Fluctuation of Brain Tissue Oxygen Partial Pressure: A Biochemical Landmark in the Arctic Ground Squirrel’s Spontaneous Arousal

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Abstract: Hibernation in the Arctic Ground Squirrel (AGS) is a regulated, adaptive response to arctic environmental conditions. Problem statement: Regional brain Blood Flow (rCBF) has been observed to increase dramatically during arousal in hibernators. However, the real time dynamic change in P$_{O_2}$ during arousal has not been studied, we hypothesized that P$_{O_2}$ is Interdependent of T$_{brain}$ and a key component in the arousal process. Approach: Simultaneous in vivo measurements of P$_{O_2}$ and brain temperature (T$_{brain}$) in conjunction with oxygen consumption (V$_{O_2}$) were conducted in the striatum of non-sedated, non-anesthetized Arctic ground squirrels during spontaneous arousal from hibernation. Results: A dramatic fluctuation of brain tissue oxygen partial pressure (P$_{O_2}$) is associated with the complex phenomena of spontaneous arousal. In this study, we observed that: (1) a P$_{O_2}$ elevation precedes changes in T$_{brain}$ and V$_{O_2}$; (2) P$_{O_2}$ changes do not correlate with changes in V$_{O_2}$ during arousal and (3), endogenous O$_2$ shift from O$_2$ enriched blood to brain in hibernating AGS induces an arousal with the pharmaceutical chemical, efaproxiral (RSR-13). Conclusion: The four turning points of P$_{O_2}$ appearing at different T$_{brain}$ during arousal suggest that changes in P$_{O_2}$ are T$_{brain}$ interdependent and support the concept that arousal from hibernation is complex process invoking different feedbacks.

Key words: Arctic Ground Squirrel (AGS), Institutional Animal Care and Use (IAUC), Central Nervous System (CNS), blood oxygen partial pressure

INTRODUCTION

Hibernation is a well regulated adaptive response to environmental conditions of low ambient temperature (T$_a$) and food shortage during winter (Ortmann and Heldmaier, 2000; Jinka et al., 2011). Body temperature, metabolic rate, heart rate, blood pressure and cerebral blood flow are greatly reduced relative to resting euthermic state in natural hibernation (Osborne et al., 2005; Toien et al., 2011). A regulated body temperature drops close to, or even below zero degree, whole body metabolism falls to only 1-2% of basal values during hibernation of Arctic Ground Squirrels (AGS). Blood flow slows in most of the vascular system to slightly above zero and the nervous system activity is reduced (Ma et al., 2005). Yet, this state is spontaneously reversible (Heller and Ruby, 2004).

AGS, a polar hibernator, is able to endure extreme changes in endogenous O$_2$ under different natural physiological states. During hibernation, AGS blood is well oxygenated with similar blood oxygen partial pressure (P$_{O_2}$) as compared to non-hibernator rat brains (Ma et al., 2005), but AGS brain tissue oxygen partial pressure (P$_{O_2}$) is low (Ma and Wu, 2008). When aroused from hibernation, AGS experiences a drop in P$_{O_2}$ to ~ 9 mm Hg, but while hypoxic there is no evidence of cellular stress, inflammatory response, neuronal pathology, or oxidative modification in brain following the period of high metabolic demand necessary for arousal (Ma et al., 2005). In their euthermic state, AGS have shown low P$_{O_2}$ and low hemoglobin oxygen saturation (sO$_2$) which was not observed in rats (Ma et al., 2005). However, the P$_{O_2}$ in the AGS euthermic state is higher than in the hibernating state (Ma and Wu, 2008; Ma et al., 2009), suggesting the changes are related to O$_2$ delivery to the brain. Regional brain blood flow (rCBF) has been observed to increase dramatically in hibernator s (Osborne and Hashimoto, 2003). However, since studies on the real time dynamic change in P$_{O_2}$ during arousal is sporadic, we hypothesized that P$_{O_2}$ is Interdependent of T$_{brain}$ and a key component in the
arousal process. The present study demonstrated that $P_{O_2}$ showed experienced dramatic fluctuations and that these changes in $P_{O_2}$ are critical factors in the complex process of arousal from hibernation.

**MATERIALS AND METHODS**

**Animals:** All procedures were performed in accordance with and approved by University of Alaska Fairbanks Institutional Animal Care and Use (IAUC) guidelines. 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°40'W; elevation 809 m) and transported to Fairbanks (permit obtained from Alaska Department of Fish and Game). Ground squirrels 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 4:20 h light: dark cycle.

**Stereotaxic surgery for brain guide cannula implantation:** The animals used in this proposal were male and female AGS (body mass: 650-840 g) at the time of the surgery. The surgeries for AGS were conducted in late September before hibernation. Animals fasted at least 12 h before the surgery. Surgeries were performed under general anesthesia with isoflurane (Halocarbon Laboratories, River Edge, NJ), induced at 5% and maintained at 1.5-3% mixed with 100% medical grade $O_2$. Animals were anesthetized at 5% and maintained at 1.5-3% mixed with 100% medical grade $O_2$ at a flow rate of 1.5 L min$^{-1}$. Under strict aseptic conditions, a skin incision was made midline on the head and the working area was exposed with a retractor. The soft tissues were scraped with a surgical 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, 13.5-14 mm; L±3.25 mm; D, -4.0 mm) for AGS 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 previously described in detail (Osborne et al., 1999; Zhou et al., 2002; Ma and Wu, 2008) and were slowly lowered 4 mm from the cortical surface. All cannula coordinates are measured from the midline and Frontal Nasal Suture. Cannulae were secured to the screws with dental cement.

Antibiotics (Baytril; Bayer Corp., Shawnee Mission, KS, dose: 5 mg kg$^{-1}$) were given twice a day, 1 day before surgery and 2 days after surgery, by subcutaneous injection in the back of the neck. After surgery, animals were allowed to recover completely at room temperature and daily cleanings were performed with 3% betadine for 10-15 days. Following recovery, AGS were housed individually in a cool chamber at 2°C under a light regime of 12:12 h light: dark.

**Calibration for $P_{O_2}$ electrode and thermocouple and temperature correction for $P_{O_2}$**

**Calibration of $P_{O_2}$ electrode and thermocouple.** For the measurement of $P_{O_2}$, a 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; CaCl$_2$:1.2; MgCl$_2$: 0.85; D-Glucose:1.4; NaHCO$_3$:24, pH 7.4) at 37°C equilibrated with air (21% $O_2$) and $N_2$ (0% $O_2$), respectively, before and after each experiment to ensure 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 an 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 -2-40°C. Compressed air containing 21% $O_2$ 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 to 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.** Because brain temperature varies at any given time, $P_{O_2}$ measurement had to be corrected to obtain the real physiological effect. The details of the principle and the procedures of temperature correction for $P_{O_2}$ measurement was described in Ma and Wu (2008) Ma et al. (2009) and Ma et al. (2011). Briefly, to perform such a temperature correction, the following steps were taken:

- 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...
The P

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The actual values of P

Under bubbling conditions of 21% O

where, the values of water vapor pressure (P

These data-P

temperatures of 0-40°C and the curve function fitted with an Excel program to find the values of A and k. After A and k values were defined, the function, P

f = P

“P

The measurement of brain temperature can more precisely reflect the regional metabolic mechanisms of the brain than the measurement of core body temperature; (2) Core body temperature (T

After animals recovered from stereotaxic surgery for brain guide cannulae implantation, the insertions of the reference electrode, the PO

The systemic metabolic rate (oxygen consumption, \( V_{\text{O}_2} \)) of AGS was recorded by indirect calorimetry in a vertical cylindrical-shaped Plexiglas metabolic chamber (I.D. 29 cm, height 32 cm) previously described (Ma and Wu, 2008; Ma et al., 2009; Ma et al., 2011). Air was drawn into a distribution tube through a vertical cylindrical-shaped Plexiglas metabolic chamber at a flow rate of 3 L min

\[ V_{\text{O}_2} = V_{\text{E}} \times (F_{\text{I_{102}}} - F_{\text{E_{102}}})/(1 - (1 - RQ) \times F_{\text{I_{102}}}), \]  

The PO

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rate of 1 min\(^{-1}\) and without further signal filtering. A PO\(_2\) 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 of the data acquisition program. A Sable interface (model: U12, Henderson, NV) was used as A/D converter for the \(\bar{V}_{O_2}\) measurement system. The output signals were then input into the interface of the data acquisition program. All experiments for PO\(_2\), T\(_{brain}\) and V\(_{\text{O}_2}\) measurement were conducted in the experimental chamber at 2\(^\circ\)C.

Data in PO\(_2\), meas. recorded originally by the PO\(_2\) electrode in one channel were calibrated online into corresponding values of PO\(_2\), cal. without the effects of temperature in another channel.

AGS in the present study used to start to enter the hibernation two to four weeks after experiment recording for PO\(_2\), T\(_{brain}\) and V\(_{\text{O}_2}\) was set up. Data in PO\(_2\), T\(_{brain}\) and V\(_{\text{O}_2}\) were recorded continuously and collected from beginning to end of first and/or second bout of the hibernation for all AGS.

Drug administration and f\(_R\) recording: The drug efaproxiral, 2-[4-[[3, 5-dimethylanilino) carbonyl]-methyl]phenoxyl]-2-methylpropionic acid (RSR-13, Allos Therapeutics, Inc, Westminster, Colorado) was given as a single injection (150 mg kg\(^{-1}\)) by i.p. The drug was provided as a solution in normal saline at concentration of 20 mg mL\(^{-1}\). Saline was used as control and administrated with the same injected volume and at the same time course as drug.

Respiratory frequency (f\(_R\)) was counted manually by direct observation during the entire experimental period with 30-min intervals beginning 60 min before drug administration and ending 120 min after drug application. Counting for f\(_R\) was blindly performed by a technician.

After PO\(_2\), T\(_{brain}\), f\(_R\) and V\(_{\text{O}_2}\) recording was completed, the animals were euthanized with isoflurane under deep anesthetic condition. The brain tissues were immediately frozen in 2-methylbutane at -40°C and kept in a freezer at -80°C. Histologic analysis of the brains using the Cresyl Violet method to identify the PO\(_2\) electrode tip location was performed for each animal, as previously described in detail (Ma and Wu, 2008) and all tips of the PO\(_2\) electrodes were confirmed to be in the striatums.

RESULTS

Dramatic PO\(_2\) fluctuation during arousal: Figure 1 compares changes in PO\(_2\), V\(_{\text{O}_2}\) and T\(_{brain}\) during a spontaneous arousal in one representative AGS, which was selected from synchronized experimental recordings of four AGS.

While a sudden drop of PO\(_2\) indicated by first dash line from appeared, no changes in T\(_{brain}\) and V\(_{\text{O}_2}\) remained unchanged. After its small downward change, PO\(_2\) increased rapidly with a larger increase in PO\(_2\) approximately 0.3 h later. V\(_{\text{O}_2}\) started to increase 2 h after the initial downward change of PO\(_2\). The second increase in PO\(_2\) occurred 4 h after the downward change reaching a maximum peak value as indicated by second dash line (Fig. 1). T\(_{brain}\) and V\(_{\text{O}_2}\) were still increasing to their maximum values.
Panel “B” in Fig. 2 shows the changes with brain temperature during arousal. No change in $V_{\text{O}_2}$ was observed during $T_{\text{brain}}$ increase from 5-10°C, then showed a small increase between 10-15°C and rapidly increased during 15-22°C. $V_{\text{O}_2}$ remained at relatively stable levels during 22-30°C but started to decline after brain temperature rose over 30°C.

Panel “C” shows no correlation between $P_{\text{O}_2}$ and $V_{\text{O}_2}$ during arousal. This result means that $P_{\text{O}_2}$ during arousal is independent of $V_{\text{O}_2}$ changes.

These relationships of $P_{\text{O}_2}$, $T_{\text{brain}}$ and $V_{\text{O}_2}$ during a natural arousal indicate that complex feedbacks were occurring.

Efaproxirial induced an arousal via $P_{\text{O}_2}$: In order to confirm that the change in $P_{\text{O}_2}$ is related to the arousal process, the pharmaceutical agent, efaproxiral, was used to cause an endogenous increase in $P_{\text{O}_2}$. Efaproxirial increases tissue oxygen delivery by decreasing the affinity of hemoglobin for oxygen. Effectively shifting the Hb-$O_2$ dissociation curve to the right has been demonstrated in animal studies (Watson et al., 1997) and in models of human stroke (Doppenberg et al., 1997). “A” in Fig. 3 shows a representative result in changes of $P_{\text{O}_2}$, $V_{\text{O}_2}$, $T_{\text{brain}}$ and respiratory rate (f$_R$) selected from five experimental recordings with three hibernating AGS before, during and after drug administration. Saline was injected (i.p.) at time showed by first dash line from left and no obvious $P_{\text{O}_2}$ change was observed for one h. Efaproxiral was then injected at time showed by second dash line. A $P_{\text{O}_2}$ jump appeared approximate 5 m after drug injection and then $P_{\text{O}_2}$ increased gradually and reached the maximum indicated by 3rd and 4th dash line. A remarkable elevation of $P_{\text{O}_2}$ values compared to control levels was obtained after drug administration. $P_{\text{O}_2}$ started to decline before $T_{\text{brain}}$ and $V_{\text{O}_2}$ reached the maximum. Similar lag of $V_{\text{O}_2}$ peak and $T_{\text{brain}}$ maximum following $P_{\text{O}_2}$ elevation were observed except f$_R$. AGS aroused after injection of efaproxirial.

Efaproxirial increased $P_{\text{O}_2}$ and induced an arousal in all AGS. This result supports that the endogenous increase of $P_{\text{O}_2}$ is a key factor in the arousal. Efaproxirial is a good enhancer of $P_{\text{O}_2}$ by increasing $O_2$ delivery to the brain. Also, to verify that saline injections had no chronic effect on the hibernation, two saline injections with the same volume and same time course as Efaproxirial administration were delivered to three hibernating AGS and no AGS aroused after saline injection (“B” in Fig. 3).
DISCUSSION

The Central Nervous System (CNS) is considered the most sensitive organ in the body to the lack of oxygen (Milledge, 2000) because of its heavy dependence on aerobic metabolism and its high resting oxygen consumption (Davis, 1975; Kontos et al., 1978). Metabolic suppression and the complex control of thermoregulatory process have been investigated in mammalian hibernators for many years (Toien et al., 2011; Jinka et al., 2011).

Very limited evidence has shown that oxygen partial pressure ($P_{O_2}$) may be involved in the modulation of CNS control of the arousal process. Oxygen levels are correlated with regulation of tissue perfusion (McCabe et al., 2003) and tissue respiration (Hogan et al., 1992) and $P_{O_2}$ was supported as a messenger in metabolic control (Platzack and Hicks, 2001). The tissue oxygen concentration is a critical biochemical variable in nuclear gene expression and mitochondrial DNA-encoded complex I gene expression (Meyron-Holtz et al., 2004; Pirurat and Lopez-Barneo, 2005). The present data suggests that $P_{O_2}$ is also involved in the complex process regulating hibernation.

The involvement of $P_{O_2}$ in the initiation of an arousal event is demonstrated by the preceding change in $P_{O_2}$ prior to both $T_{brain}$ and $V_{o2}$ in Fig. 1. The arousal begins with a sudden $P_{O_2}$ decrease followed by a rapid jump in $P_{O_2}$ with an increase in $P_{O_2}$ levels. A maximum peak value of $V_{o2}$ occurring during arousal supports the observations of Toien et al. (2001). In the
The rapid jump in \( P_{O_2} \) during arousal; the increase in \( rCBF \) can be related to the change in \( T_{brain} \); in a previous study, the change in oxygen partial pressure in arterial blood (\( P_{O_2} \)) in hibernating AGS suggested that blood \( O_2 \) was shunted to the brain (Ma et al., 2005). Osborne and Hashimoto (2003) observed that \( T_{brain} \) varied with \( rCBF \) (Hayward and Baker, 1968; 1969).

Four turning points of \( P_{O_2} \) appearing at \( T_{brain} \) values of 5-35°C during arousal may represent the critical points for \( P_{O_2} \) at which some physiological and/or biochemical metabolic mechanisms were triggered and/or switched (“A” in Fig. 2). When \( P_{O_2} \) reaches the maximum, \( T_{brain} \) and \( V_{al} \) are still only at half of their maximum values. The increase in \( P_{O_2} \) Precedes \( V_{al} \) and \( T_{brain} \) during arousal. All these results support that \( P_{O_2} \) is a biochemical landmark for brain activity during the arousal event. Thus \( P_{O_2} \) reflects an increase in CNS activity which, in turn, is a physiological prelude to whole animal arousal from torpor. The effect of efaproxiral on \( P_{O_2} \) (“A” in Fig. 3) provides additional support to the idea that \( P_{O_2} \) levels are involved in regulating the complex process of arousal. Efaproxiral leads to the CNS enriched in \( O_2 \) which is redistributed from blood (\( P_{O_2} \) (“A” in Fig. 3).

In summary, \( P_{O_2} \) plays a key role in triggering and regulating a spontaneous arousal, which is demonstrated by: (1) a preceding \( P_{O_2} \) elevation before changes in \( T_{brain} \) and \( V_{al} \); (2) \( P_{O_2} \) changes do not correlate with changes in \( V_{al} \) during arousal; (3) endogenous \( O_2 \) shift from \( O_2 \) enriched blood to brain in hibernating AGS induces an arousal with the pharmaceutical chemical, efaproxiral (RSR-13); (4) four turning points of \( P_{O_2} \) appearing at different \( T_{brain} \) during arousal suggest that changes in \( P_{O_2} \) are \( T_{brain} \) dependent.

CONCLUSION

The four turning points of \( P_{O_2} \) appearing at different \( T_{brain} \) during arousal suggest that changes in \( P_{O_2} \) are \( T_{brain} \) interdependent and support the concept that \( P_{O_2} \) plays a key role in triggering and regulating a spontaneous arousal.

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