature changes heterogeneously and continuously in different organs of hibernators (Tøien et al, 2001; Ma et al, 2005). Therefore, some physiological studies using P_{O_2} electrodes have even reported P_{O_2} values in tissues without temperature correction because of the lack of a proper real-time correction method. The present study provides, for the first time, a real-time online temperature correction method. To our knowledge, the results shown in Figure 6 are also the first simultaneous measurement of P_tO_2 , T_{brain} , and VO_2 in non-sedated and non-anesthetized animals. Those results demonstrate that the real values of P_tO_2 without accounting for temperature effects (panel C in Figure 6) in the striatum of AGS are significantly different from P_tO_2 values with temperature effects (panel B) during arousal. Our results in VO_2 during arousal (panel D) are similar to other reported results in VO_2 (Tøien et al, 2001). Although changes in T_{brain} of AGS during arousal have not yet been reported, our results in AGS are similar to the reported value in golden-mantled ground squirrels (*Spermophilus lateralis*) during arousal (Trachsel et al, 1991). All the results shown in Figure 6 and Figure 7 demonstrate that this method is a valid and practical method to correct temperature effects on P_{O_2} online and that it is suitable for both general physiologic and hibernation studies, in which a change in temperature is involved.

P_tO_2 of AGS in hibernation and euthermia

Brain P_{O_2} is a measure of the tissue's oxygenation status (Maas et al., 1993; Dings et al., 1996b; Kiening et al., 1996; Van Santbrink et al., 1996; Hoffman et al., 1996). P_tO_2 measures true substrate delivery and represents regional CBF (Zauner et al., 1997). All of the reported studies on P_tO_2 have been conducted under conditions of anesthesia or restraint. The P_tO_2 recorded in non-sedated and non-anesthetized animals has not been previously reported. In this study, we selected the striatum as the recording site of P_tO_2 for two reasons. First, the striatum of AGS, a brain structure thought to reflect global changes rather than region-specific effects (Drew et al, 2007), is vulnerable to ischemia/hypoxia but is tolerant to penetration damage caused by implanted dialysis cannula during hibernation (Zhou et al, 2001, 2002). The neurons in the cerebral cortex and the Purkinje cells in the cerebellum and the pyramidal cells in the

hippocampus are also vulnerable to ischemia/hypoxia and thus could be alternative recording sites, but we do not have any evidence that these two tissues of AGS are also tolerant to damage by electrode penetration during hibernation. Second, electrode measurements can be made more precisely in the striatum because it is a relatively large structure. The P_{tO_2} recorded from the striatum in the present study were extracellular and interstitial values. Care was taken to avoid the damage of blood vessels. If the electrode was in a pool of blood, an abnormally higher P_{O_2} was obtained and the tip location was confirmed with later histological verification. These values, when they occurred, were excluded.

Figure 7 shows pioneering data in P_{tO_2} in the striatum of euthermic and hibernating AGS under non-sedated and non-anesthetized conditions. The P_{tO_2} in the striatum of AGS is significantly higher during euthermia than during hibernation. Our previous study (Ma et al, 2005) showed that blood oxygen partial pressure (PaO_2) in AGS during euthermia is significantly lower than during hibernation. PaO_2 recorded were actually hypoxic in euthermia and normoxic in hibernation. However, the present results in P_{tO_2} suggest that brain oxygenation is normoxic in euthermia and relatively hypoxic in hibernation. In addition, our present results in P_{tO_2} also do not support our previous reported results regarding expression of hypoxia inducible factor-1 α (HIF-1 α) in the brains of euthermic AGS, in which we found that HIF-1 α expression in the forebrain tissue was significantly higher in euthermia than in hibernation (Ma et al, 2005). According to those previous results in HIF-1 α , it appeared that brain tissues are hypoxic in euthermia and normoxic in hibernation. Higher expression of HIF-1 α in euthermia in AGS brain can account for the fact that the expression of HIF-1 α in euthermic AGS brains is not induced by real tissue hypoxia. It is more likely that the expression of HIF-1 α in euthermic AGS brain is induced by other factors, for instance, by undetermined nitric oxide (NO) (Mateo et al, 2003) or by an abnormal, higher concentration of $CoCl_2$ in tissue (Sharp et al., 2001). For a further explanation of those results, determination of the concentration of NO and $CoCl_2$ in the striatum will be necessary.

This intriguing difference of P_{O_2} between brain tissue and blood during two physiologic states suggests that regional protection mechanisms in the brain play a role in maintaining tissue oxygenation against tissue hypoxia during hibernation. This protection mechanism may also explain the morphologic cellular results of brain tissue in our previous study (Ma et al, 2005), in which the neuronal pathology or evidence of intra-neuronal oxidative stress was absent in the brain of both euthermic and hibernating AGS. However, the details about such a mechanism to protect normal brain oxygenation during hibernation are not yet understood.

In summary, this study investigated a novel method that allows online correction of the effect of temperature on P_{O_2} , a method that can be used to measure real-time in vivo P_{tO_2} and brain temperature simultaneously in conjunction with VO_2 in non-sedated and non-anesthetized freely moving animals. This method fills an important research gap—the lack of a suitable method for physiologic studies of tissue P_{O_2} in hibernating or other cool-blooded species. The technique could also be of use in studies of hypoxic ischemic brain injury and other states of metabolic dysfunction as well.

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Figure 1.

Water vapor pressure changes with temperature. The dashed line with black dots shows the relation between $P_{\text{H}_2\text{O}}$ and temperature. The solid line shows the fitting curve plotted with use of an Excel power function.

Figure 2.

Measured P_{O_2} values ($P_{O_2, \text{ meas. }}$) in ACSF plots against temperature. The data in $P_{O_2, \text{ meas. }}$ were recorded with one O_2 electrode at 37°C in ACSF solution bubbled with 21% O_2 .

Figure 3.
The calculated P_{O_2} values ($P_{O_2, \text{cal.}}$) without temperature effects corresponding to measured P_{O_2} values ($P_{O_2, \text{meas.}}$) shown in Figure 2.

Figure 4.

$P_{O_2,cal.} / P_{O_2, meas.}$ plots against temperature for defining correction factor of P_{O_2} electrode. The dashed line with solid triangles shows the relation between $P_{O_2,cal.} / P_{O_2, meas.}$ and temperature. The solid line shows the fitting curve plotted with an Excel power function.

Figure 5.

Experimental chamber. A euthermic AGS is in recording for P_{tO_2} , T_{brain} and VO_2 . The open-flow experimental chamber are set on the rotator plat. The air goes in upper plastic tubing and comes out from bottom tubing. All wires from P_{O_2} electrode, reference electrode, and micro-thermocouple are come out from the middle top of metal cover through a sprint tubing and connect to P_{O_2} and thermocouple meter, respectively.

Figure 6.

The representative plotting of simultaneous measurements of P_{tO_2} , T_{brain} , and VO_2 during arousal from hibernation of AGS in a cold chamber at 2 $^{\circ}\text{C}$. First and second dashed line from left shows the time when arousal and euthermia started. A) Changes in brain temperature during arousal. B) Changes in P_{tO_2} without temperature correction ($P_{\text{O}_2, \text{ meas.}}$). C) Changes in online-calculated P_{tO_2} with temperature correction ($P_{\text{O}_2, \text{ cal.}}$). D) Changes in VO_2 .

Figure 7. P_{tO_2} and T_{brain} in the striatums of AGS brains and VO_2 during euthermic and hibernating states (N=5), measured in non-sedated and non-anesthetized AGS at 2 °C. The dashes on the top of bars show the significant differences between two experimental groups.

Figure 8.

A representative tip location of P_O₂ electrode in the left striatum of AGS brain. The upper arrow points to the guide cannula tube and the lower arrow points to the tip location of the P_O₂ electrode in the left striatum of AGS.

A. P_{O_2} electrode stability in ACSF at 37 °C

B. P_{O_2} electrode stability in the striatums of euth AGS at 37 °C

Figure 9.

Stability tests of P_{O_2} electrodes in ACSF solution and in tissue fluids of the striatums of winter euthermic AGS with brain temperature of around 36.5 °C in a cold chamber of 2 °C. A) Stability of P_{O_2} electrode in ACSF ($n=5$, mean of P_{O_2} drift = 5.41 mm Hg/hr with bubbling 21% O_2 ; 0.50 mm Hg/hr without bubbling of 21% O_2). Arrow shows the time when 21% O_2 was off. B) Stability of P_{O_2} electrodes in the striatums of AGS brains during euthermia (average values in P_tO_2 were used for plotting, $n=3$, mean of P_{O_2} drift = -0.02661 mm Hg/hr).