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Thermoregulatory and metabolic responses to hypoxia in the oviparous lizard, *Phrynocephalus przewalskii*

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ABSTRACT

The effects of hypoxia on behavioral thermoregulation, rate of heating and cooling, hysteresis of heart rate, and standard metabolic rate (SMR) were investigated in *Phrynocephalus przewalskii*, a small size toad headed lizard. Preferred temperature (T_b) descended when lizards were exposed to severe hypoxia (8% O_2 and 6% O_2) for 22 h, and lizards were able to maintain preferred T_b after one week at 12% and 8% O_2 respectively. The period of heating increased after being treated with hypoxia (12% and 8% O_2) for one week. Hysteresis of heart rate appeared at any given body temperature and oxygen level except at 39 °C and 40 °C at 8% O_2. SMR significantly increased after one-week acclimatization to 12% and 8% O_2 when ambient temperature (Ta) was 25 °C, however, it did not change at 35 °C. Thus, we suggest that *P. przewalskii* has special thermoregulatory and metabolic mechanisms to acclimatize to the hypoxic environment.

1. Introduction

Hypoxia elicits a number of compensatory responses, such as a regulated decrease in body temperature (T_b) and the associated metabolic depression, in order to decrease oxygen consumption (Steiner and Branco, 2002). Thermoregulatory responses to hypoxia have been extensively studied in ectothermic models (Malvin and Wood, 1992; Wood, 1995). Generally, ectotherms with a low endogenous heat production mainly maintain suitable body temperature (T_b) by behavioral selection of microenvironments with appropriate temperatures (Huey and Kingsolver, 1989; Angilletta et al., 2002). When exposed to hypoxic environment, ectotherms can reduce their preferred T_b by behavioral action, which is called hypothermia (Hicks and Wood, 1985). The phenomenon was initially described in teleost fish (Bryan et al., 1984), and then discovered in reptiles in the laboratory (Hicks and Wood, 1985) as well as in the field (Rollinson et al., 2008). Reduced T_b via behavioral thermoregulation is a beneficial stress response. Firstly, it can protect tissues against depletion, particularly in life-sustaining organs such as the brain and heart. Moreover, it reduces oxygen consumption and blunts the energetic cost through a combination of lowered metabolic rate via Q_{10} effects (Hicks and Wang, 2004), an increase in the oxygen-loading capacity of the lungs, and an accompanying reduction in ventilatory costs (Wood and Gonzales, 1996).

Besides behavioral thermoregulation, ectotherms can control rates of transient heat transfer between the environment and their core by altering peripheral circulation and heart rate (Dzialowski and O'Connor, 1999). Cutaneous blood flow is reported to increase during warming and decrease during cooling in several species of reptiles (Weinheimer et al., 1982; Dzialowski and O'Connor, 2001). Changes in heart rate have been used as an indicator of changes in peripheral blood flow in several species of reptiles (Seebacher, 2000; Smith et al., 1978). Therefore, heart rate increases during heating, and decreases during cooling, such that the heart rate is significantly higher during heating than during cooling at a given T_b, which is referred to as heart-rate hysteresis (Grigg et al., 1979; Galli et al., 2004). Accelerated and decreased heart rates lead to a faster warming and a slower cooling, respectively (Grigg et al., 1979). Currently, studies investigating the physiological thermoregulation response to hypoxia are fairly limited in ectotherms. It is just reported that hypoxia abolished the difference between rate of heating and rate of cooling in the iguana, thereby increasing the rate of cooling during hypoxia and shortening the time required for T_b to drop after hypoxic animals select a lower ambient temperature (Hicks and Wood, 1989). It is still unknown whether hypoxia leads to disappearance of heart-rate hysteresis.

Metabolic depression is another potential physiological adaption to hypoxia in ectotherms (Hicks and Wang, 1999), and it appears that reptiles can reduce overall metabolic demands during periods of moderate to severe hypoxia (Hochachka et al., 1996; Hochachka and Lutz, 2001). Generally, it is accomplished by two strategies. Firstly, behavioral reduction of the preferred body temperature through the Q_{10} effects decreases aerobic demands of the tissues (Hochachka and

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Lutz, 2001; Hicks and Wang, 2004). In addition at any given temperature, exposure to hypoxia can result in a down-regulation of aerobic metabolism (Buck et al., 1993; Hicks and Wang, 1999). However, some studies (Wood and Malvin, 1991; Bishop et al., 2000) showed that hypoxic exposure did not lead to changed metabolism at consistent temperature and even lead to an increase in metabolism.

The purpose of this study was to investigate the effects of hypoxia on thermoregulation and standard metabolic rate in the lizard *Phrynocephalus przewalskii* and to provide some experimental evidence about acclimating mechanisms to hypoxia in reptiles.

2. Materials and methods

2.1. Animals

P. przewalskii, a terrestrial, ectothermic vertebrate, was collected from Minqin (38°38′N, 103º05′E), Gansu province, China. This area is semi-desert on the southwest edge of Tengger Desert and the altitude is 1480 m. Adult males were captured by hand from the end of May to avoid any confounding effects of female reproduction and possible effects of seasonal changes (Ashby, 1985). After transport to laboratory in Lanzhou University, where the altitude is 1520 m, lizards were randomly divided into nine groups. The first six groups (15 lizards each) were exposed to six different levels of oxygen content (28%, 21%, 17%, 12%, 8% and 6% O_2) for 22 h respectively, to test the effect of hypoxia and hyperoxia on behavioral thermoregulation; the last three groups (18 lizards each) were kept at normoxia, 12% O_2, and 8% O_2 for one week to measure physiological thermoregulation and standard metabolic rate (SMR). All the lizards in the group were exposed to the different levels of oxygen content at the same time. There were no significant differences in mean initial body mass (5.24 ± 0.93 g) and snout-vent lengths (SVL: 52.5 ± 3.3 mm) among groups. All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee at Lanzhou University.

2.2. Nonpressurized hypoxia chamber

A nonpressurized hypoxic chamber (1.20 m long, 0.65 m high and 0.45 m wide) was used to continually provide hypoxia for lizards, in which the bottom was covered with 10 mm depth of silver sand and there were no special shelters. The chamber walls were made of transparent polymethyl methacrylate acrylic plastic, facilitating the observation of the animal. To measure the preferred T_b, we built a temperature gradient. One end of this enclosed chamber was heated (to approximately 50 °C) with a 100-W light bulb and the other end cooled to room temperature (about 24 °C) using air conditioning. Lizards were exposed to normoxic condition in the chamber for 24 h before acclimated to different oxygen content. Chamber gas composition was controlled by influx of O_2 or N_2 using an oxygen controller (HCD-2B, Mei Cheng Oxygen Analysis Instruments Plant) connected to an electromagnetic valve to control nitrogen and oxygen flow. The self-indicating silica gel and dry soda lime (CaO and NaOH) were used to absorb redundant moisture and CO_2 in the chamber. The chamber was opened for 15 min every day to clean the inside, and to replenish food and water. The lizards were subjected to a 14L: 10D photoperiod and heat was available from 0850 to 1850 h every day, the central 10 h of the light cycle.

2.3. Preferred T_b

After 22 h acclimatization in different oxygen contents respectively, we measured their body temperatures by using an electronic thermometer (TM6801 b; Zhangzhou Weihua Electronic Instrument, Zhang zhou, China), which probe was inserted 3 mm into cloaca of those lizards to test. In addition, to determine whether behavioral thermoregulation exhibits plasticity, we tested selected T_b after one-week acclimatization to 12% and 8% O_2. Before each experiment, electronic thermometer was calibrated against a mercury thermometer at three different temperatures.

2.4. Rate of heating and cooling and heart rate

Body temperature was monitored by inserting a 1 cm soft flexible temperature probe into the cloaca. Heart rate was obtained from measurements of the electrocardiogram (ECG). The ECG was recorded by inserting subcutaneously three 2 mm long needles. The ECG signal was recorded with a BL-420F Data Acquisition & Analysis System (Chengdu TME Technology Co, Ltd, China). To maximize the signal, one needle was placed close to the right forelimb, the second needle was placed to the left posterior limb, and the third needle was placed to the right posterior limb. Insertion of these needles should be performed within less than 2 min in order to minimize the stress to the animals.

During experiments, animals were restrained on plastic board back upward in the original acclimatized chamber. Animals were heated from 25 °C to 40 °C with a 100 W heat lamp suspended 25 cm above the plastic board and allowed to cool by convection of air of 24 °C. Heart rate and body temperature were recorded continuously during heating and cooling. Heart rate was quantified at each degree Celsius and was based on the average of 10–15 consecutive beats.

2.5. Standard metabolic rate (SMR) and Q_{10} for SMR

SMR was measured under resting conditions by an animal respiration measuring system (RP1LP, Qubit, Canada) and expressed by the production of carbon dioxide (CO_2) (Fen Yue et al., 2012). SMR of lizards was determined after one-week acclimatization of normoxia, 12% O_2, and 8% O_2, respectively, at two constant temperatures (25 ± 0.1 °C and 35 ± 0.1 °C) controlled by water bath. The oxygen contents during SMR measurements were the same as these of acclimatization. Prior to each measurement, all lizards were fasted for at least 48 h to ensure a postabsorptive state (Coulson and Hernandez, 1980) and body mass was measured individually. To minimize activity, SMR measurements were made in a dark room from 2000 h to 0600 h.

Q_{10} for SMR at different oxygen level was calculated from the following equation:

$$\text{Log}\ Q_{10} = (\log\ R_{35} - \log\ R_{25}) \times 10/(35\text{–}25)$$

where R_{35} is the SMR at 35 °C; and R_{25} is the SMR at 25 °C.

2.6. Statistical analysis

All analysis was performed using SPSS release 16.0 (SPSS Inc., Chicago, IL, USA). The data were tested for normality and homogeneity of variances to meet the assumptions of Para metric testing prior to analysis and no significant deviations from these assumptions were evident in the data. One-way ANOVA was used to evaluate the effect of hypoxia on preferred T_b. In addition, heart rate and the rates of heating and cooling were analyzed with a general linear model three-way ANOVA for repeated measures with the Minitab statistical package. Data were tested for differences among different hypoxic acclimatized animals, and whether the animals were heated or cooled. The independent variables were T_b, oxygen content, and heating vs. cooling. The effect of turning off the heat lamp on heart rate for hypoxic acclimatized lizards was evaluated with a two-way ANOVA for repeated measures. Finally, the SMRs at 25 °C and 35 °C were used in multivariate ANOVA to analyze the differences among different hypoxic acclimatized lizards, with body mass as the covariate. All data were presented as mean ± SD, and all hypotheses were tested for statistical significance at the $P < 0.05$ level.