VENTILATORY RESPONSES TO HYPOXIA IN THE TOAD BUFO PARACNEMIS BEFORE AND AFTER A DECREASE IN HAEMOGLOBIN OXYGEN-CARRYING CAPACITY

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Summary

The ventilatory and cardiovascular effects of a decreased O₂-carrying capacity of the blood were evaluated in the toad Bufo paracnemis. Pulmonary ventilation was monitored using a pneumotachographic technique. Chronic arterial catheters served to record both cardiac frequency and blood pressure and enabled the withdrawal of blood samples for analysis of pH and partial pressure of O₂ (P₂O₂). Haemoglobin concentrations were determined by the cyanmethaemoglobin method. The ventilatory response to hypoxia was not affected by the reduction in blood O₂-carrying capacity, which suggests that P₂O₂ rather than O₂ content is the regulated variable. The reduction in capacity is accompanied by an increased normoxic heart rate and by a reversal of the hypoxic tachycardia normally observed.

Introduction

Amphibians and reptiles increase pulmonary ventilation in response to hypoxia (Jackson, 1973; Benchetrit et al. 1977; Glass et al. 1983; Kruhøffer et al. 1987), but the exact O₂ stimulus (whether P₂O₂, haemoglobin O₂-saturation or O₂ content of the blood) has not yet been identified. Some effects of temperature on ventilatory responses to hypoxia suggest a dependence on haemoglobin O₂-saturation or on O₂ content. Over a wide temperature range, the turtle Chrysemys picta increased ventilation when haemoglobin O₂-saturation declined to about 50% (Glass et al. 1983). Toads and lizards move to lower temperatures when exposed to hypoxia (Hicks and Wood, 1985). The decrease in temperature increases blood O₂-affinity and a fairly constant O₂ saturation and O₂ content are maintained. Reduction of blood O₂-carrying capacity by bleeding also induces behavioural hypothermia in lizards and toads (Hicks and Wood, 1985; Wood, 1991). Previous studies have primarily addressed conditions at different temperatures (Glass et al. 1983; Dupre et al. 1989), where O₂ content seems to be rather closely

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regulated, even if the specific stimulus for increased ventilation is partial pressure of O₂ (cf. Wood and Glass, 1991).

The present study evaluates the relationships between O₂ content of the blood and ventilation in normoxic and hypoxic *Bufo paracnemis*. Ventilation was measured directly on unrestrained specimens, and blood samples were obtained via arterial catheters to measure \( P_{O_2} \), haemoglobin concentration ([Hb]) and pH. Additionally, cardiac frequency and blood pressures were recorded. Measurements were performed before and after bleeding to reduce [Hb] and, thereby, O₂-carrying capacity.

**Materials and methods**

**Experimental animals**

Toads, *Bufo paracnemis* (Lutz), weighing 300–450 g were collected close to Ribeirão Preto (São Paulo State, Brazil) a few weeks before experimentation. The toads were kept in containers equipped with dry areas and running water. Temperature was maintained at approximately 27 °C and the daily photoperiod was 12 h:12 h light:dark. Food was withheld for 4 days before surgery.

**Surgical procedure**

The right femoral artery was chronically cannulated (PE 50) as described by Boutilier *et al.* (1979), using ether vapour anaesthesia. Catheters were filled with heparinized amphibian Ringer (de la Lande *et al.* 1962) and were flushed daily. Within a few hours, all toads had recovered from anaesthesia and measurements were initiated 24 h after the conclusion of surgery.

**Blood analysis**

Arterial blood was analyzed for \( P_{O_2} \) and pH by means of a FAC 204A O₂ analyzer (FAC Instruments, São Carlos, Brazil) and a Micronal B374 pH meter. All electrodes were kept at the temperature of the experimental animal. The O₂ electrode was calibrated with pure N₂ and atmospheric air and the pH electrode was adjusted using high-precision buffers (S1500 and S1510, Radiometer, Denmark). Haemoglobin concentration was determined by the cyanometahaemoglobin method, assuming an extinction coefficient of 11.0 per mmol Hb of human blood (Zilstra *et al.* 1983).

**Ventilation and blood pressure measurements**

Ventilation was measured directly by a pneumotachographic method, which is based on the Poiseuille principle that the laminar flow of a gas is proportional to the pressure gradient across a tube. A lightweight transparent face mask provided an air-tight connection between the nostrils and a Fleisch tube. Inspiratory and expiratory gas flows were monitored by means of a highly sensitive differential air pressure transducer (Statham, model 12123) connected to a Narco coupler (type 7179; Narco Bio-systems, Inc., Houston, Texas, USA). The method was described by Glass *et al.* (1978) and has
been used to measure ventilation in *Bufo paracnemis* (Kruhoff et al. 1987; Branco et al. 1992). Arterial blood pressure was measured by connecting the catheter to a Statham pressure transducer (P23Db) kept at the level of the toad’s heart. A vertical water column was applied for frequent calibration. Heart rate was determined by counting pressure pulses. A Narco Physiograph (model Four-A; Narco Instruments, Inc.) displayed ventilation and blood pressure.

**Gas mixtures**

Hypoxic gases were prepared by mixing pure N₂ and atmospheric air. Flowmeters stabilized delivery of gases to a humidifying mixing chamber connected to the animal container (total flow 5–7 l min⁻¹). Oxygen concentrations within the container (2.5 l) were monitored continuously, using a Beckman OM-11 O₂ analyzer calibrated with pure N₂ and dry air.

**Experimental protocol**

Repeated blood sampling may eventually decrease the blood oxygen-carrying capacity. The purpose of this study required this effect to be kept to a minimum. Accordingly, measurements were divided into two series that were identical as to levels of hypoxia and exposure schedules. One group of toads (A) provided data on ventilation, \( P_{\text{a}O_2} \) and [Hb], whereas the second group (B) provided blood pressure, pHa and [Hb] data. The main purpose of this second group was to ascertain that a reduction of O₂-carrying capacity did not affect the changes in pH occurring during hypoxia, thereby altering the magnitude of the ventilatory responses to hypoxia. This second group was also masked to maintain identical experimental conditions.

Stable ventilation and blood pressures were usually obtained a few hours after placement of the toad into the experimental chamber. Subsequently, hypoxic gas mixtures were applied in the following sequence of fractional O₂ concentrations (\( F_{O_2} \)): 0.150, 0.100, 0.075 and 0.050; each exposure was maintained for 30 min. Ventilation and blood pressures normally reached stable values during the first 10 min. Blood samples were withdrawn during the last minute of each exposure.

As the next step, the blood O₂-carrying capacity was reduced by bleeding. Blood volume was restored by injection of amphibian saline. The protocol was repeated 24 h after this treatment. All experiments were carried out at 27±1°C.

**Data analysis**

Lung ventilation was distinguished by the presence of a biphasic flow profile during expiration (Jones, 1982). Ventilation, blood pressure and heart rate were calculated for 10 min periods. Tidal volume was obtained from the square area of the inspired flow signal above the zero line. This relationship was calibrated by injection of standard volumes. Effects of hypoxia and bleeding were tested for statistical significance using a two-way analysis of variance (ANOVA) and differences between means were evaluated by Tukey’s test. A fiducial limit of \( P \leq 0.05 \) was applied. All values are given as mean ±1 S.E.M.
Results

Blood haemoglobin concentrations were 1.04±0.09 and 0.90±0.16 mmol Hb l⁻¹ blood for groups A and B (both N=6), respectively. Bleeding and subsequent replacement by Ringer reduced these values to 0.65±0.12 mmol Hb l⁻¹ for group A and 0.46±0.12 mmol Hb l⁻¹ for group B. Throughout the experiments, [Hb] remained constant in spite of repeated blood sampling.

Ventilation rate increased significantly during hypoxia both before and after reduction of haemoglobin oxygen-carrying capacity (Fig. 1). Ventilation rate in toads with normal capacity was 100±19 ml kg⁻¹ min⁻¹ (N=6) during normoxia and increased to 256±46 ml kg⁻¹ min⁻¹ (N=6) when FO₂ was reduced to 0.05. Following reduction of O₂ capacity, the corresponding values for ventilation rate were 92±4 and 202±29 ml kg⁻¹ min⁻¹ (N=6), respectively (Fig. 1). There was no significant effect of reduced blood O₂-carrying capacity on the ventilatory response to hypoxia. Moreover, reduction of capacity did not affect the values of pHₐ or PAO₂ during exposures to hypoxia (Table 1).

Toads with a normal O₂-carrying capacity significantly increased their heart rate from a normoxic value of 23±2.5 beats min⁻¹ to 37±2.5 beats min⁻¹ (N=6) when FO₂ was reduced to 0.05 (Fig. 2). Bleeding almost doubled the normoxic heart rate to 38±5 beats min⁻¹ (N=6) and abolished the effect of hypoxia. Following a reduction in haemoglobin oxygen-carrying capacity, breathing gas with an FO₂ of 0.05 resulted in a

![Graph](image_url)
lower heart rate in two out of six toads. At normal capacity, the normoxic blood pressures were 5.2±0.5 (systolic)/3.2±0.4 (diastolic) kPa (N=6). These pressures increased to 6.1±0.4 (systolic)/4.0±0.2 (diastolic) kPa (N=6) when \( F_O^2 \) was reduced to 0.05. Reduction in the blood \( O_2 \)-carrying capacity caused a marginally significant (\( P<0.3 \)) decrease of systolic pressure.

### Table 1. Effects of reduction in haemoglobin \( O_2 \)-carrying capacity and hypoxia on arterial \( P_O^2 \) in \( B. p. \)

<table>
<thead>
<tr>
<th>( F_O^2 )</th>
<th>( P_O^2 ) (kPa)</th>
<th>( P_O^2 ) (kPa)</th>
</tr>
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<tbody>
<tr>
<td>0.21</td>
<td>7.83±0.04</td>
<td>7.82±0.02</td>
</tr>
<tr>
<td>0.15</td>
<td>7.88±0.05</td>
<td>7.88±0.03</td>
</tr>
<tr>
<td>0.10</td>
<td>7.94±0.04*</td>
<td>7.93±0.03*</td>
</tr>
<tr>
<td>0.075</td>
<td>7.96±0.04*</td>
<td>7.95±0.04*</td>
</tr>
<tr>
<td>0.05</td>
<td>7.96±0.02*</td>
<td>7.95±0.04*</td>
</tr>
</tbody>
</table>

No effect of reduced haemoglobin \( O_2 \)-carrying capacity was detected. A significant change (\( P<0.05 \)) from the value at \( F_O^2 = 0.21 \) is indicated with an asterisk.

Values are mean ± s.e.m. (N=6).

Fig. 2. Heart rate versus inspired \( P_O^2 \) before (filled squares) and after (open circles) reductions in blood \( O_2 \)-carrying capacity. Mean values ± s.e.m. (N=6). Significant (\( P<0.05 \)) increases of heart rate relative to the normoxic control are indicated by an asterisk, whereas increases of heart rate caused by reduced capacity are marked by a dagger.
There are several studies reporting ventilatory responses to hypoxia in amphibians (Toews, 1971; Boutilier and Toews, 1977; Kruhøffer et al. 1987; Smatresk and Smits, 1991; Van Vliet and West, 1992). Increases in respiratory frequency were reported for hypoxic *Bufo marinus*, on the basis of pressure changes within the buccal cavity and the lung (Boutilier and Toews, 1977). Later, ventilation was measured directly by Kruhøffer et al. (1987), who studied ventilatory responses to hypoxia in *Bufo paracnemis* at various temperatures. The present data on ventilation, \( P_{aO_2} \) and pH are consistent with the results of Kruhøffer et al. (1987) and also agree well with blood gas values reported by de Castro-e-Silva et al. (1992), who studied hypoxia-induced hyperglycaemia in *Bufo paracnemis*.

*Bufo vulgaris* possesses oxygen-sensitive chemoreceptors at the carotid labyrinth (Ishii et al. 1966) and at the aortic arch (Ishii et al. 1985). Chemoreceptors may also be present on the pulmocutaneous trunk, since perfusion with lobeline induces intensive ventilatory responses in conscious toads (Hoffmann and Souza, 1982).

Van Vliet and West (1992) recorded the discharge characteristics of aortic and carotid nerves in pithed unidirectionally ventilated *Bufo marinus* and reported an inverse relationship between discharge frequency and \( P_{aO_2} \) below 15 kPa. Moreover, reductions in blood O\(_2\)-carrying capacity of 35–89% had virtually no effect on the discharge characteristics of the carotid chemoreceptors. Their study cannot exclude the possibility that additional O\(_2\) receptors monitor the O\(_2\) content of the blood and, eventually, dominate the O\(_2\)-related ventilatory drive. The present data are, however, consistent with the discharge characteristics reported by Van Vliet and West (1992) and indicate that pulmonary ventilation in *Bufo paracnemis* is not altered by decreases in blood O\(_2\)-carrying capacity.

Lahiri et al. (1981) emphasized that the response characteristics of O\(_2\) receptors may depend on their blood supply. The Fick principle states that O\(_2\) consumption is equal to blood flow minus arteriovenous O\(_2\) concentration difference. Accordingly, an increased blood flow to a receptor tissue decreases its arteriovenous O\(_2\) concentration difference, which reduces stimulation of the receptors. *Bufo paracnemis* increased its cardiac frequency after a reduction in blood O\(_2\)-carrying capacity. This tachycardia might have partially restored \( O_2 \) delivery to receptor tissues. Therefore, it is possible that receptors sensitive to O\(_2\) content exist in *Bufo paracnemis* but were not detected because of the tachycardia.

The arterial O\(_2\) chemoreceptors in various groups of vertebrates are probably homologous (Comroe, 1974), but the available data do not support the idea of a common specific stimulus. Most recent studies on mammalian O\(_2\) receptors agree that the carotid bodies monitor \( P_{aO_2} \), whereas the aortic bodies are sensitive to blood O\(_2\) content as well. This difference probably correlates with a very high ratio of perfusion to O\(_2\) consumption for the carotid bodies (Lahiri et al. 1980, 1981). In mammals, the aortic chemoreceptors contribute little to normoxic and hypoxic ventilation, and studies comparing individuals with different O\(_2\) affinities show that ventilation depends on \( P_{aO_2} \) and not on O\(_2\) content (Santiago et al. 1975; Hebbel et al. 1977; Birchard and Tenney, 1986).
Some studies on non-mammalian vertebrates suggest that ventilatory responses to hypoxia depend on blood O₂-content or haemoglobin O₂-saturation. In birds, inhalation of carbon monoxide provokes increases in ventilation brought about by changes in tidal volume (Tschorn and Fedde, 1974). Moreover, the ventilatory responses to hypoxia correlate with saturation rather than with $P_{O_2}$ when species of different O₂ affinity are compared (Boggs and Birchard, 1983). Smith and Jones (1982) reported a direct relationship between gill ventilation and the O₂ content of the blood in trout (*Oncorhynchus mykiss*). Oxygen content also seems to be closely regulated in amphibians and reptiles exposed to temperature changes (see Wood and Glass, 1991). Nevertheless, the present data suggest that $P_{O_2}$ is the predominant specific stimulus for hypoxia-induced ventilatory responses in *Bufo paracnemis*.

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**References**


