Interaction between temperature and hypoxia in the alligator

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Branco, L. G. S., H. O. Pörtner, and S. C. Wood. Interaction between temperature and hypoxia in the alligator. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R1339-R1343, 1993.—Hypoxia elicits behavioral hypothermia in alligators. Under normoxic conditions, the selected body temperature is 27.8 ± 1.2°C. However, when inspired O_2 is lowered to 4%, selected body temperature decreases to 15.4 ± 1.0°C. The threshold for the behavioral hypothermia is between 4 and 5% inspired O_2, the lowest threshold measured so far in terrestrial vertebrates. This study assessed the physiological significance of the behavioral hypothermia. The body temperature was clamped at 15, 25, and 35°C for measurements of ventilation, blood gases, metabolic rate, plasma lactate, and acid-base status. Hypoxia-induced changes in ventilation, acid-base status, oxygen consumption, and lactate were proportional to body temperature, being pronounced at 35°C, less at 25°C, and absent at 15°C. The correlation between selected body temperature under severe hypoxia and the measured parameters show that behavioral hypothermia is a beneficial response to hypoxia in alligators.

crocodilian; *Alligator mississippiensis*; lactate; hypoxia; thermoregulation; control of breathing; metabolism; acid-base regulation

HYPOXIA ELICITS a number of physiological responses in reptiles and other ectothermic vertebrates. Active responses to hypoxia such as increased ventilation and cardiac output are, however, O_2 consuming, which sets a limit to their usefulness.

Therefore a voluntary decrease of body temperature may be more efficient, due to reduced metabolic demand and reduced sensitivity to hypoxia (7, 12). Such behavioral hypothermia has previously been shown in a wide variety of animal species (28). Crocodilians had not previously been studied in this context despite their high degree of tolerance to hypoxia (5). The present paper provides data for the American alligator. Moreover, we provide an integrated evaluation, including effects on blood gases, ventilation, and O_2 consumption as well as on pH and lactate, which were measured both for plasma and for some intracellular compartments.

MATERIALS AND METHODS

Ten juvenile alligators, *Alligator mississippiensis*, were studied. Body mass was 697.5 ± 97.4 (SE) g. They were housed in a temperature-controlled room at 25°C with free access to water. They had access to a heat lamp and dry area for basking and were fed dry cat food until 7 days before the experiments.

Animals were anesthetized with a mixture of 5% halothane and 95% oxygen using a small animal anesthesia machine (Summit Hill Laboratories). Anesthesia was maintained using a mixture of 3% halothane. The femoral artery was cannulated using a catheter (PE-50) filled with heparinized saline solution. All animals recovered promptly from the halothane anesthesia. The cannulated alligators were equipped with face masks to measure ventilation and were left undisturbed for 48 h at the experimental temperature of 15, 25, or 35°C (environmental chamber, Lab Line). The animal chamber (length 62 cm; diam 12 cm) was continuously flushed with a humidified gas mixture (25% O_2 and 75% N_2) at a rate of 700 ml/min using a gas mixing pump (301 a/F; Wösthoff, Bochum, Germany). At the end of 48 h, ventilation was recorded for 30 min and arterial blood was sampled (after a ventilatory period) for analysis of blood gases, pH, and plasma lactate. Hypoxic gas mixtures (inspired O_2 of 10, 7, 5, and 3%) were then applied for 3 h. Equivalent altitudes for these oxygen levels in Albuquerque (1,600 m altitude) are 6,500, 8,400, and 12,700 m. Ventilation was recorded and arterial blood withdrawn at the end of each experimental condition.

Blood gases, ventilation, and O_2 consumption. Because alligators are periodic breathers, blood gases show large cyclic variations. We sampled blood immediately after the last breath of a breathing cycle and presume to have measured maximum P_{O_2} and minimum P_{CO_2}. Arterial blood samples were analyzed immediately for P_{O_2} and pH (Radiometer blood gas analyzer; BMS 3 MK2, Westlake, OH). The oxygen electrode was calibrated using precision gas mixtures (CCS gas, Radiometer). The pH electrode was calibrated with Radiometer precision buffer solutions (S1510 and S1500). Electrodes were calibrated at the experimental temperature.

Ventilation was measured with a pneumotachographic technique (8). Before analysis, a face mask was constructed for each animal. After cooling the animal to 4°C for 1 h, a head-negative impression was made using alginate resin to which a plaster model was constructed. Mouthguard resin (Healthco) was molded into a face mask using the plaster models. Signals from a differential air transducer (Validyne, model MP45-1) were displayed on a paper recorder (Lafayette, model 76102 B).

Oxygen consumption was measured by flow-through respirometry using an oxygen analyzer (model S-3A; Electrochemistry, Sunnyvale, CA). Flow through the animal chamber was achieved by means of a gas mixing pump (Wösthoff 301 a/F) and measured with a flow meter (Gilmont, model F11).

Intracellular pH and lactate measurements. Intracellular pH was measured by a previously described method (18). In brief, tissue samples were collected from anesthetized animals (methohexital sodium; Brevital sodium, 3 ml/kg, via arterial cannulas) within 10 min after onset of anesthesia. The freeze-clamped samples were wrapped in aluminum foil and stored in liquid nitrogen until analyzed. Tissue powder was prepared under liquid N_2 using a mortar and pestle. For intracellular pH measurements part of the tissue powder was resuspended in ice-cold media, which contained 130 mM of KF and 5 mM of Na_2-nitrilotriacetic acid. The suspension was then spun down and the supernatant pH was measured.

For tissue lactate measurements, part of the tissue powder was subjected to extraction in ice-cold 0.6 M perchloric acid. The supernatant was neutralized by the addition of 5 M KOH (10% of the volume of perchloric acid) and a mixture of solid $K_2CO_3/KHCO_3$ (1/6, wt/wt) (17). Samples were analyzed using standard enzymatic procedures (2). Plasma samples were also subjected to perchloric acid extraction (final concentration of 0.6 M), neutralized, and analyzed as described above.

Calculations and statistics. All values in this paper are given as means ± SE. Analysis of variance was used to test for the differences between means. Multiple comparisons of group means were tested using a step-down multiple F test (Ryan-Einot-Gabriel-Welsch multiple range test; SAS User's Guide,

1985). Values of $P < 0.05$ were considered significant differences.

RESULTS

As shown in a separate study (5), the preferred body temperature of alligators drops from 27.8 ± 1.2°C under normoxia to 15.4 ± 1.0°C with 4% inspired O_2 (5; Fig. 1). One physiological consequence associated with this drop in body temperature is the ventilatory responses to hypoxia. This was evaluated in and above the respective temperature range (Fig. 2). Hyperventilation was greater at higher temperatures. At the body temperature selected under 4% inspired O_2 (15°C) there was no significant increase in ventilation. The hyperventilation caused a respiratory alkalosis both at 25 and 35°C, whereas at 15°C, as expected, there was no significant change in arterial pH (Fig. 3). Inspired O_2 of 3% was tolerated at 15 and 25°C, but was lethal at 35°C.

Figure 4 shows the relationships between oxygen consumption and inspired O_2 at 15, 25, and 35°C. Alligators are oxyregulators with increasing critical Po_2 at higher temperatures. At 35°C, a significant decrease in O_2 uptake occurred at 10, 7, and 5% inspired O_2. At 25°C, there was a significant decrease in O_2 uptake at 5 and 3% inspired O_2. At 15°C there was no effect of hypoxia on O_2 uptake. These results correlated quite closely with the effect of temperature on the release of lactate into the blood. As shown in Fig. 5, at 35°C an accumulation of lactate occurred between 10 and 15% inspired O_2, whereas at 25°C elevated lactate levels were measured at 7, 5, and 3% inspired O_2. There was no increase in arterial plasma lactate levels at 15°C.

Several potential tissue sources of lactate were sampled (tail muscle, liver, heart ventricle, and brain) under normoxic and hypoxic conditions (Fig. 6). These experiments were performed at 25°C. There was no significant difference between tissue lactate levels under normoxic conditions. Hypoxia caused tissue lactate to increase significantly in cardiac and skeletal muscles, whereas no significant change was detected in the brain or liver.

Intracellular pH analysis revealed a general trend to develop an acidosis with hypoxia but only the cardiac and nervous tissues showed a significant drop in pH. This drop was largest in the ventricle (Fig. 7).

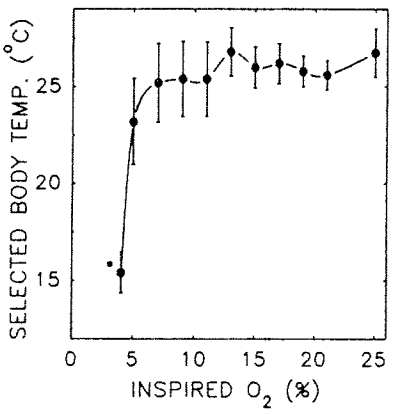

Fig. 1. Behavioral hypothermia in the American alligator, *Alligator mississippiensis*. Preferred body temperature is kept constant until the inspired O_2 fell below 4% O_2. *Significant difference from control (25% O_2). Data from Ref. 5.

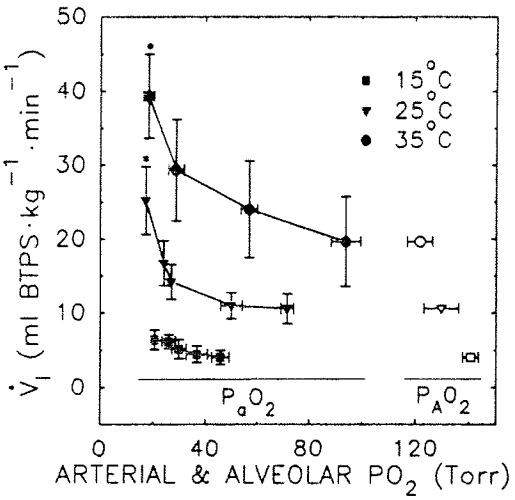

Fig. 2. Relationships between pulmonary ventilation and arterial Po_2 (Pa_{O_2}). No change in ventilation was measured at 15°C. *Significant increase in ventilation occurred under 3% inspired O_2 at 25°C and under 5% inspired O_2 at 35°C. Pa_{O2} was significantly reduced at 3, 5, and 7% inspired O_2 at 15°C, and already from 10% on at 25 and 35°C. The calculated values for alveolar Po_2 (PA_{O_2}) are on right.

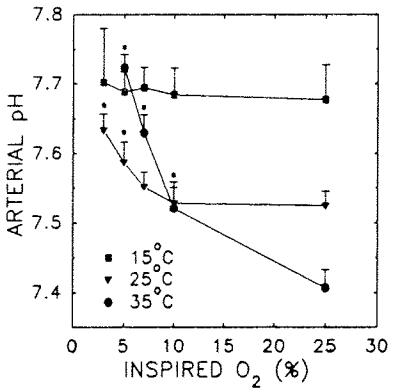

Fig. 3. Relationships between arterial pH and inspired O_2. Respiratory alkalosis occurred at 25°C and was more severe at 35°C. No significant change was detected at 15°C.

DISCUSSION

Hypoxia-induced behavioral hypothermia. Exposure to hypoxic environments elicits behavioral hypothermia in a variety of homeothermic and ectothermic species (15, 28). The American alligator, *A. mississippiensis*, is well adapted for long diving. This fact may be related to the lowest threshold for the onset of behavioral hypothermia (between 4 and 5% O_2) measured so far in a wide range of vertebrates. The threshold for the hypoxia-induced hypothermia has been determined in the following terrestrial vertebrates: 10% in *Bufo marinus* (29), 8% in mice (cf. 28), and 10% in lizards (10).

Ventilation. In most reptiles, pulmonary ventilation increases with increasing body temperature. Turtles have been most extensively studied. Funk and Milsom (6), using *Chrysemys picta*, reported a threefold increase in pulmonary ventilation from 10 to 30°C. Similar responses were found in *Pseudemys floridana* (13) and in *C. picta* (7). On the other hand, the freshwater turtle *Pseudemys scripta* shows no change in pulmonary ventilation over a

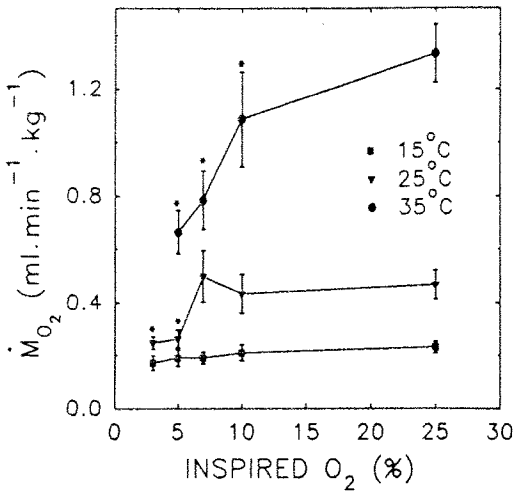

Fig. 4. Relationship between oxygen uptake ($\dot{M}$) and inspired O_2. There is a clear effect of temperature on O_2 uptake. There is no significant change in O_2 uptake at 15°C. At 25°C there was a significant decrease at 5 and 3% inspired O_2, while at 35°C the decrease was detected already at inspired O_2 of 7%.

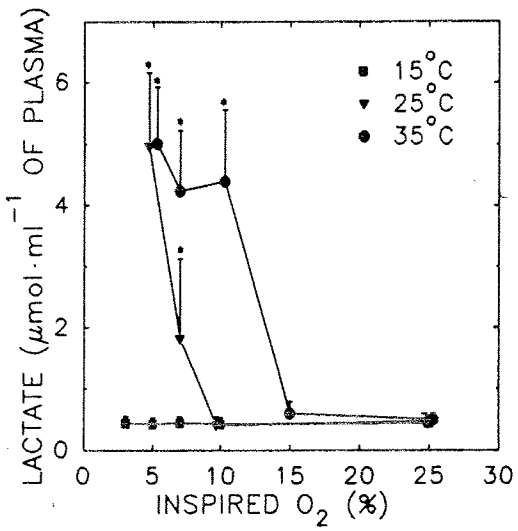

Fig. 5. Lactate levels in arterial plasma under different inspired O_2 levels. No change was measured at 15°C, whereas an increase was detected under 7 and 10% inspired O_2 at 25 and 35°C, respectively.

wide range of temperatures (11). In alligators, the present study found a 2.8-fold increase in ventilation with increasing temperature from 15 to 35°C. Lizards also show an increase in ventilation with increasing body temperature, as was reported for *Varanus gouldii* (1) and *Sauromalus obesus* (3).

Respiratory response to the levels of hypoxia we tested was abolished at 15°C (the preferred body temperature under 4% inspired O_2). The absence of an increase in pulmonary ventilation at 15°C was also reported for hypoxic toads (14). Hypoxia-induced hyperventilation was present at 25°C and more so at 35°C, indicating a temperature-dependent response as previously shown for turtles (12).

Blood gases. The effect of temperature on arterial P_{O_2} and pH under normoxic conditions is consistent with previous reports on ectothermic vertebrates. Arterial P_{O_2} was lower at lower temperatures and the alveolar-arterial P_{O_2} difference was greater (Fig. 2). These results are consistent with the model proposed by Wood (27) for animals with intracardiac shunts, where arterial P_{O_2} is a dependent variable of arterial content and blood oxygen affinity.

Arterial pH varied inversely with temperature (pH/T = −0.016 U/°C, a higher value than found in most vertebrates species; Ref. 9). This value is similar to that previously reported by Davies et al. (4), who suggested that the control of pulmonary ventilation is based on maintenance of a constant ionization of histidine-imidazole groups (21). Changes in arterial pH with temperature result from a relative hypoventilation; i.e., oxygen consumption increased 5.8-fold from 15 to 35°C, whereas ventilation increased approximately threefold (data not shown).

Oxygen consumption, lactate, and intracellular pH measurements. Under normoxic conditions, O_2 uptake increased with increasing temperature with a Q_{10} effect of

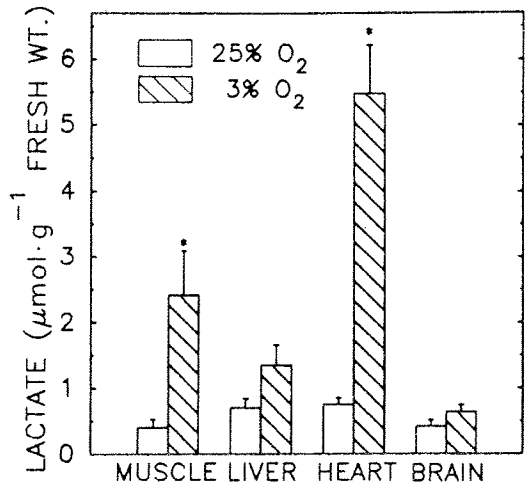

Fig. 6. Tissue lactate measured at 25 and 3% inspired O_2. A significant increase was measured in tail muscle and heart ventricle. Experiments were performed at 25°C.

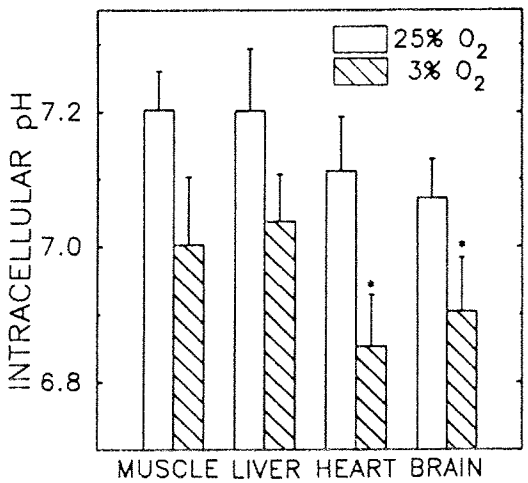

Fig. 7. Intracellular pH measurements under 25 and 3% inspired O_2. An acidification was measured in heart ventricle and brain tissue. Experiments were performed at 25°C.

2.6 from 35 to 25°C and 2.5 from 25 to 15°C. The relationship between O_2 uptake and hypoxia was also influenced by temperature. Oxygen uptake stayed constant with graded hypoxia at 15°C, whereas it decreased significantly below 7% inspired O_2 at 25°C and 10% inspired O_2 at 35°C. Pörtner et al. (19, 20) studied the shift from aerobic to anaerobic metabolism that occurs simultaneously with an increase in O_2 uptake in toads. They suggested that lactate appearance at the critical P_{O_2} acts as a metabolic signal to stimulate metabolism. Below this threshold the O_2 uptake decreases. In alligators, the decrease in O_2 uptake was preceded by lactate accumulation in plasma. This also happened in Pörtner's study of toads. Their experiments were performed only at 20°C. The present data, like that for turtles (25), show that the shift from aerobic to anaerobic metabolism is temperature-dependent, i.e., the threshold falls with decreasing body temperature.

Many aspects of temperature dependence of respiratory function remain to be studied in more detail. It is presently clear that a correlation exists between a larger oxygen consumption at high temperature and right-shifts of the critical thresholds for aerobic metabolism.

Lactate accumulation was large in the ventricle, a working muscle, whereas it was modest in tail muscle and not significant in liver and brain. High brain lactate levels have been reported for anoxic frogs (24). In our study, inspired O_2 of 3% did not cause lactate accumulation, suggesting that this organ is hypoxia-tolerant in alligators.

Hypoxia caused the intracellular pH to drop in heart ventricle and brain, whereas there was no significant change in tail muscle and liver. The lack of correlation between lactate and pH data for tail muscle and brain may reflect relative buffer capacity of these tissues. Acid-base balance in the brain has been studied after hypercapnia in toads (22), fish (26), and mammals (16). In all these experiments, the brain tissue was found to be poorly buffered. On the other hand, the turtle brain intracellular pH shows the same magnitude of change when anoxia was applied with or without accompanying hypercapnia, indicating a high intracellular nonbicarbonate buffer capacity (23).

Low intracellular pH values in the ventricle have also been measured in hypoxic toads by Pörtner et al. (20), who suggested it would be due to high rates of lactate acid formation and accumulation.

Conclusions. Behavioral hypothermia in response to hypoxia in *Alligator mississippiensis* shifts the threshold for compensatory mechanism to lower oxygen levels. At 15°C (preferred temperature during hypoxia), all the parameters measured are kept constant regardless of the severity of hypoxia we tested. Furthermore, the present study provides additional evidence that the threshold of lactate formation in ectothermic vertebrates is a temperature-dependent variable.

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