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Adaptive response of brain tissue oxygenation to environmental hypoxia in non-sedated, non-anesthetized arctic ground squirrels

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Abstract

The present study examined the physiological mechanisms of the responses of brain tissue oxygen partial pressure (P_{tO_2}), brain temperature (T_{brain}), global oxygen consumption $\dot{V}_{O_2}$, and respiratory frequency (f_R) to hypoxia in non-sedated and non-anesthetized arctic ground squirrels (*Spermophilus parryii*, AGS) and rats. We found that 1) in contrast to oxygen partial pressure in blood (P_{aO_2}), the baseline value of P_{tO_2} in summer euthermic AGS is significantly higher than in rats; 2) both P_{tO_2} and P_{aO_2} are dramatically reduced by inspired 8% O_2 in AGS and rats, but AGS have a greater capacity in P_{tO_2} to cope with environmental hypoxia; 3) metabolic rate before, during, and after hypoxic exposure is consistently lower in AGS than in rats; 4) the respiratory responding patterns to hypoxia in the two species differ in that f_R decreases in AGS but increases in rats. These results suggest that 1) AGS have special mechanisms to maintain higher P_{tO_2} and lower P_{aO_2} , and these levels in AGS represent a typical pattern of adaptation of heterothermic species to and a brain protection from hypoxia; 2) AGS brain responds to hypoxia through greater decreases in P_{tO_2} and decreased f_R and ventilation. In contrast, rat brain responds to hypoxia by less reduction in P_{tO_2} and increased f_R and ventilation.

Keywords

hypoxia; arctic ground squirrels; brain oxygenation; brain tissue O_2 partial pressure; brain temperature; O_2 consumption; ventilation

Introduction

The fossorial heterothermic hibernators live in a naturally hypoxic environment (McArthur MD and Milson WK, 1991), and the fractional concentration of oxygen in a burrow could be as low as 10% (Kuhnen, 1986). Heterothermic species tolerate anoxia and hypoxia to a greater degree than do non-heterotherms (Bullard, 1960; Burington et al., 1969; D'Alecy et al., 1990; Drew et al., 2004) and survive hypoxia better than do non-hibernating species (Hiestand et al., 1950). The tolerance of hypoxia in heterothermic species has been attributed to the

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adaptation to chronic hypoxia associated with the burrow-dwelling lifestyle (Boggs et al., 1984; Tenney and Boggs, 1986; Boggs and Birchard, 1989; Mortala, 1991; Frappell and Mortola, 1994).

Arctic ground squirrels (AGS, *Spermophilus parryii*), an extreme heterothermic endotherm and distinct from homeothermic endotherms, experience the most severe overwinter environment ($< -20^{\circ}\text{C}$), the longest hibernation season (7–8 months), and consequently the shortest active season of any hibernator (Buck and Barnes, 1999). AGS experience endogenous hypoxia regularly during periodically arouse to euthermia from hibernation (Drew et al., 2004; Ma et al., 2005). Histological, metabolic, and electrophysiological studies conducted under exposure to hypoxic conditions provide evidence that heterothermic species are distinct in hypoxic tolerance from non-heterotherms and that the heterothermic state is profoundly different from euthermia.

We have recently demonstrated that AGS during hibernation are well oxygenated in blood, as compared with rats, and there is no evidence of cellular stress, inflammatory response, neuronal pathology, or oxidative modification following the period of high metabolic demand that is necessary for arousal. In contrast, euthermic AGS have low blood oxygen partial pressure (P_{aO_2}) and hemoglobin oxygen saturation (sO_2), thus indicating a natural state of mild, chronic hypoxia (Ma et al., 2005). These observations suggest that AGS experience and tolerate chronic endogenous hypoxia during euthermia, and hypoxia may serve to precondition these animals for neuroprotection. In contrast to the P_{aO_2} data in euthermic AGS, brain tissue oxygen partial pressure (P_{tO_2}) in winter interbout euthermic AGS is normal and is higher than that in hibernating AGS (Ma and Wu, 2008). To our knowledge, there have been no investigations that demonstrate 1) the difference between P_{tO_2} and P_{aO_2} in summer euthermic AGS; 2) differences in P_{tO_2} and P_{aO_2} between AGS and rats; 3) how brain tissue oxygenation responds to environmental hypoxia in AGS. We hypothesized that 1) P_{tO_2} of summer euthermic AGS differs from P_{aO_2} and is higher than that of rats; 2) P_{tO_2} and P_{aO_2} in AGS respond to and tolerate environmental hypoxia differently from non-hibernating species in that AGS have better adaptive mechanisms to preserve functions during hypoxia. The present investigation was conducted to address those issues at physiological levels using *in vivo* measurements of brain tissue oxygenation in non-sedated and non-anesthetized, free-moving animals.

Materials and methods

Animals

All procedures were performed in accordance with and approved by University of Alaska Fairbanks Institutional Animal Care and Use guidelines. Arctic ground squirrels (*Spermophilus parryii*, AGS) and Sprague Dawley rats (*Rattus norvegicus*) of both sexes were used for these experiments. Adult AGS were trapped during mid-July in the northern foothills of the Brooks Range, Alaska, approximately 40 miles south of the Toolik Field Station ($68^{\circ}38'\text{N}$, $149^{\circ}38'\text{W}$; elevation 809 m) of the University of Alaska Fairbanks (UAF) and transported to Fairbanks under permit from the Alaska Department of Fish & Game. AGS were housed individually at $16\text{--}18^{\circ}\text{C}$ and fed rodent chow, sunflower seeds, 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 (a typical resident temperature in burrow for AGS) and 4:20 h light:dark cycle for winter. Sprague Dawley rats (6 to 7 months of age at time of experiment) were purchased from Simonsen Laboratories (Gilroy, CA, USA) and transported by air to UAF. Rats were housed at $20\text{--}21^{\circ}\text{C}$, 12:12 h light:dark cycle and fed rodent chow *ad libitum*.

Surgery for brain guide cannula implantation and abdominal cannulation

A total of 10 AGS (body mass: 897.2 ± 33.2 g) and 12 rats (body mass: 480.5 ± 21.7 g) were used in present study. Five summer euthermic AGS and eight rats were used in stereotaxic surgery for guide cannula implantation and P_{iO_2} measurement in brain. The surgeries for AGS and rats were conducted between March and April. Animals were fasted at least 12 h before the surgery. Surgery was performed at room temperature under general anesthesia with isoflurane (Halocarbon Laboratories, River Edge, NJ, USA), induced at 5%, and maintained at 1.5% to 3% mixed with 100% medical grade O_2 at a flow rate of 1.5 L/min. During surgery, the animals' heads were held 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, USA).

Under strictly aseptic conditions, skin incision was made midline on the head and 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) for AGS, and another four holes were made with a steel burr (0.5-mm diameter) for anchor screws near the cannulae holes (two in front of and two in back of the guide cannula holes) on the skull for the screw foundation of dental cement. After completing the drilling process, four bone screws (BAS, West Lafayette, IN, USA) were driven into the skull through the screw holes. Two guide cannulae (CMA11, Acton, MA, USA) 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; Zou et al., 2002; Ma & Wu, 2008), and were slowly lowered 4 mm from the cortical surface. The surgical procedures for rats were similar to those for AGS. Guide cannulae for rats were stereotaxically positioned above the right and left striatum (A, +0.6 from bregma; L, ± 3.5 ; v, 5.5) with the incisor bar set at -2.4 mm as described previously (Drew et al., 1990). Cannulae were secured to the screws with dental cement.

Another five summer euthermic AGS and four rats were used for the abdominal cannule implantation and sampling blood for gas analysis. For monitoring blood gas, rats and AGS were fit with indwelling femoral arterial catheters as described by Toien et al. (2001). Surgery was performed at room temperature under general anesthesia with isoflurane (Halocarbon Laboratories, River Edge, NJ, USA), induced at 5%, and maintained at 1.5 to 3% mixed with 100% medical grade O_2 at a flow rate of 1.5 L/min. Under strictly aseptic conditions, catheters (Tygon tubing; 0.375 mm i.d., 0.75 mm o.d.; Norton, Akron, OH, USA) were inserted into the right femoral artery, externalized in the neck area on the back, and sealed.

Antibiotics (Baytril; Bayer Corp., Shawnee Mission, KS, USA, dose: 5 mg/kg) 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 to 15 days. After complete recovery, AGS were housed individually in a cool chamber at 2 °C for a natural polar resident temperature under a light regime of 12:12 h light:dark; rats were maintained at room temperature in the recovery room until use for experiment.

Blood gas sampling and monitoring

To understand the correlation between P_{iO_2} and P_{aO_2} before, during, and after hypoxic gas exposure, blood samples were taken from another five summer euthermic AGS and four rats with the implanted abdominal cannule. These samples were obtained from May through August in an open vertical cylindrical-shaped Plexiglas metabolic chamber, described in our previous study (Ma and Wu, 2008). For monitoring blood gas, an extension was added to the arterial catheter on the day of sampling so that blood could be taken without touching the animal. Approximately 100 μ L of blood for each measurement was sampled at 30-min intervals from

rats and from euthermic AGS, and was analyzed immediately after sampling. Total of 13 samples were taken during 6-hour experiment (2 before, 8 during, and 3 after 8% O₂ exposure). Blood gases were measured for P_aO₂, P_aCO₂, and pH at 37 °C using an I-Stat blood gas analyzer and G3+ or G4+ cartridges (East Windsor, NJ). Temperature corrections for blood gases (analyzed at 37 °C) were performed according to the methods of Severinghaus (1966) for comparison with reported results of blood gases.

Calibration for P_{O2} electrode and thermocouple, and temperature correction for P_tO₂

Calibration of P_{O2} electrode and thermocouple—For the measurement of P_tO₂, a P_{O2} 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 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 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_{O2} 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_{O2} value as 0 mm Hg on the P_{O2} 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_{O2} measurement—During hypoxic exposure, brain temperatures in both AGS and rats dropped dramatically so that temperature effects on P_tO₂ measurement had to be corrected properly to get the real physiological effect. The details of the principle and the procedures of temperature correction for P_tO₂ measurement was described previously (Ma and Wu, 2008). Briefly, to perform such a temperature correction, the following steps were taken:

1. The P_{O2} meter was initially calibrated to a P_{O2} 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% O₂) at 37 °C, and to a P_{O2} value of zero when ACSF was equilibrated with compressed nitrogen gas (0% O₂) at 37 °C.
2. Under the condition of bubbling with 21% O₂ in ACSF, the signal outputs of the P_{O2} electrode (P_{O2, meas.}) were determined in ACSF solutions ranging in temperature from 0 to 40 °C.
3. 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}), which were obtained from the literature (Weast, 1968), were appropriate for a given temperature.

4. These data—P_{O2, meas.}, P_{O2, cal.}, and temperature in Celsius (t)—were used to derive a temperature correction factor ($f = A e^{-k t}$, 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:

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

“ $P_{O_2, cal.} / P_{O_2, meas.}$ ” were plotted against t at temperatures of 0 to 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, $P_{O_2, cal.} = (A e^{-k t})(P_{O_2, meas.})$, 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} and T_{brain} in the striatum of AGS in conjunction with $\dot{V}_{O_2}$ and f_R —After animals recovered from stereotaxic surgery for brain guide cannulae implantation, 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, USA) 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_{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 preimplanted 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 AGS and rats. 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 and reference electrode to P_{O_2} meter, and from thermocouple to thermocouple meter went through a sprint tube and came out the experimental chamber in the middle of the top.

Systemic metabolic rate (whole animal oxygen consumption), $\dot{V}_{O_2}$ of AGS and rats was recorded by indirect calorimetry in a vertical cylindrical-shaped Plexiglas metabolic chamber (I.D. 29 cm, height 32 cm) described previously (Ma and Wu, 2008). 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, USA). 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, USA), which was calibrated with air. $\dot{V}_{O_2}$ 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 sampling 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 $\dot{V}_{O_2}$ 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, USA) 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, USA) was used as A/D converter for the $\dot{V}_{O_2}$ measurement system, and the signals of the outputs were then input into the interface to 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 18 °C. Data in $P_{O_2, meas.}$ recorded originally by the P_{O_2} electrode in one channel were calibrated online into corresponding values of $P_{O_2, cal.}$ without the effects of temperature in another channel.

Respiratory frequency (f_R) was counted manually by direct observation during the entire experimental period with 30-min intervals beginning 60 min before 8% O_2 exposure and ending 60 min after hypoxic exposure. Counting for f_R was performed blindly by the same technician who did not know experimental conditions of gas exposure. Each f_R value was a mean of two counts.

Hypoxic exposure

When the recorded values in P_tO_2 and T_{brain} were stable for at least 1 hour under fresh air, air was replaced with 8% O_2 (approximate 57 mmHg) in N_2 , and hypoxic gas was delivered into the experimental chamber for 3 h, followed by a return to ambient air. P_tO_2 and T_{brain} were recorded continuously for at least an additional 2 h after the return to ambient air. $\dot{V}_{O_2}$ and f_R were measured throughout the experimental period, beginning to end.

For the first 30-min interval immediately after the beginning of 8% O_2 exposure and after the end of exposure (See panel A, Figure 3), precise $\dot{V}_{O_2}$ values were not able to be calculated. That is because of the unavailability of a stable O_2 standard in the respiratory system for online calculation of $\dot{V}_{O_2}$ during these two transition periods.

After the experiment was completed for the measurements of P_tO_2 , T_{brain} , and $\dot{V}_{O_2}$ after 8% O_2 exposure, the animals were euthanized under deep anesthetic condition with isoflurane. 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 was performed to identify the P_{O_2} electrode tip location for each animal, as previously described in detail (Ma and Wu, 2008).

Data Analysis

Values in this study are given as mean $\pm$ SEM. Data shown in Figure 1 and Figure 2 for P_tO_2 , P_aO_2 , P_aCO_2 , and pH during the periods before, during, and after 8% O_2 exposure were averaged to represent the value in each of those three experimental periods. Data for P_tO_2 , P_aO_2 , P_aCO_2 , pH, and $\dot{V}_{O_2}$ shown in the bar graphs of Figure 1, Figure 2, and Figure 3 were analyzed with two-way ANOVA (SigmaStat, version 3.5, Systat Software, Inc. Chicago, IL, USA) using two factors: animal group (AGS and rats) and experimental conditions (before, during, and after 8% O_2 exposure). Significant differences ($p < 0.05$) were determined by means of All Pairwise Multiple Comparison Procedure with Holm-Sidak's method. P values < 0.05 were taken to represent significant differences. The horizontal lines above the tops of the bars in Figure 1, Figure 2, and Figure 3 represent significant differences either between two groups or between two experimental treatments.

Results

The response of P_tO_2 to 8% O_2 in the brain

The baseline value of P_tO_2 in summer euthermic AGS (28.3 ± 1.1 mm Hg; $n=5$) was significantly higher ($P < 0.001$) than in rats (15.3 ± 0.1 mm Hg, $n=8$) before 8% O_2 exposure. The P_tO_2 dropped significantly during 8% O_2 exposure in both AGS (5.2 ± 0.4 mm Hg, $n=5$) and rats (7.2 ± 0.2 mm Hg, $n=8$) as compared with baseline values, but there was no difference

in P_tO_2 between the two groups during 8% O_2 exposure. The P_tO_2 recovered to baseline levels in both AGS (23.8 ± 0.8 mm Hg, $n=5$) and rats (16.4 ± 0.2 mm Hg, $n=8$). There were no differences of P_tO_2 between pre- and post- 8% O_2 exposure in both AGS and rats. The P_tO_2 values were significantly higher in AGS than in rats before and after 8% O_2 exposure ($P=0.002$).

The response of P_aO_2 , P_aCO_2 , and pH to 8% O_2 in the blood

Response of P_aO_2 —In contrast to the results in P_tO_2 , the mean baseline value of P_aO_2 in AGS (60.4 ± 6.8 mm Hg, $n=5$) was significantly lower than in rats (83.3 ± 0.5 mm Hg, $n=4$) before 8% O_2 exposure (Panel A and B, Figure 2). During 8% O_2 exposure, P_aO_2 in both AGS and rats dropped significantly as compared with the baseline values, but the value of P_aO_2 in AGS (15.2 ± 2.9 mm Hg, $n=5$) was significantly lower ($P=0.002$) than in rats (28.7 ± 1.3 mm Hg, $n=4$). After 8% O_2 exposure and return to ambient air, P_aO_2 recovered completely and significantly in both AGS and rats ($P=0.000$), as compared with the values during exposure. P_aO_2 in rats (102.1 ± 12.2 mm Hg, $n=4$) was significantly higher than in AGS (54.5 ± 2.2 mm Hg, $n=5$) after 8% O_2 exposure. P_aO_2 did not differ between the two periods of *before* and *after* 8% O_2 exposure in AGS. In rats, however, the P_aO_2 after 8% O_2 exposure was significantly higher than the value before exposure ($P=0.000$).

Response of P_aCO_2 —The baseline values of P_aCO_2 in AGS (64.0 ± 2.1 mm Hg, $n=5$) were significantly higher than in rats (42.2 ± 1.3 mmHg, $n=4$) before 8% O_2 exposure (Panel C and D, Figure 2). During 8% O_2 exposure, P_aCO_2 in both AGS and rats dropped significantly as compared with the baseline values ($P=0.000$), and P_aCO_2 did not differ between AGS and rats. After cessation of 8% O_2 exposure and return to ambient air for 90 minutes, P_aCO_2 in AGS was significantly lower than before exposure ($P=0.000$), but in rats P_aCO_2 did not differ before and after 8% exposure. After 8% O_2 exposure, P_aCO_2 in AGS was still significantly higher than in rats.

Response of pH—The baseline values of blood pH did not differ between AGS (7.42 ± 0.01 , $n=5$) and rats (7.46 ± 0.02 , $n=4$) ($P=0.069$) before 8% O_2 exposure (Panel E and F, Figure 2). During 8% O_2 exposure, pH in the blood of both AGS and rats increased significantly ($P=0.000$) as compared with their baseline values, but pH was significantly higher in AGS (7.74 ± 0.03 , $n=5$) than in rats (7.67 ± 0.04 , $n=4$) ($P=0.006$). After cessation of hypoxic exposure, blood pH decreased significantly to near baseline values in both AGS (7.47 ± 0.05 , $n=5$) and rats (7.47 ± 0.04 , $n=4$), and there was no difference in pH between AGS and rats ($P=0.793$). For rats, there was no difference in the pH levels before and after 8% O_2 exposure ($P=0.706$), but for AGS pH before 8% O_2 exposure was significantly lower than that after exposure ($P=0.012$).

Response of systemic oxygen consumption to 8% O_2

Before 8% O_2 exposure the baseline value of $\dot{V}_{O_2}$ of AGS (1.1 ± 0.1 mL O_2 /g/hr, $n=7$) was significantly lower ($P<0.001$) than in rats (1.5 ± 0.2 mL O_2 /g/hr, $n=8$). During hypoxic exposure, $\dot{V}_{O_2}$ dropped significantly ($P=0.00001$) in both AGS and rats, as compared with values before hypoxic exposure. During the hypoxic period $\dot{V}_{O_2}$ in AGS (0.7 ± 0.1 mL/g/hr) was significantly lower than in rats (1.0 ± 0.1 mL/g/hr) ($P<0.001$). During the period after 8% O_2 exposure, $\dot{V}_{O_2}$ in AGS (1.4 ± 0.2 mL/g/hr) was also significantly lower than in rats (2.0 ± 0.3 mL/g/hr) ($P<0.001$). For both AGS and rats, $\dot{V}_{O_2}$ after 8% O_2 exposure was significantly higher than baseline values ($P=0.007$).

Response of brain temperature to 8% O₂

Brain temperature in both AGS and rats declined immediately after exposure to 8% O₂ (Figure 4). Hypoxic exposure (8% O₂) for 3 hours induced the drop of T_{brain} from 36.5 to 29 °C in AGS and from 37 to 30 °C in rats. There was no statistical difference, however, in the pattern of change in T_{brain} between the two species (P=0.167). After cessation of 8% O₂ exposure, T_{brain} recovered to the baseline within 1 hour in both AGS and rats.

Response of respiratory frequency to 8% O₂

The respiratory frequency (f_R) immediately decreased in AGS with the onset of hypoxia (8% O₂ exposure), continued to decline for 2 hours, and then remained at a relatively stable level for the next hour. The f_R started to recover in AGS immediately after their return to ambient air. In contrast to AGS, the f_R in rats increased rapidly during the first 90 minutes of 8% O₂ exposure and started to decline during the last 90 minutes. After rats were returned to air, f_R continued to decline and recovered to the baseline value in 1 hour. The mean values in f_R of AGS responses (decrease in f_R) during 8% O₂ exposure are significantly different from those in rats (increase in f_R) (P<0.001).

Discussion

Hypoxic response of brain tissue oxygenation

Most reported studies of hypoxic exposure have been done in anesthetized animals. In anesthetized rats, brain tissue oxygen falls immediately when the partial pressure of inspired oxygen is reduced even moderately (Mettzger et al., 1971; Leniger-Frllert et al., 1975; Kreisman et al., 1981; LaManna et al., 1992; Hou et al., 2005). The anesthetic condition has a remarkable influence on brain oxygenation (Hoffman et al., 1997; Hoffman and Edelman, 2000). The reported baseline values of tissue oxygen pressures in rats vary with 1) heterogeneity of P_tO₂ distribution in different area of the brain; 2) methodology used; 3) different anesthesia and anesthetized conditions; 4) whether or not the temperature effects on PO₂ were corrected properly. Little is known about the natural profile of P_tO₂ and hypoxic response of P_tO₂ in non-sedated, non-anesthetized mammals. The present study represents the first time that data on the effects of hypoxic exposure on P_tO₂ in the striatum have been recorded from non-sedated, non-anesthetized, freely moving animals. These data provide intriguing insights into brain oxygenation with a number of new findings.

The first finding is that the baseline P_tO₂ (28.3 mm Hg) in the striatum of summer euthermic AGS brain was significantly higher than the P_tO₂ (15.4 mm Hg) in rat brains, which is similar to the value (14 mm Hg) in rat cortex as determined by electron paramagnetic resonance oximetry (Hou et al., 2005). The P_tO₂ in summer euthermic AGS in the present study is also comparable with the P_tO₂ value (21.2 mm Hg) in winter interbout euthermic AGS (Ma and Wu, 2008). The discrepancy in P_tO₂ values between summer euthermic and winter interbout euthermic AGS may be accounted for by different physiological states. We are not able to offer a more details about the mechanism that is responsible for such a difference at present time. This finding that baseline P_tO₂ is higher in summer euthemic AGS brain than in rat brain suggests that brain oxygenation in AGS has a greater capacity to cope environmental hypoxia. In contrast to P_tO₂, the baseline value of P_aO₂ in AGS was lower than in rats (Panel B, Figure 2). The baseline values of P_aO₂ in euthermic AGS blood in the present study are consistent with our previous results (Mat et al., 2005) and with those of other investigators who have reported resting P_aO₂ values for euthermic mammalian heterotherms that are frequently below 80 mmHg (11 kPa) (Burlington et al., 1969; Musacchia and Volkert, 1971; Frerichs et al., 1995; Kudej and Vatner, 2003). Decreases in P_aO₂ are a strong stimulus for increased cerebral blood flow (CBF), presumably to defend the brain against the reduced O₂ supply (Borgstrom et al., 1975; Buck et al., 1998). Hypoxia has been shown to induce cerebral vasodilation and

a prolonged increase in cerebral blood flow (Borgstrom et al., 1975; Hudetz et al., 1998; Pearce, 1995; Williams et al., 1998; Hunter et al., 2003). Based on these reported observations and our results (lower P_aO_2 and higher P_tO_2 in AGS than in rats), we suggest that 1) the blood oxygenation at the euthermic state in AGS is chronically and mildly hypoxic; 2) more O_2 and blood may be shunt to brain tissues by increasing CBF in euthermic AGS through local adaptive mechanisms, which may not exist in rats; 3) endogenous hypoxia in blood of euthermic AGS may precondition this species to challenge the natural extreme conditions caused by long polar winter hibernation, periodical arousal, and burrowing hypoxia (7–8 months). From these data, we can conclude that the elevated basal P_tO_2 and reduced systemic P_aO_2 in AGS represent a typical pattern of adaptation of heterothermic species to and a brain protection from chronic environmental hypoxia.

The second finding in the present investigation is that there were some differences in response to sustained environmental hypoxia between the brain tissue and blood oxygenation in AGS and rats. First, the hypoxia-induced level of P_tO_2 during exposure did not differ between AGS and rats, but hypoxia-induced reduction of P_tO_2 in AGS is greater than in rat. The greater reduction of P_tO_2 in AGS than in rats during 8% O_2 exposure could be accounted for by a higher capacity for P_tO_2 and better adaptation to ischemia/hypoxia as displayed by dramatic swings of CBF during hibernation/arousal in ground squirrels (Frerichs et al., 1994). The hypoxia-induced level of P_aO_2 during 8% O_2 exposure was significantly lower in AGS than in rats. This result suggests that AGS may possess special physiological function to obtain a stronger stimulus caused by lower P_aO_2 for the increase in CBF during environmental hypoxia (Borgstrom et al., 1975; Buck et al., 1998). Second, after cessation of hypoxic exposure and return to normal inspiration with air, P_tO_2 in both AGS and rats were rapidly recovered to baseline values with no difference in P_tO_2 between the two periods, before and after 8% O_2 exposure. P_aO_2 in AGS recovered to baseline value after cessation of hypoxic exposure, but P_aO_2 in rats recovered to a significantly higher value than its baseline. These results suggest that brain has regional protection mechanisms to restore normal tissue oxygen levels after environmental hypoxia in both AGS and rats, but AGS show better recovery ability and adaptation in blood after hypoxia exposure.

The baseline value of P_aCO_2 was higher in AGS than in rats (Panel D, Figure 2), and baseline pH did not differ between summer euthermic AGS and rats (Panel F, Figure 2). These results are consistent with our earlier results and further support our previous finding that euthermic AGS are naturally hypoxic (Ma et al., 2005).

Comparing the basal values and hypoxia-induced values of P_tO_2 , P_aO_2 , P_aCO_2 , and pH, we could conclude that 1) AGS are a special heterothermic model and are different from non-hibernating species; 2) both AGS and rats share some similar mechanisms to respond to environmental hypoxia, but some differences in the responses of P_tO_2 , P_aO_2 , P_aCO_2 , and blood pH exist between these two species. Unfortunately, we are not able to compare brain tissue response of CO_2 partial pressure (P_tCO_2) and pH to environmental hypoxia with those responses of P_aCO_2 and pH in blood in the present study due to the difficulty in methodology. If those data could be obtained, it would provide more physiological insight into the mechanisms by which brain tissue responds to environmental hypoxia.

Hypoxic response in brain temperature

Hypoxia that leads to a drop in body temperature occurs in newborns and adults of many species, including human (Cross et al., 1958, Atchley et al., 2008). A reduction of core body temperature (T_b) induced by hypoxia (7% O_2) in golden-mantled ground squirrels has been reported (Barros et al., 2001). Initiating a fall in metabolism and T_b during chronic hypoxia, at the appropriate time, is thought to contribute to the entrance into hibernation in heterothermic species (Barros et al., 2001).

The present study shows, for the first time, the response of brain temperature to environmental hypoxia in both freely moving AGS and rats. There were no differences between these two species in terms of changes of T_{brain} during hypoxic exposure. These results suggest that the decline of brain temperature could be a common mechanism used by both hibernating and non-hibernating species to protect them from brain hypoxia in a hypoxic environment. A reduction in core body temperature (T_b) has been shown to be a beneficial response to hypoxia in mammals. For example, a hypoxic reduction in T_b increases survival in rats (Wood and Stabenau, 1998), and a 1 °C drop in brain temperature in rats is adequate to prevent a fall in brain ATP levels at low O_2 partial pressure (20 mm Hg) (Berntman et al., 1981).

T_{brain} is positively correlated with regional metabolic rate (Bacher et al., 1998). A regulated reduction in T_b during chronic hypoxia will reduce both resting O_2 demands and the energetically costly increases in cardiac output and ventilation induced by acute hypoxia (Barros et al., 2001). In addition, the reduction in T_{brain} could increase dissolved oxygen content in brain tissues and the binding ability of hemoglobin-oxygen in blood. All of these measures could significantly enhance brain oxygenation and protect brain from chronic deterioration during environmental hypoxia.

Hypoxic response in metabolic rate

In the present study the basal values of $\dot{V}_{O_2}$ in euthermic AGS, which are similar to those in previous reports (Tøien et al., 2001; Ma et al., 2005), are significantly lower than in rats. We calculated the a-values of empirically derived factor in allometric equation ($Y = aM^b$, Nevill, 1994) and found that rat and AGS had different a-values (rat: 1.9; AGS: 1.05). These different a-values demonstrate that the difference of $\dot{V}_{O_2}$ between these two species is not caused by body mass scaling and is induced by real physiological effect. The lower $\dot{V}_{O_2}$ in AGS represents another adaptive pattern under hypoxic burrowing conditions in fossorial heterothermic species.

Hypoxic hypometabolism has been observed in many species (Frappell et al., 1992, Atchley et al., 2008). The systemic metabolic rates in AGS and rats decreased rapidly during 8% O_2 exposure and then recovered to a higher level than the basal level (Fig. 3). This result suggests that metabolic depression is a common adaptive mechanism used by both hibernators and non-hibernators. The reduced metabolic rate was maintained throughout hypoxic exposure in AGS, whereas in rats the reduced metabolic rate showed a gradual increase during that period (Panel A, Figure 3). The difference of hypoxic response in $\dot{V}_{O_2}$ of AGS demonstrates that this burrowing species is better able to maintain hypometabolism during hypoxia and adapt to environmental hypoxia than surface-dwelling rats.

Hypoxic ventilatory response in f_R

Changes in breathing pattern associated with sustained hypoxia differ between AGS and rats. With the onset of hypoxia, f_R decreased in AGS but increased in rats. The decline in f_R during hypoxia was also observed in our previous study (Ma et al., 2004). The differences in the response pattern of f_R between these two species may represent distinct hypoxic ventilatory responses. This opposite response in f_R to sustained hypoxic exposure may reflect differences in the protecting mechanisms of the two species.

Ventilation (V_E) is determined by both respiratory frequency and tidal volume. Lacking a validated method, we were unable to measure tidal volumes under hypoxic conditions in these two species. However, a big difference in ventilation has been demonstrated between golden-mantled ground squirrels (*Spermophilus lateralis*) and rats: V_E is 2.5-fold greater in rats than in the ground squirrels in normothermia at 36 °C, and this species difference in ventilation is mainly due to the difference in f_R (Osborne and Milson, 1993). Their study demonstrated that,

in both species, V_E and f_R decreased but the tidal volume (V_T) was kept unchanged while body temperature was dropping. Thus, f_R is the main contributor to ventilation when the body temperature is dropping in these two species. The mean V_E decreases in golden-mantled ground squirrels (72%) were much greater than in rats (44%) during progressive hypothermia. In addition, ventilations were increased by hypoxic exposure in both golden-mantled ground squirrels and rats, but V_E increases were much less in ground squirrels than in rats. These two opposite effects of hypothermia and hypoxia on ventilation interacted and the combined effects were dependent on which factor was a dominant one. The regulated body temperature reduction during hypoxic exposure may be a predominant factor that resulted in a ventilation drop in golden-mantled ground squirrels, which induced a reduction in f_R in ground squirrels. The hypoxic effect, however, may be a main effect on rats during hypoxia exposure, which induced the increases of both ventilation and f_R in rats. These two physiological states (hypothermia and hypoxia) coexist during hypoxic exposure in both AGS and rats in the present study. AGS and golden-mantled ground squirrels are similar fossorial hibernators. Therefore, their finding (Osborne and Milson, 1993) helps to explain the differences in f_R response to hypoxia that we saw between AGS and rats. Elevated ventilation induces an increase of f_R in rats and reduced ventilation induces a decrease of f_R in AGS during 8% O_2 exposure.

In summary, the results of the present investigation suggest that heterothermic species, euthermic AGS, maintain normoxic brain oxygenation even with chronic hypoxia in blood and possess specific adaptations that facilitate greater responses to hypoxia as compared with a non-heterothermic species, the rat. AGS use specific mechanisms to maintain brain oxygenation and respond to hypoxia through greater decreases in P_tO_2 and decreased f_R and ventilation. Homeothermic species, rats, maintain their cerebral oxygenation against hypoxia by less reduction in P_tO_2 and increased f_R and ventilation. The cellular and molecular basis of this mechanism remains to be explored.

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A. P_{tO_2} before, during, and after 8% O_2

Figure 1.

Changes in P_tO_2 before, during, and after 8% O_2 exposure in non-sedated and non-anesthetized summer euthermic AGS (n=5) and rats (n=8) measured by PO_2 microelectrode at 18 °C. Panel A: real-time changes in P_tO_2 before, during, and after 8% O_2 exposure. The horizontal bar in Panel A indicates the duration of 8% O_2 exposure. Panel B: averaged P_tO_2 before, during, and after 8% O_2 exposure. The horizontal bars show the statistical differences between two groups or two experimental conditions.

Figure 2.

Changes in P_aO_2 , P_aCO_2 , and pH before, during, and after 8% O₂ exposure in non-sedated and non-anesthetized summer euthermic AGS (n=5) and rats (n=4) at 18 °C. Panel A, C, E: real-time changes in mean P_aO_2 , P_aCO_2 , and pH before, during, and after 8% O₂ exposure. The horizontal bars in Panel A, C, E indicate the duration of 8% O₂ exposure. Panel B, D, and F: averaged values of P_aO_2 , P_aCO_2 , and pH in the period before, during, and after 8% O₂ exposure. The horizontal bars show the statistical differences between two groups or two experimental conditions.

Figure 3.

Changes in oxygen consumption rate in non-sedated, non-anesthetized summer euthermic AGS (n=5) and rats (n=8) before, during, and after 8% O₂ exposure at 18 °C. Panel A: real-time changes in $\dot{V}O_2$ before, during, and after 8% O₂ exposure. The horizontal bar in Panel A indicates the duration of 8% O₂ exposure. Panel B: averaged changes in $\dot{V}O_2$ in the periods before, during, and after 8% O₂ exposure. The horizontal lines indicate the significant differences between either the two groups or the two experimental conditions.

Figure 4.

Real-time changes of brain temperature in non-sedated, non-anesthetized summer euthermic AGS (n=5) and rats (n=8) before, during, and after 8% O₂ exposure at 18 °C. The horizontal bar indicates the duration of 8% O₂ exposure.

Figure 5.

Changes in respiratory frequency before, during, and after 8% O₂ exposure in non-sedated, non-anesthetized summer euthermic AGS (n=5) and rats (n=8) at 18 °C. The horizontal bar indicates the duration of 8% O₂ exposure.