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 O_2-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 O_2 (P_{O_2}). Haemoglobin concentrations were determined by the cyanmethaemoglobin method. The ventilatory response to hypoxia was not affected by the reduction in blood O_2-carrying capacity, which suggests that P_{O_2} rather than O_2 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 O_2 stimulus (whether P_{O_2}, haemoglobin O_2-saturation or O_2 content of the blood) has not yet been identified. Some effects of temperature on ventilatory responses to hypoxia suggest a dependence on haemoglobin O_2-saturation or on O_2 content. Over a wide temperature range, the turtle *Chrysemys picta* increased ventilation when haemoglobin O_2-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 O_2-affinity and a fairly constant O_2 saturation and O_2 content are maintained. Reduction of blood O_2-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 O_2 content seems to be rather closely

regulated, even if the specific stimulus for increased ventilation is partial pressure of O_2 (cf. Wood and Glass, 1991).

The present study evaluates the relationships between O_2 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, O_2-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 O_2 analyzer (FAC Instruments, São Carlos, Brazil) and a Micronal B374 pH meter. All electrodes were kept at the temperature of the experimental animal. The O_2 electrode was calibrated with pure N_2 and atmospheric air and the pH electrode was adjusted using high-precision buffers (S1500 and S1510, Radiometer, Denmark). Haemoglobin concentration was determined by the cyanomethaemoglobin 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* (Kruhøffer *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 N_2 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 O_2 analyzer calibrated with pure N_2 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, Pa_{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 O_2-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 O_2 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 O_2-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 $27\pm1\,^{\circ}C$.

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 $P\leqslant0.05$ 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 $Hb_4 l^{-1}$ 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 $Hb_4 l^{-1}$ for group A and 0.46 ± 0.12 mmol $Hb_4 l^{-1}$ 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 F_{O_2} was reduced to 0.05. Following reduction of O_2 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 O_2-carrying capacity on the ventilatory response to hypoxia. Moreover, reduction of capacity did not affect the values of pHa or Pa_{O_2} during exposures to hypoxia (Table 1).

Toads with a normal O_2-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 F_{O_2} 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 F_{O_2} of 0.05 resulted in a
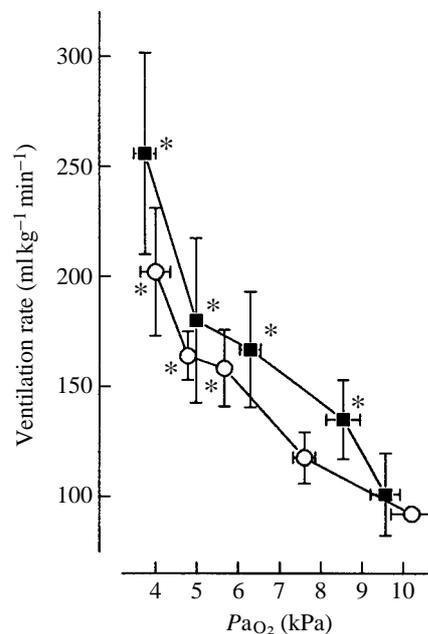

Fig. 1. Relationship between ventilation rate and Pa_{O_2} before (filled squares) and after (open circles) reduction in blood O_2-carrying capacity. The curves are statistically identical. Mean values $\pm$ S.E.M. ($N=6$). Significant ($P\leqslant0.05$) increases in ventilation relative to the normoxic control are indicated by an asterisk.

Table 1. *Effects of reduction in haemoglobin O_2-carrying capacity and hypoxia on arterial* P_{O_2} *in* Bufo paracnemis

F_{O_2}	Before reduction		After reduction	
	pH	P_{O_2} (kPa)	pH	P_{O_2} (kPa)
0.21	7.83±0.04	10.2±0.5	7.82±0.02	9.6±0.4
0.15	7.88±0.05	7.6±0.3*	7.88±0.03*	8.5±0.4*
0.10	7.94±0.04*	5.7±0.1*	7.93±0.03*	6.3±0.3*
0.075	7.96±0.04*	4.8±0.2*	7.95±0.04*	5.0±0.3*
0.05	7.96±0.02*	4.0±0.4*	7.95±0.04*	3.7±0.3*

No effect of reduced haemoglobin O_2-carrying capacity was detected. A significant change ($P{\leqslant}0.05$) from the value at $F_{O_2} = 0.21$ is indicated with an asterisk.

Values are mean ± S.E.M. ($N=6$).

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.
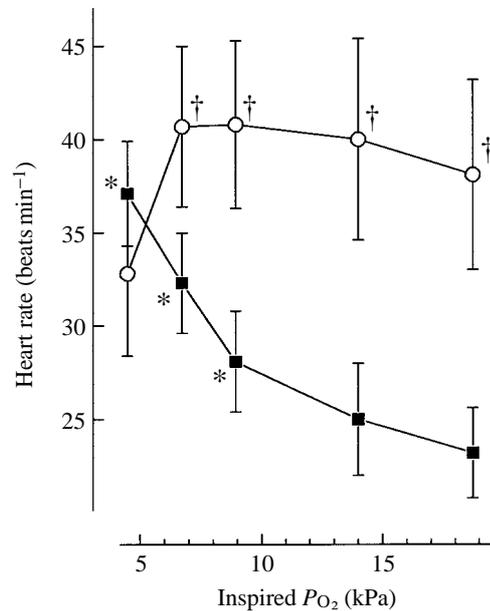

Fig. 2. Heart rate *versus* inspired P_{O_2} before (filled squares) and after (open circles) reductions in blood O_2-carrying capacity. Mean values ± S.E.M. ($N=6$). Significant ($P{\leqslant}0.05$) increases of heart rate relative to the normoxic control are indicated by an asterisk, whereas increases of heart rate caused by reduced capacity are marked by a dagger.

Discussion

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, Pa_{O_2} and pHa 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 Pa_{O_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 Pa_{O_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 Pa_{O_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 O_2-content or haemoglobin O_2-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 O_2 affinity are compared (Boggs and Birchard, 1983). Smith and Jones (1982) reported a direct relationship between gill ventilation and the O_2 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.*

References

BENCHETRIT, G., ARMAND, J. AND DEJOURS, P. (1977). Ventilatory chemoreflex drive in the tortoise *Testudo horsfieldi*. *Respir. Physiol*. **31**, 183–191.

BIRCHARD, G. F. AND TENNEY, S. M. (1986). The hypoxic ventilatory response of rats with increased blood oxygen affinity. *Respir. Physiol*. **66**, 225–233.

BOGGS, D. F. AND BIRCHARD, G. F. (1983). Relationship between haemoglobin O_2 affinity and the ventilatory response to hypoxia in the rhea and pheasant. *J. exp. Biol*. **102**, 347–352.

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. AND TOEWS, D. P. (1979). Acid–base relationships in the blood of the toad *Bufo marinus*. I. The effects of environmental CO_2. *J. exp. Biol*. **82**, 331–344.

BOUTILIER, R. G. AND TOEWS, D. P. (1977). The effect of progressive hypoxia on respiration in the toad, *Bufo marinus. J. exp. Biol*. **68**, 99–107.

BRANCO, L. G. S., GLASS, M. L. AND HOFFMANN, A. (1992). Central chemoreceptor drive to breathing in unanesthetized toads, *Bufo paracmenis*. *Respir. Physiol*. **87**, 195–204.

COMROE, J. H., JR (1974). *Physiology of Respiration*, 2nd edn. Chicago: Year Book Medical Publ., Inc.

DE CASTRO-E-SILVA, E., BRANCO, L. G. S. AND GLASS, M. L. (1992). Autonomic basis for hypoxia-induced hyperglycaemia in toads (*Bufo paracnemis*). *Comp. Biochem. Physiol*. **102**, 731–734.

DE LA LANDE, I. S., TYLER, M. J. AND PRIDMORE, B. J. (1962). Pharmacology of the heart of *Tiliqua* (*Trachysaurus*) *rugosa* (the sleepy lizard). *Aust. J. exp. Biol. med. Sci*. **40**, 129–137.

DUPRE, K. R., HICKS, J. W. AND WOOD, S. C. (1989). Effect of temperature on chemical control of ventilation in Mexican black iguanas. *Am. J. Physiol*. **257**, R1258–R1263.

GLASS, M. L., BOUTILIER, R. G. AND HEISLER, N. (1983). Ventilatory control of arterial P_{O_2} in the turtle *Chrysemys picta bellii*: Effects of temperature and hypoxia. *J. comp. Physiol*. **151**, 145–153.

GLASS, M. L., WOOD, S. C. AND JOHANSEN, K. (1978). The application of pneumotachography on small unrestrained animals. *Comp. Biochem. Physiol*. **59**A, 425–427.

HEBBEL, R. P., KRONENBERG, R. S. AND EATON, J. W. (1977). Hypoxic ventilatory response in subjects with normal and high oxygen affinity hemoglobins. *J. clin. Invest.* **60**, 1211–1215.

HICKS, J. W. AND WOOD, S. C. (1985). Temperature regulation in lizards: effects of hypoxia. *Am. J. Physiol.* **248**, R595–R600.

HOFFMANN, A. AND SOUZA, M. B. C. (1982). Cardiovascular reflexes in conscious toads. *J. auton. nerv. Syst.* **5**, 345–355.

ISHII, K., HONDA, K. AND ISHII, K. (1966). The function of the carotid labyrinth in the toad. *Tohoku J. exp. Med.* **88**, 103–116.

ISHII, K., ISHII, K. AND KUSAKABE, T. (1985). Chemo- and baroreceptor innervation of the aortic trunk of the toad *Bufo vulgaris. Respir. Physiol.* **60**, 365–375.

JACKSON, D. C. (1973). Ventilatory response to hypoxia in turtles at various temperatures. *Respir. Physiol.* **18**, 178–187.

JONES, R. M. (1982). How toads breathe: Control of air flow to and from the lung by the nares in *Bufo marinus. Respir. Physiol.* **49**, 251–265.

KRUHØFFER, M., GLASS, M. L., ABE, A. S. AND JOHANSEN, K. (1987). Control of breathing in an amphibian *Bufo paracnemis:* effects of temperature and hypoxia. *Respir. Physiol.* **69**, 267–275.

LAHIRI, S., MULLIGAN, E. , NISHINO, T., MOKASHI, A. AND DAVIES, R. O. (1981). Relative responses of aortic body and carotid body chemoreceptors to carboxyhemoglobinemia. *J. appl. Physiol.* **50**, 580–586.

LAHIRI, S., NISHINO, T., MOKASHI, A. AND MULLIGAN, E. (1980). Relative responses of aortic body and carotid body chemoreceptors to hypotension. *J. appl. Physiol.* **48**, 781–788.

SANTIAGO, T. D., EDELMAN, N. H. AND FISHMAN, A. F. (1974). The effect of anemia on the ventilatory response to transient and steady-state hypoxia. *J. clin. Invest.* **55**, 410–418.

SMATRESK, N. J. AND SMITS, A. W. (1991). Effects of central and peripheral chemoreceptor stimulation on ventilation in the marine toad, *Bufo marinus. Respir. Physiol.* **83**, 223–238.

SMITH, F. M. AND JONES, D. R. (1982). The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **97**, 325–334.

TOEWS, D. P. (1971). Factors affecting the onset and termination of respiration in the salamander, *Amphiuma tridactylum. Can. J. Zool.* **49**, 1231–1237.

TSCHORN, R. R. AND FEDDE, M. R. (1974). Effects of carbon monoxide on avian intrapulmonary carbon dioxide-sensitive receptors. *Respir. Physiol.* **20**, 303–311.

VAN VLIET, B. N. AND WEST, N. H. (1992). Functional characteristics of arterial chemoreceptors in an amphibian (*Bufo marinus*). *Respir. Physiol.* **88**, 113–127.

WOOD, S. C. (1991). Effect of hematocrit on behavioral temperature regulation of the toad, *Bufo marinus. Am. J. Physiol.* **258**, R848–R851.

WOOD, S. C. AND GLASS, M. L. (1991). Respiration and thermoregulation of amphibians and reptiles. In *Physiological Strategies for Gas Exchange and Metabolism* (ed. A. J. Woakes, M. K. Grieshaber and C. R. Bridges), pp. 107–124. Cambridge: Cambridge University Press.

ZILSTRA, W. G., BUURMSMA, A. AND ZWART, A. (1983). Molar absorptions of human haemoglobin in the visible range. *J. appl. Physiol.* **52**, 1287–1291.