

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

Yi Long Ma, Shu Fen Wu and Lawrence K. Duffy

Alaska Neuroscience Program, Institute of Arctic Biology,
University of Alaska Fairbanks, 902 N Koyukuk Dr, Fairbanks, 99775-7000, Alaska

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_{iO_2} during arousal has not been studied, we hypothesized that P_{iO_2} is Interdependent of T_{brain} and a key component in the arousal process. **Approach:** Simultaneous *in vivo* measurements of P_{iO_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_{iO_2}) is associated with the complex phenomena of spontaneous arousal. In this study, we observed that: (1) a P_{iO_2} elevation precedes changes in T_{brain} and V_{O_2} ; (2) P_{iO_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_{iO_2} appearing at different T_{brain} during arousal suggest that changes in P_{iO_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

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_aO_2) as compared to non-hibernator rat brains (Ma *et al.*, 2005), but AGS brain tissue oxygen partial pressure (P_{iO_2}) is low (Ma and Wu, 2008). When aroused from hibernation, AGS experiences a drop in P_aO_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_aO_2 and low hemoglobin oxygen saturation (sO_2) which was not observed in rats (Ma *et al.*, 2005). However, the P_{iO_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 hibernators (Osborne and Hashimoto, 2003). However, since studies on the real time dynamic change in P_{iO_2} during arousal is sporadic, we hypothesized that P_{iO_2} is Interdependent of T_{brain} and a key component in the

Corresponding Author: Yi Long Ma, Alaska Neuroscience Program, Institute of Arctic Biology, University of Alaska Fairbanks, 902 N Koyukuk Fairbanks, 99775-7000, Alaska Fax: 907-474-7827

arousal process. The present study demonstrated that P_tO_2 showed experienced dramatic fluctuations and that these changes in P_tO_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 (IACUC) 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°38'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 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; $L \pm 3.25$ 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; $L \pm 3.25$ 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_tO_2 : 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; $CaCl_2$: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 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 $\pm 0.1^\circ C$ in a water circulating bath before gas sterilization.

Temperature correction for P_{O_2} measurement. Because brain temperature varies at any given time, P_tO_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_tO_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

equilibrated with compressed nitrogen gas (0% O₂) at 37°C

- Under bubbling conditions of 21% O₂ in ACSF solutions ranging in temperature from 0-40°C, the signal outputs of the P_{O₂} electrode (P_{O₂,meas.}) were measured
- The actual values of P_{O₂} (P_{O₂,cal.}), independent of temperature effects, were calculated at each given temperature according to the equation:

$$P_{O_2, cal.} (\text{mm Hg}) = (P_{atm} - P_{H_2O}) * FO_2,$$

where, the values of water vapor pressure (P_{H₂O}) (Weast, 1968; Withers, 1977), were appropriate for a given temperature.

These data-P_{O₂,meas.}, P_{O₂,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_{O₂} of tissues measured at various temperatures:

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

“P_{O₂,cal./P_{O₂,meas.}” were plotted against t at 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_{O₂,cal. = (Ae^{-kt}) (P_{O₂,meas.), was programmed into the data acquisition program (PowerLab) for online temperature correction of P_{O₂} measurements.}}}

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

Real-time measurement of P_tO₂ and T_{brain} in the striatum of AGS in conjunction with \dot{V}_{O_2} . The P_tO₂ in the striatum was measured by the implanted PO₂ electrode, T_{brain} was measured by needle micro thermocouple with a tip diameter of 0.22 mm (HYPO-33-1, Omega, Stamford, CT, USA) through guide cannula. The core body temperature was not measured in the present study because: (1) 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_b) could not be used for online calculation of P_tO₂ because the T_b changes lag behind T_{brain}.

After animals recovered from stereotaxic surgery for brain guide cannulae implantation, the insertions of the reference electrode, the P_{O₂} 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; a general anesthetic procedure with isoflurane gas was performed before insertion of the P_{O₂} electrode and micro-thermocouple. After implantation of the reference electrode, the calibrated and sterilized P_{O₂} electrode and needle micro-thermocouple for T_{brain} were inserted through the pre-implanted guide cannulae into the left and right striatum, respectively and secured by medical tape. The tips of the P_{O₂} electrode and needle micro-thermocouple extended about 2 mm beyond the guide cannulae tubes. Buprenorphine (0.03 mg kg⁻¹), a pain reliever, was given by subcutaneous injection on the back, with one single dose after insertions of the P_{O₂} electrode and thermocouple for 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_{O₂} electrode and reference electrode to P_{O₂} meter and from thermocouple to thermocouple meter went through a sprint tube and came out the experimental chamber in the middle of the top.

The systemic metabolic rate (oxygen consumption, \dot{V}_{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⁻¹ 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 with molecular sieves and connected in a series with a Nafion drying column. O₂ extraction was measured with an O₂ analyzer (Model: FoxBox 2.0, Sable System International, Inc, Las Vegas, NV), which was calibrated with air. \dot{V}_{O_2} was calculated with an Excel program on the basis of the O₂ fraction differences between fresh air (before being used by an animal) and expired air (after being used by an animal), flow rate of sampling air, animal body weight and a known respiratory quotient value from previous experiments with AGS (Tøien *et al.*, 2001; Ma *et al.*, 2005), according to Withers principles of Eq. 1:

$$\bar{V}_{O_2} = V_E * (F_{IO_2} - F_{EO_2}) / ((1 - (1 - RQ) * F_{IO_2})), \quad (1)$$

The P_tO₂, T_{brain} and \dot{V}_{O_2} were recorded simultaneously from 20 m after buprenorphine injection to the end of experiment 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^{-1} 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 of the data acquisition program. A Sable interface (model: U12, Henderson, NV) was used as A/D converter for the \dot{V}_{O_2} measurement system. The output signals were then input into the interface of the data acquisition program. All experiments for P_{tO_2} , T_{brain} and \dot{V}_{O_2} measurement were conducted in the experimental chamber at 2°C . Data in $P_{O_2, \text{meas}}$, recorded originally by the P_{O_2} electrode in one channel were calibrated online into corresponding values of $P_{O_2, \text{cal}}$, 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 P_{tO_2} , T_{brain} and \dot{V}_{O_2} was set up. Data in P_{tO_2} , T_{brain} and \dot{V}_{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]phenoxy]-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 administered 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 m before drug administration and ending 120 m after drug application. Counting for f_R was blindly performed by a technician.

After P_{tO_2} , T_{brain} , f_R and \dot{V}_{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 P_{O_2} electrode tip location was performed for each animal, as previously described in detail (Ma and Wu, 2008) and all tips of the P_{O_2} electrodes were confirmed to be in the striatum.

RESULTS

Dramatic P_{tO_2} fluctuation during arousal: Figure 1 compares changes in P_{tO_2} , \dot{V}_{O_2} and T_{brain} during a spontaneous arousal in one representative AGS, which was selected from synchronized experimental recordings of four AGS.

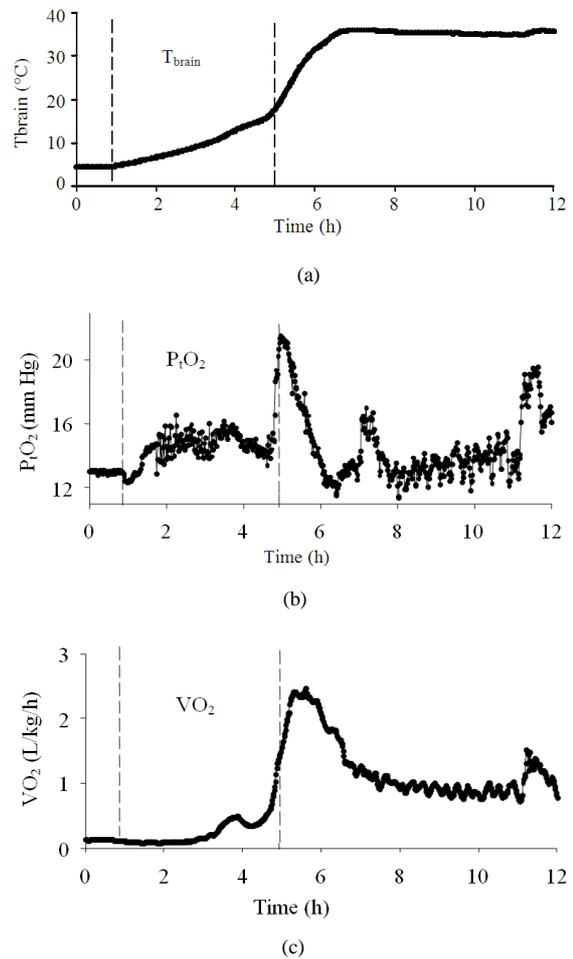


Fig. 1: P_{tO_2} fluctuates during arousal of AGS. The vertical dashed lines indicate the synchronized time course for P_{tO_2} , T_{brain} and \dot{V}_{O_2} . The first dash line identifies a sudden decrease in P_{tO_2} from baseline at the initiation of arousal and the second vertical dashed line shows a P_{tO_2} peak before T_{brain} and \dot{V}_{O_2} peak

While a sudden drop of P_{tO_2} indicated by first dash line from appeared, no changes in T_{brain} and \dot{V}_{O_2} were observed at that point. Right after a decrease in P_{tO_2} , T_{brain} began to increase gradually and \dot{V}_{O_2} remained unchanged. After its small downward change, P_{tO_2} increased rapidly with a larger increase in P_{tO_2} approximately 0.3 h later. \dot{V}_{O_2} started to increase 2 h after the initial downward change of P_{tO_2} . The second increase in P_{tO_2} occurred 4 h after the downward change reaching a maximum peak value as indicated by second dash line (Fig. 1). T_{brain} and \dot{V}_{O_2} were still increasing to their maximum values.

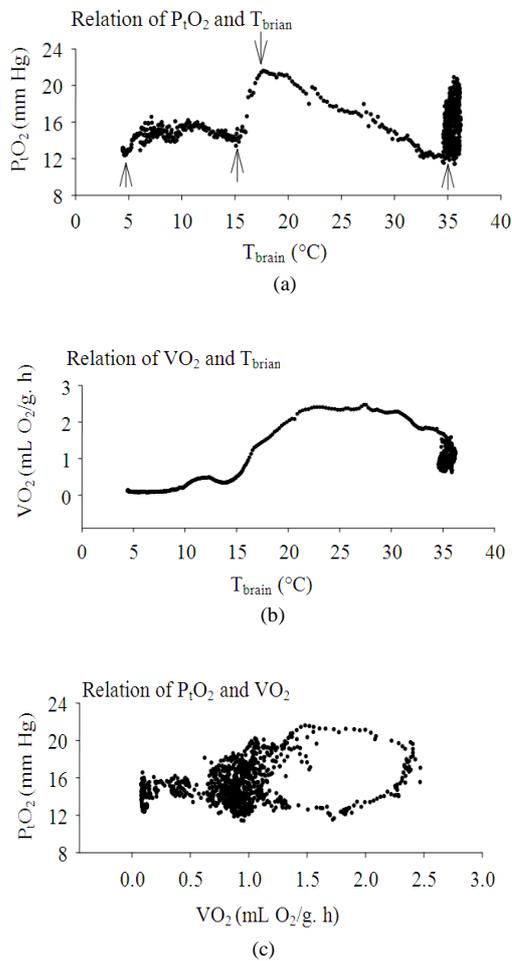


Fig. 2: Relations between P_tO₂, V_{O₂} and T_{brain} during a spontaneous arousal. P_tO₂ and T_{brain}; B. V_{O₂} and T_{brain}; C. P_tO₂ and V_{O₂}

The maximum V_{O₂} peak value reached about 0.5 h after the maximum P_tO₂ peak value.

The maximum body temperature (36.5°C) was reached almost 1 h after maximum P_tO₂ peak value.

Using the same AGS as in Fig. 1, the correlation among P_tO₂, T_{brain} and V_{O₂} during a natural arousal is shown in Fig. 2. The top panel, “A”, in Fig. 2 shows the P_tO₂ changes with T_{brain} during the arousal. P_tO₂ was gradually increased while brain temperature rose from 5-15°C. After brain temperature reached 15-18°C, P_tO₂ increased rapidly. After brain temperature was over 18°C, P_tO₂ declined continuously until the temperature reached 35°C.

Panel “B” in Fig. 2 shows the V_{O₂} changes with brain temperature during arousal. No change in V_{O₂} was observed during T_{brain} increase from 5-10°C, then showed a small increase between 10-15°C and rapidly increased during 15-22°C. V_{O₂} 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_tO₂ and V_{O₂} during arousal. This result means that P_tO₂ during arousal is independent of V_{O₂} changes.

These relationships of P_tO₂, T_{brain} and V_{O₂} during a natural arousal indicate that complex feedbacks were occurring.

Efaproxiral induced an arousal via P_tO₂: In order to confirm that the change in P_tO₂ is related to the arousal process, the pharmaceutical agent, efaproxiral, was used to cause an endogenous increase in P_tO₂. Efaproxiral increases tissue oxygen delivery by decreasing the affinity of hemoglobin for oxygen. Effectively shifting the Hb-O₂ 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_tO₂, V_{O₂}, T_{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_tO₂ change was observed for one h. Efaproxiral was then injected at time showed by second dash line. A P_tO₂ jump appeared approximate 5 m after drug injection and then P_tO₂ increased gradually and reached the maximum indicated by 3rd and 4th dash line. A remarkable elevation of P_tO₂ values compared to control levels was obtained after drug administration. P_tO₂ started to decline before T_{brain} and V_{O₂} reached the maximum. Similar lag of V_{O₂} peak and T_{brain} maximum following P_tO₂ elevation were observed except f_R. AGS aroused after injection of efaproxiral.

Efaproxiral increased P_tO₂ and induced an arousal in all AGS. This result supports that the endogenous increase of P_tO₂ is a key factor in the arousal. Efaproxiral is a good enhancer of P_tO₂ by increasing O₂ 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 Efaproxiral administration were delivered to three hibernating AGS and no AGS aroused after saline injection (“B” in Fig. 3).

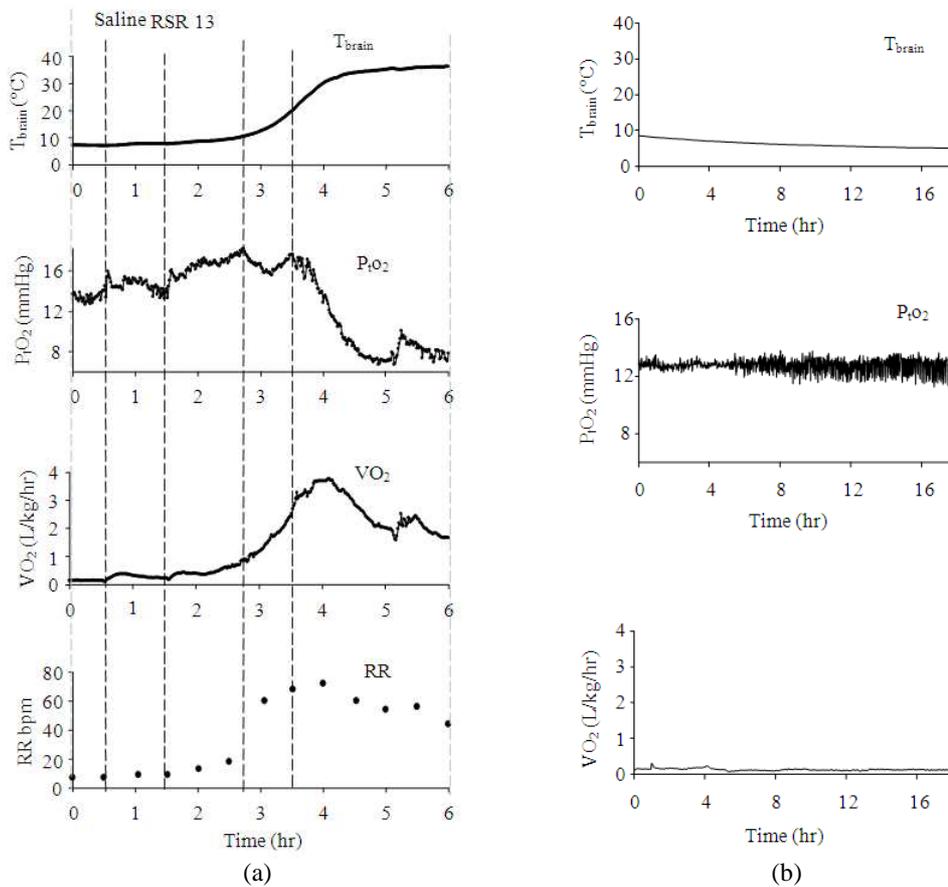


Fig. 3: A representative induced arousal by drug efaproxiral (RSR-13). A. Enhanced O_2 delivery to brain with efaproxiral induces an arousal by increasing P_{tO_2} . Saline (control) and efaproxiral are given at time showed by first and second dash line from left. The peaks in P_{tO_2} are showed by 3rd and 4th line. B. Saline injections have no effect on hibernating AGS. Salines with the same volume at the same time course as efaproxiral were administrated twice and no arousal was observed in 18 h

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; Piruat and Lopez-Barneo, 2005). The present data suggests that PO_2 is also involved in the complex process regulating hibernation.

The involvement of P_{tO_2} in the initiation of an arousal event is demonstrated by the preceding change in P_{tO_2} prior to both T_{brain} and \dot{V}_{O_2} in Fig. 1. The arousal begins with a sudden P_{tO_2} decrease followed by a rapid jump in P_{tO_2} with an increase in P_{tO_2} levels. A maximum peak value of \dot{V}_{O_2} occurring during arousal supports the observations of Toien *et al.* (2001). In the

present study, the delay times of the \dot{V}_{O_2} maximum showed small variations in each AGS. These delay times of the \dot{V}_{O_2} maximum are partially explained by the dead volume of the experimental Plexiglas chamber (7.058 L), which had a calculated delayed time that is 4.3 min at flushing flow rate of 3.0 L min^{-1} using reported method (Bartholomew *et al.*, 1981). However, the consistent existence of this \dot{V}_{O_2} delay suggests it is a true physiological consequence of an active P_tO_2 feedback during arousal in AGS. The lag time (30-45 m) of \dot{V}_{O_2} reaching the maximum value compared to P_tO_2 peak is shorter than the lag time for maximum T_{brain} , which agrees with Milsom *et al.* (2001) observation in which ventilation rate and metabolism increased rapidly prior to any changes in body temperature after animal become committed to the arousal process.

The correlation analysis among P_tO_2 , \dot{V}_{O_2} and T_{brain} during arousal (Fig. 2) gives solid support to P_tO_2 feedback in the regulation of the arousal process. "C" in Fig. 2 shows that no correlation exists between P_tO_2 and \dot{V}_{O_2} during arousal. The rapid jump in P_tO_2 can be attributed to cardiovascular/CNS regulation of heart rate and a resulting arrhythmia during arousal. In a previous study, the change in oxygen partial pressure in arterial blood (P_aO_2) in hibernating AGS suggested that blood O_2 was shunted to the brain (Ma *et al.*, 2005). Osborne and Hashimoto (2003) observed that critical Cerebral Blood Flow (rCBF) increased dramatically during arousal. The increase in rCBF can be related to the change in T_{brain} by the demonstration that brain temperature varied with rCBF (Hayward and Baker, 1968; 1969).

Four turning points of P_tO_2 appearing at T_{brain} values of 5-35°C during arousal may represent the critical points for P_tO_2 at which some physiological and/or biochemical metabolic mechanisms were triggered and/or switched ("A" in Fig. 2). When P_tO_2 reaches the maximum, T_{brain} and \dot{V}_{O_2} are still only at half of their maximum values. The increase in P_tO_2 precedes \dot{V}_{O_2} and T_{brain} during arousal. All these results support that P_tO_2 is a biochemical landmark for brain activity during the arousal event. Thus P_tO_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_tO_2 ("A" in Fig. 3) provides an additional support to the idea that P_tO_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_tO_2) ("A" in Fig. 3).

In summary, P_tO_2 plays a key role in triggering and regulating a spontaneous arousal, which is

demonstrated by: (1) a preceding P_tO_2 elevation before changes in T_{brain} and \dot{V}_{O_2} ; (2) P_tO_2 changes do not correlate with changes in \bar{V}_{O_2} 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_tO_2 appearing at different T_{brain} during arousal suggest that changes in P_tO_2 are T_{brain} dependent.

CONCLUSION

The four turning points of P_tO_2 appearing at different T_{brain} during arousal suggest that changes in P_tO_2 are T_{brain} interdependent and support the concept that P_tO_2 plays a key role in triggering and regulating a spontaneous arousal.

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