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Cardiovascular responses to hypoxia and anaemia in the toad *Bufo marinus*

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Summary

Amphibians exhibit cardiorespiratory responses to hypoxia and, although several oxygen-sensitive chemoreceptor sites have been identified, the specific oxygen stimulus that triggers these responses remains controversial. This study investigates whether the cardiovascular response to oxygen shortage correlates with decreased oxygen partial pressure of arterial blood ($P_{a_{O_2}}$) or reduced oxygen concentration ($[O_2]$) in toads. Toads, equipped with blood flow probes and an arterial catheter, were exposed to graded hypoxia [fraction of oxygen in the inspired air ($F_{I_{O_2}}$)=0.21, 0.15, 0.10, 0.07 and 0.05] before and after reductions in arterial $[O_2]$ by isovolemic anaemia that reduced haematocrit by approximately 50%. Toads responded to hypoxia by increasing heart rate (f_H) and pulmocutaneous blood flow ($\dot{Q}_{pc}$) and reducing the net cardiac right-to-left-shunt. When arterial $[O_2]$ was reduced by anaemia, the toads exhibited a similar cardiovascular response to that observed in hypoxia. While arterial CO_2 partial pressure ($P_{a_{CO_2}}$) decreased significantly during hypoxia, indicative of increased alveolar ventilation, anaemia did not alter $P_{a_{CO_2}}$. This suggests that reductions in $[O_2]$ mediate cardiovascular adjustments, while ventilatory responses are caused by reduced $P_{a_{O_2}}$.

Key words: amphibian, *Bufo*, cardiovascular, respiratory, anaemia, hypoxia, cardiac shunt.

Introduction

Several studies have shown that anurans (frogs and toads) increase ventilation, heart rate (f_H) and pulmocutaneous blood flow ($\dot{Q}_{pc}$) in response to low oxygen levels (Boutilier and Toews, 1977; Kruhøffer et al., 1987; Wang et al., 1994; Branco and Glass, 1995; Gamperl et al., 1999; Andersen et al., 2001). These cardiovascular and ventilatory adjustments serve to protect oxygen delivery in response to hypoxia. The single ventricle of anurans is undivided and allows for mixing of systemic and pulmonary blood within the heart, although a large blood flow separation has been documented (Johansen and Ditadi, 1966; Tazawa et al., 1979; Shelton, 1985). The ability to alter $\dot{Q}_{pc}$ independently of systemic blood flow ($\dot{Q}_{sys}$) enables anurans to control arterial blood gas composition by altering pulmonary ventilation and/or by changing the magnitude of the right-to-left (R–L) cardiac shunt whenever an elevated oxygen delivery is required during anaemia, hypoxia or exercise (Wang and Hicks, 1996; Gamperl et al., 1999; Hedrick et al., 1999).

The specific oxygen stimulus that triggers the cardiorespiratory response to hypoxia remains controversial. At issue is the degree to which arterial oxygen tension [physically dissolved oxygen in arterial blood ($P_{a_{O_2}}$)], haemoglobin oxygen saturation [the percentage of haemoglobin molecules with bound oxygen (HbO_2sat)] and/or oxygen concentration {the sum of dissolved and haemoglobin bound oxygen, ($[O_2]$)} represent the primary signal for stimulating these homeostatic adjustments. For example, several studies on reptiles have suggested that HbO_2sat is the primary stimulus for the ventilatory response to hypoxia because ventilation is well-correlated with arterial HbO_2sat (Glass et al., 1983; Dupré et al., 1989). Other studies on amphibians have noted cardiovascular changes in response to anaemia, by either anaemia or carbon monoxide exposure, without associated changes in ventilation (Wang et al., 1994; Branco and Glass, 1995). This suggests that different oxygen signals – partial pressure and concentration – may exist for cardiovascular and ventilatory adjustments to hypoxia and anaemia.

In the present study, we investigate the cardiovascular response to reductions in both arterial $[O_2]$ and $P_{a_{O_2}}$ by measuring blood flows and arterial blood gases during exposure to hypoxia (progressively reducing inspired oxygen from 0.21 to 0.05) before and after inducing anaemia that reduced haematocrit by approximately 50%.

Materials and methods

Experimental animals

Cane toads, *Bufo marinus* L., of undetermined sex and with a body mass ranging from 200 g to 504 g (mean ± S.E.M.,

366±60 g) were obtained from a commercial supplier (Lemberger, Oshkosh, WI, USA) and kept at the University of Aarhus for several months before being used in this study. The toads were maintained at a temperature of 23–28°C in large containers with access to running water and dry areas. They were fed mealworms five times a week, but food was withheld at least three days prior to surgery.

Surgical procedures

Toads were anaesthetised by immersion into a 1.0 g l^{-1} benzocaine solution (ethyl *p*-amino benzoate, Sigma E 1501), and surgery started when the corneal reflex disappeared. The lungs were deflated by opening the glottis using a small piece of plastic tubing, and the animal was placed on a surgical table and covered with wet paper towels. Toads were artificially ventilated with air every 15 min through a small piece of soft rubber tubing inserted through the glottis. A left lateral incision (approximately 2–3 cm) was made in the body wall ventral to the parotoid gland. From this incision, the left systemic and pulmocutaneous arteries were exposed by blunt dissection of connective tissue between the abdominal and forelimb muscles (see Hedrick et al., 1999). Transonic 2S blood flow probes (Transonic Systems Inc., Ithaca, NY, USA) were placed around the left systemic and left pulmocutaneous arteries, and the space between probe and artery was filled with an acoustic coupling gel (Berner Lab, Helsinki, Finland). The leads from the flow probes were threaded back through the incision and tied to the skin at several positions on the dorsal surface of the toad to ensure that the probes remained in position. The right femoral artery was occlusively cannulated through an incision in the hind leg to sample arterial blood and to measure systemic arterial blood pressure. The catheter was secured to the dorsal surface of the animal by silk sutures.

Surgery normally lasted less than 60 min and all toads regained normal righting reflexes within 45 min of being placed under running tapwater. All toads were treated with enrofloxacin (Baytril Bayer AG, Leverkusen, Germany; 2 mg kg^{-1}, intramuscular) to prevent infections. When the toads had regained normal reflexes, each individual animal was transferred to an experimental chamber (40 cm×30 cm×20 cm) containing wet paper towels and a dry area. These experimental chambers were maintained within a climatic chamber at a constant temperature of 25°C, which is close to the preferred body temperature (24°C) of *Bufo marinus* in the laboratory (Johnson, 1972). Toads were visually and audibly shielded from disturbances during measurements and withdrawal of arterial blood.

Experimental protocol

Experiments began 24–48 h after surgery; arterial blood gases and acid–base parameters of *Bufo marinus* stabilise within 24 h of surgery (Andersen and Wang, 2002). The blood flow probe leads and catheter were connected to blood flow and pressure measuring equipment located outside the experimental chamber housing the toad, and haemodynamic variables were allowed to stabilise for 30–90 min. Thereafter, initial cardiovascular measurements were made, a control (normoxic) blood sample was withdrawn, and the toads were exposed to progressive hypoxia [fraction of oxygen in the inspired air (F_{IO_2})=0.15, 0.10, 0.07 and 0.05]. The hypoxic gas mixtures were prepared by mixing pure N_2 and air with a Dameca gas mixer, and the P_{O_2} of the mixed gases entering and leaving the chamber was monitored by a gas analyser (model 602; Criticare Systems, Inc., Waukesha, WI, USA). The incoming gas mixtures passed through a water column placed inside the climatic chamber to ensure the appropriate temperature and high humidity in the experimental chamber. Each gas level was maintained for 30 min, and a blood sample was removed for analysis at the end of each level. Following the exposure to hypoxia, toads were bled to reduce [O_2] by approximately 50%. Plasma was reinfused with toad Ringer (composition given by Prosser, 1970) in order to maintain blood volume. After approximately 24 h, the hypoxic protocol above was repeated with the anaemic animals.

A second group of control animals (N=6) were exposed to hypoxia on successive days without anaemia exposure. These animals were equipped with flow probes to measure haemodynamic variables as described above, but were not equipped with an arterial catheter, so measurements of blood gases were not performed.

Measurements of blood flows and blood pressures and calculations of cardiac shunt patterns

The blood flow probes were connected to a dual-channel flow meter (model T201; Transonic Systems Inc., Ithaca, NY, USA). The femoral catheter was connected to a disposable pressure transducer (model PX600; Baxter Edward, Irvine, CA, USA), and the signal was amplified using an in-house-built preamplifier. The transducer was calibrated daily against a static water column. Signals from the blood pressure transducer and the blood flow meter were collected digitally using an AcqKnowledge MP 100 data-acquisition system (version 3.2.3; BioPac Systems, Inc., Santa Barbara, CA, USA) at 50 Hz.

The left and right side of the truncus arteriosus and the pulmocutaneous arteries in *Bufo marinus* are of similar diameter, and blood flows are similar when probes are placed in ipsilateral or bilateral positions (West and Burggren, 1984). Therefore, we assumed that flows were bilaterally equal. Total blood flow in the pulmocutaneous ($\dot{Q}_{pc}$) and systemic ($\dot{Q}_{sys}$) arteries was obtained by doubling measured values in the left pulmocutaneous artery and left systemic artery, and total blood flow ($\dot{Q}_{tot}$) was calculated as $\dot{Q}_{pc}+\dot{Q}_{sys}$. Heart rate ($f_H$) was calculated directly from the blood flow trace, and stroke volume (V_S) was calculated as $\dot{Q}_{tot}/f_H$. The net shunt flow (net $\dot{Q}_{shunt}$) was calculated as $\dot{Q}_{pc}-\dot{Q}_{sys}$, and the cardiac shunt pattern was also expressed as $\dot{Q}_{pc}/\dot{Q}_{sys}$.

Blood gas analysis

Arterial blood was analysed for oxygen tension (P_{aO_2}), pH, haematocrit, blood haemoglobin concentration ([Hb_4]), oxygen content ([O_2]) and total carbon dioxide content of plasma ([CO_2]). P_{O_2} and pH were measured with Radiometer (Copenhagen, Denmark) electrodes maintained in a BMS 3 electrode set-up at 25°C, and the output from the electrodes was displayed on a Radiometer PHM 73. Haematocrit was determined in duplicate as the fractional red cell volume after centrifugation (12 000 r.p.m. for 3 min), and [Hb_4] was measured in triplicate after conversion to cyanmethaemoglobin and applying a millimolar extinction coefficient of 10.99 at 540 nm (Zijlstra et al., 1983). Arterial [O_2] was measured as described by Tucker (1967), with a correction described by Bridges et al. (1979), and plasma [CO_2] was measured according to Cameron (1971). The Tucker and Cameron chambers were maintained at 40°C. Haemoglobin-bound oxygen (HbO_2) was calculated as arterial [O_2]–($\alpha_{O_2}\times P_{aO_2}$), where α_{O_2} is the blood oxygen solubility (Christoforides and Hedley-Whyte, 1969). Haemoglobin saturation (HbO_2sat) was calculated as HbO_2/[Hb], under the assumption that all haemoglobin was functional.

Arterial carbon dioxide tension (P_{aCO_2}) was calculated from pH and plasma [CO_2] using the rearranged Henderson–Hasselbalch equation, and the plasma solubility of CO_2 (α_{CO_2}) was provided by Boutilier et al. (1979). Assuming that the carbonate concentration is negligible, plasma [HCO_3^-] was calculated as [CO_2] – ($\alpha_{CO_2}\times P_{CO_2}$).

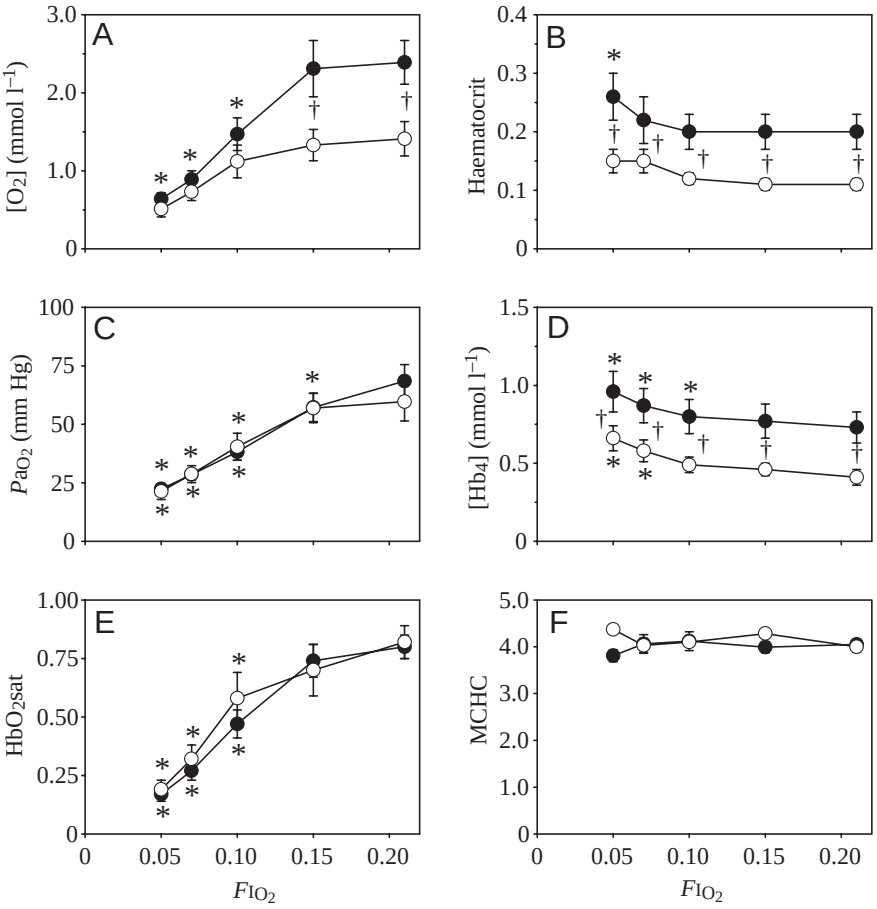

Fig. 1. Effects of hypoxia (F_{IO_2}=fraction of oxygen in the inspired air) on arterial oxygen levels before and after reducing haematocrit (filled and unfilled circles, respectively). (A) Oxygen concentration ([O_2]); (B) haematocrit; (C) arterial oxygen tension (P_{aO_2}; 1 mm Hg=133.3 Pa); (D) haemoglobin concentration ([Hb_4]); (E) haemoglobin oxygen saturation (HbO_2sat) and (F) mean cellular haemoglobin concentration (MCHC). Significant effects of hypoxia within each of the two groups (normal and low haematocrit, respectively) are marked with an asterisk, and significant differences between the groups are marked with a dagger. Data are presented as means ± 1 S.E.M. (N=6).

Data analysis, statistical analysis and presentation

For each hypoxic level, a continuous recording of 3–8 min was analysed for mean blood flows ($\dot{Q}_{sys}$ and $\dot{Q}_{pc}$), mean blood pressure and heart rate (obtained from the systemic blood flow trace). All recordings were analysed using AcqKnowledge data-analysis software (version 3.5.7).

A two-way analysis of variance with repeated measures (RM-ANOVA) was used to identify significant effects of hypoxia and anaemia on measured variables. Because anaemia and exposure to an F_{IO_2} of 0.10 in toads with normal haematocrit resulted in similar reductions in arterial [O_2] (Fig. 1), we performed an additional one-way RM-ANOVA to evaluate the specific effects of anaemia and hypoxia on the measured variables. In all analyses, differences among means were analysed *post hoc* using a Student–Newman–Keuls (SNK) multiple comparison test. All statistical analyses were performed using SigmaStat statistical software (version 2.03; SPSS Science, Chicago, IL, USA), and the level of significance was chosen at the P<0.05 level. All data are presented as means ± 1 S.E.M.

Results

Effects of hypoxia and anaemia on arterial blood gases and haematological parameters

The effects of hypoxia on arterial oxygen levels and haematological parameters of toads with normal and reduced haematocrit are shown in Fig. 1. Bleeding and replacement of plasma reduced haematocrit from 0.20±0.03 to 0.11±0.01, with an attendant decrease in [Hb_4] from 0.73±0.10 mmol l^{-1} to 0.41±0.05 mmol l^{-1}, whereas mean cellular haemoglobin concentration (MCHC) was unaffected. This reduced arterial [O_2] in normoxia from 2.39±0.28 mmol l^{-1}

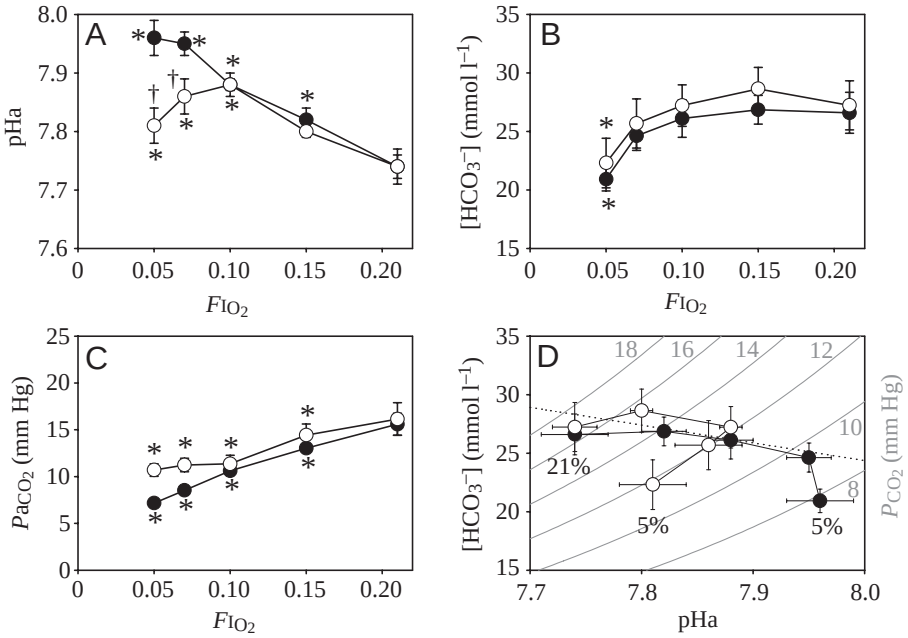

Fig. 2. Effects of hypoxia (F_{IO_2}=fraction of oxygen in the inspired air) on acid–base status before and after reducing haematocrit (filled and unfilled circles, respectively). (A) Arterial pH (pHa); (B) plasma bicarbonate concentration; (C) arterial carbon dioxide tension (P_{aCO_2}; 1 mm Hg=133.3 Pa) and (D) Davenport diagram. In the Davenport diagram, an *in vitro* buffer line previously determined by Andersen et al. (2001) is shown as a dotted line, and isobars for carbon dioxide tension have been added (grey lines). Significant effects of hypoxia within each of the two groups (normal and low haematocrit, respectively) are marked with an asterisk, and significant differences between the groups are marked with a dagger. Data are presented as means ± 1 S.E.M. (N=6).

to 1.41±0.22 mmol l^{-1}, but there were no changes in P_{aO_2} or HbO_2sat. Hypoxia significantly decreased $[O_2]$, P_{aO_2} and HbO_2sat, whereas haematocrit and $[Hb_4]$ were elevated.

In the toads with normal haematocrit, hypoxia elicited an increase in arterial pH (pHa; Fig. 2A) that was associated with a reduction in P_{aCO_2} (Fig. 2C) and followed the non-bicarbonate buffer line (Fig. 2D) until the most severe hypoxic exposure, where a metabolic acidosis contributed to a reduction in pHa. Arterial acid–base parameters were not affected by anaemia during normoxia (Fig. 2; Table 1), but the anaemic toads exhibited a larger reduction in pHa than did toads with normal haematocrit during hypoxia. The more severe acidosis was due to a metabolic acidosis and a smaller reduction in P_{aCO_2} during hypoxia (Fig. 2).

Effects of hypoxia and anaemia on distribution of blood flows and cardiac parameters

In toads with normal haematocrit, $\dot{Q}_{pc}$ was lower than $\dot{Q}_{sys}$ during normoxia, which is indicative a net R–L cardiac shunt. Hypoxia caused an almost threefold increase in $\dot{Q}_{pc}$ (Fig. 3A,B). $\dot{Q}_{sys}$, however, did not change (Fig. 3C,D), and exposure to hypoxia was, therefore, associated with a pronounced increase in $\dot{Q}_{pc}/\dot{Q}_{sys}$ (Fig. 3E,F) and a reversal to a net L–R cardiac shunt (Fig. 3G,H). The increase in $\dot{Q}_{tot}$ was primarily caused by an increased f_H, but an increased V_S also contributed (Fig. 4). Blood pressure increased slightly at the most severe hypoxic exposures, but these changes were not statistically significant.

Anaemia was associated with a doubling of $\dot{Q}_{pc}$ in normoxic

Table 1. *Effects of hypoxia and anaemia on arterial blood gases and haematological parameters in* Bufo marinus

Haematocrit F_{IO_2}	Normal 0.21	Normal 0.10	Anaemic 0.21	Significance
$[O_2]$ (mmol l^{-1})	2.39±0.28	1.47±0.21	1.41±0.22	a,b
P_{aO_2} (mm Hg)	68.5±7.0	38.2±3.6	59.7±8.3	a,c
HbO_2sat	0.80±0.05	0.47±0.06	0.82±0.07	a,c
Haematocrit	0.20±0.03	0.20±0.03	0.11±0.01	b,c
$[Hb_4]$ (mmol l^{-1})	0.73±0.10	0.80±0.11	0.41±0.05	b,c
MCHC	4.05±0.11	4.12±0.20	4.00±0.12	
Plasma pH	7.74±0.03	7.88±0.02	7.74±0.02	a,c
P_{aCO_2} (mm Hg)	15.6±1.2	10.6±0.5	16.2±1.7	a,c
$[HCO_3^-]$ (mmol l^{-1})	26.6±1.8	26.1±1.6	27.2±2.1	

Fraction of inspired oxygen (F_{IO_2}), oxygen concentration ($[O_2]$), oxygen tension (P_{aO_2}), haemoglobin oxygen saturation (HbO_2sat), haemoglobin concentration ($[Hb_4]$), mean cellular haemoglobin concentration (MCHC), plasma carbon dioxide tension (P_{aCO_2}) and bicarbonate concentration ($[HCO_3^-]$).

'a' denotes a significant difference between normal normoxia and normal hypoxia; 'b' denotes a significant difference between normal normoxia and anaemia and 'c' denotes a significant difference between normal hypoxia and anaemia.

Values are means ± 1 S.E.M. 1 mm Hg=133.3 Pa.

toads, but $\dot{Q}_{sys}$ was not affected (Fig. 3). Anaemia, therefore, caused an increase in $\dot{Q}_{pc}/\dot{Q}_{sys}$ and a reduction in the net R–L cardiac shunt. The increased $\dot{Q}_{tot}$ caused by anaemia was attributed to a combination of an increased f_H and an increased V_S (Fig. 4). When exposed to hypoxia, the anaemic toads exhibited further increases in $\dot{Q}_{pc}$, so $\dot{Q}_{pc}$ remained elevated in comparison with toads with normal haematocrit at any given level of hypoxia. As in toads with normal haematocrit, $\dot{Q}_{sys}$ did not increase during hypoxia, and hypoxia was associated with a progressive increase in the net L–R shunt and a large increase in $\dot{Q}_{pc}/\dot{Q}_{sys}$ (Fig. 3). Blood pressure did not change after anaemia and remained stable during hypoxia in the anaemic toads. The control group, which was exposed to two periods of hypoxia with no manipulation of haematocrit, exhibited a decrease in $\dot{Q}_{sys}$ (P=0.04) between the first and second day (Fig. 5), while all other parameters were unaffected by the repeated hypoxic exposure.

As shown in Fig. 1A, toads with normal haematocrit at F_{IO_2}=0.10 had an arterial [O_2] that is similar to the arterial oxygen concentration of anaemic animals in normoxia (1.47±0.21 mmol l^{-1} and 1.41±0.22 mmol l^{-1}, respectively; see Table 1 for an additional comparison of haematology and blood gases between these groups). It is illustrative, therefore, to compare the cardiovascular status of anaemic animals with the status of toads with normal haematocrit during normoxia and when exposed to an F_{IO_2} of 0.10. These comparisons are shown by the bar graphs inserted on the right panel of Figs 3 and 4.

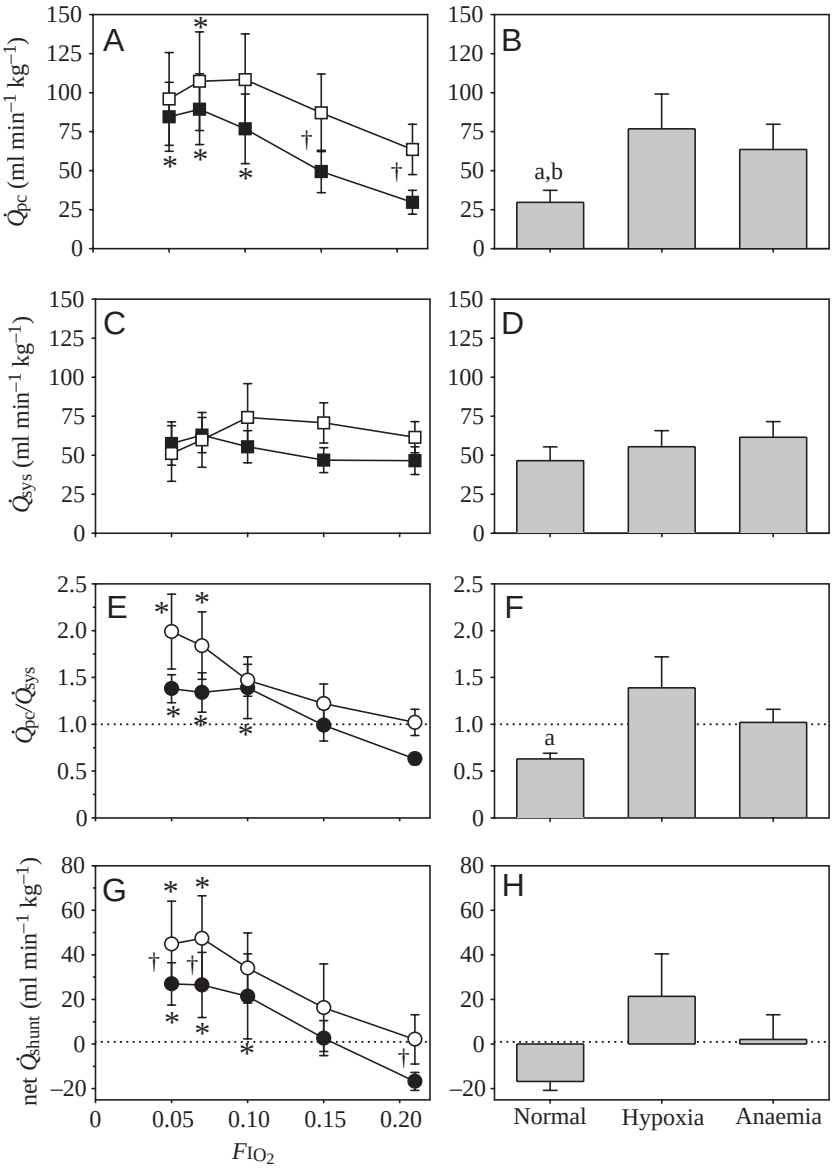

Fig. 3. Effects of hypoxia (F_{IO_2}=fraction of oxygen in the inspired air) on blood flows before and after reducing haematocrit (filled and unfilled circles, respectively). (A,B) Pulmocutaneous blood flow ($\dot{Q}_{pc}$); (C,D) systemic blood flow ($\dot{Q}_{sys}$); (E,F) net shunt fraction ($\dot{Q}_{pc}/\dot{Q}_{sys}$) and (G,H) net shunt flow ($\dot{Q}_{shunt}$). The dotted lines represent the condition where there is no net shunt. Significant effects of hypoxia within each of the two groups (normal and low haematocrit, respectively) are marked with an asterisk, and significant differences between the groups are marked with a dagger. B, D, F and H show data for toads with normal [O_2] during normoxia, toads with normal [O_2] during hypoxia (F_{IO_2}=0.10), and anaemic toads during normoxia. Significant differences between normoxic and hypoxic animals are marked with 'a', and differences between normoxic and anaemic animals are marked with 'b'. Data are presented as means ± 1 S.E.M. (N=6).

Discussion

Critique of methods and comparison with other studies

Blood gases and acid–base status during normoxia and the changes during hypoxia are similar to previous studies on *Bufo marinus* (Fig. 1; Boutilier and Toews, 1977; Wang et al., 1994; Branco and Glass, 1995; Malvin et al., 1995; Andersen et al., 2001). Hypoxia and the reductions in arterial oxygen levels were associated with a respiratory alkalosis, but severe hypoxia caused a metabolic acidosis that was most pronounced in the anaemic animals (Fig. 2).

We did not measure blood flows in all arteries leaving the heart, but the left and right truncus arteriosus have similar diameters, and blood flows are similar on the two sides (West and Burggren, 1984). Therefore, we calculated $\dot{Q}_{pc}$ and $\dot{Q}_{sys}$ by doubling the values measured on the left pulmocutaneous artery and the left aortic arch. The probe on the pulmocutaneous artery was placed before the small cutaneous artery branches off the larger pulmonary artery, so we cannot distinguish flows between these two circuits. Cutaneous flow ranges between 10% and 20% of $\dot{Q}_{pc}$ in *Bufo*, and absolute changes in flows are small compared with pulmonary flow

(West and Burggren, 1984). On the systemic side, we underestimate $\dot{Q}_{sys}$ because the probe was positioned after the point where the carotid arteries emerge from the systemic arch. The flows in the carotid arteries are less than 6% (West and Smits, 1994) but will cause some underestimation of $\dot{Q}_{pc}/\dot{Q}_{sys}$ relative to the study by Gamperl et al. (1999).

The heart rates reported for *Bufo marinus* differ enormously among studies depending on the degree of instrumentation. In our study, f_H of undisturbed normoxic toads was elevated compared with previous studies on toads instrumented with ECG leads or a femoral cannula (e.g. Dumsday, 1990; Wang et al., 1994). Nevertheless, our values for f_H, $\dot{Q}_{tot}$ and net cardiac shunt patterns are consistent with values reported previously on toads instrumented with flow probes (Gamperl et al., 1999; Hedrick et al., 1999).

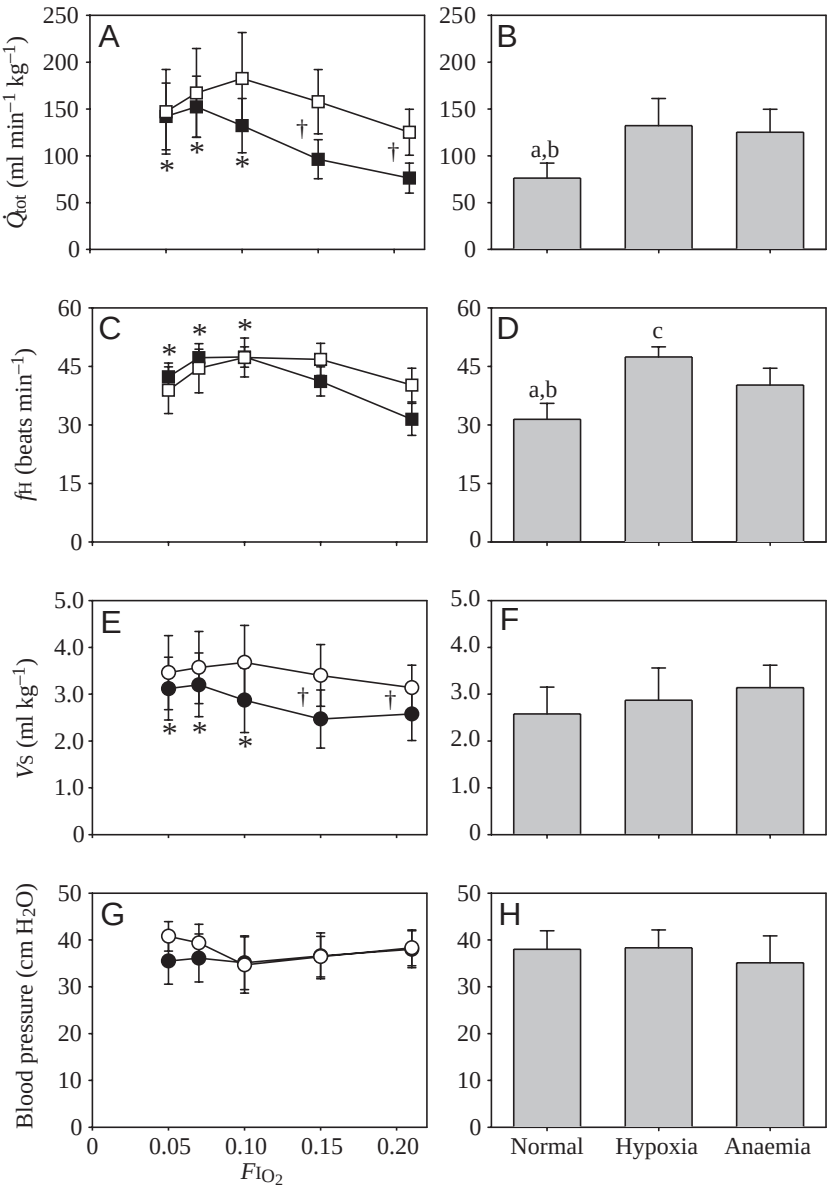

Fig. 4. Effects of hypoxia (F_{IO_2}=fraction of oxygen in the inspired air) on heart parameters before and after a 50% reduction in haematocrit (filled and unfilled circles, respectively). (A,B) Total cardiac output ($\dot{Q}_{tot}$); (C,D) heart rate (f_H); (E,F) stroke volume (V_S) and (G,H) systemic blood pressure (1 cm H_2O=98.1 Pa). Significant effects of hypoxia within each of the two groups (normal and low haematocrit, respectively) are marked with an asterisk, and significant differences between the groups are marked with a dagger. B, D, F and H show data for toads with normal [O_2] during normoxia, toads with normal [O_2] during hypoxia (F_{IO_2}=0.10), and anaemic toads during normoxia. Significant differences between normoxic and hypoxic animals are marked with 'a', and differences between normoxic and anaemic animals are marked with 'b'. Data are presented as means ± 1 S.E.M. (N=6).

Ventilatory response to reduced oxygen levels

Bufo marinus exhibits a vigorous ventilatory response to hypoxia (e.g. Boutilier et al., 1979; Kruhøffer et al., 1987; Wang et al., 1994), which is believed to stem from stimulation of oxygen-sensitive receptors on the major arteries (see West and Van Vliet, 1992; cf. Jones and Chu, 1988). The hypoxic ventilatory response of toads is mediated by reductions in P_{aO_2}, as toads breathing normoxic air do not increase ventilation when blood [O_2] is reduced by anaemia or inhalation of CO (Wang et al., 1994; Branco and Glass, 1995). Furthermore, afferent nerve activity from the carotid nerve of *Bufo marinus* is not affected by blood [O_2] (Van Vliet and West, 1992). Our study is consistent with P_{aO_2} being the major determinant for hypoxic ventilatory responses because anaemia did not alter P_{aCO_2} during normoxia, suggesting that lung ventilation did not change with reduced haematocrit (Fig. 2C; see also Katz, 1980).

Cardiovascular responses to hypoxia and anaemia

The toads with normal haematocrit were characterised by a large net R–L cardiac shunt during normoxia, which is consistent with previous studies on resting and undisturbed toads (Gamperl et al., 1999; Hedrick et al., 1999). When exposed to hypoxia, the toads with normal haematocrit responded with an increased f_H and a large increase in $\dot{Q}_{pc}$, while $\dot{Q}_{sys}$ did not change. This response resulted in progressive reduction in the R–L cardiac shunt and a reversal to a net L–R shunt during severe hypoxia. A large reduction in the net R–L shunt has previously been documented in *Bufo marinus* (Gamperl et al., 1999) and serves to increase systemic oxygen transport because arterial [O_2] is maximised by elimination of R–L cardiac shunts (e.g. Wang and Hicks, 1996). Cholinergic blockade by infusion of atropine results in similar cardiovascular changes to those observed during hypoxia (Gamperl et al., 1999), and circulating catecholamines increase only during exposure

to severe hypoxia (Andersen et al., 2001). Thus, most of the cardiovascular response to hypoxia is probably caused by release of vagal tone on the heart and pulmonary artery (de Saint-Aubain and Wingstrand, 1979; West and Burggren, 1984).

Our study shows that toads respond to reduced haematocrit by increasing f_H and $\dot{Q}_{pc}$ and by reducing the net R–L cardiac shunt (Figs 3B,F,H, 4D). Thus, even though anaemia did not affect $P_{a_{O_2}}$, the cardiovascular response was qualitatively similar to the response elicited by hypoxia. When exposed to hypoxia, the anaemic toads exhibited an additional increase in $\dot{Q}_{pc}$ and also developed a large net L–R cardiac shunt. The cardiovascular response to anaemia cannot be ascribed to habituation or exposure to hypoxia on the previous day, because all haemodynamic variables in normoxia and hypoxia were similar (although $\dot{Q}_{sys}$ was slightly elevated) during the second experimental day of the control animals, where haematocrit was not manipulated (Fig. 5). To evaluate the extent to which the cardiovascular response correlates with $P_{a_{O_2}}$ *versus* arterial $[O_2]$, we compared the responses of toads with normal haematocrit exposed to an $F_{I_{O_2}}$ of 0.10 with those induced by a similar reduction in $[O_2]$ caused by anaemia at normoxic $P_{a_{O_2}}$ (bar graphs in Figs 3 and 4). This analysis indicates that reduced arterial $[O_2]$ can explain most of the cardiovascular response that is observed when hypoxia reduces both arterial $[O_2]$ and $P_{a_{O_2}}$. However, the cardiovascular response was more pronounced when similar reductions in arterial $[O_2]$ were achieved by hypoxia. This may be explained by an additive effect of reduced $P_{a_{O_2}}$, which would be consistent with the more pronounced cardiovascular response when the anaemic toads were exposed to hypoxia. It is also possible that the increased ventilation during hypoxia, as opposed to the lack of ventilatory response to anaemia (Wang et al., 1994), may be associated with increased $\dot{Q}_{pc}$ through stimulation of stretch receptors in the lungs and feed-forward mechanisms (Wang et al., 1999). Finally, the higher $P_{a_{CO_2}}$ of anaemic toads during hypoxia may have augmented $\dot{Q}_{pc}$, because hypercapnia is associated with increased $\dot{Q}_{pc}$ (West and Smits, 1994; Gamperl et al., 1999).

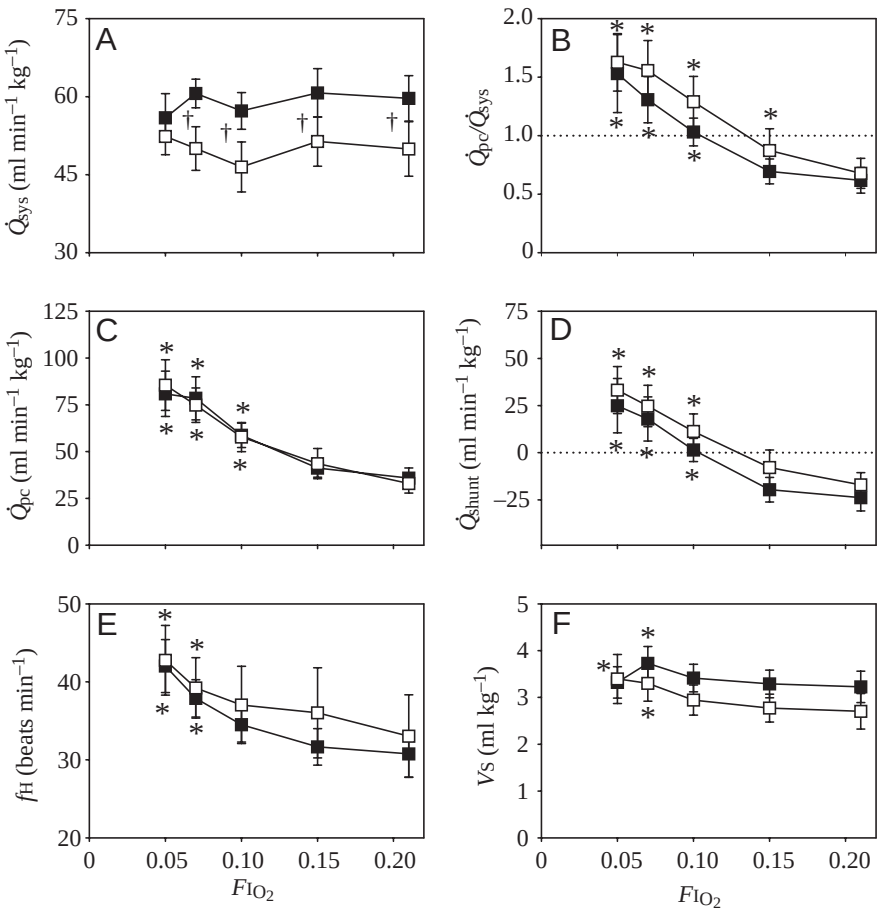

Fig. 5. Effects of hypoxia ($F_{I_{O_2}}$=fraction of oxygen in the inspired air) on the distribution of blood flows and cardiac parameters on two consecutive days exposure to hypoxia (filled and unfilled circles, respectively). (A) Systemic blood flow ($\dot{Q}_{sys}$); (B) net shunt fraction ($\dot{Q}_{pc}/\dot{Q}_{sys}$); (C) pulmocutaneous blood flow ($\dot{Q}_{pc}$); (D) net shunt flow ($\dot{Q}_{shunt}$); (E) heart rate (f_H) and (F) stroke volume (V_S). The dotted lines represent the condition where there is no net shunt. Significant effects of hypoxia during either the first or second exposure are marked with an asterisk. Significant effects between first and second exposure are marked with a dagger. Data are presented as means ± 1 S.E.M. (N=6).

The cardiovascular response to reduced arterial $[O_2]$ at normal $P_{a_{O_2}}$ may be explained by the presence of oxygen-sensitive chemoreceptors that specifically affect the cardiovascular system and are stimulated by reductions in blood $[O_2]$. A receptor that can sense oxygen bound to haemoglobin within the red cells is highly unlikely, but P_{O_2}-sensitive chemoreceptors located in an under-perfused tissue would be able to sense reduced arterial $[O_2]$ as reductions in P_{O_2}. This is believed to be the case for the aortic bodies in mammals (Lahiri et al., 1980, 1981a,b) that primarily affect the cardiovascular system (Daly and Ungar, 1966; Daly, 1997; Jones and Daly, 1997). Alternatively, the P_{O_2}-sensitive chemoreceptor could be located in the venous circulation or, as suggested previously, on the pulmocutaneous artery, which is perfused predominantly by venous systemic blood (Wang et al., 1997, in press). In addition, receptors have been located on the pulmocutaneous artery (Ishii et al., 1985), but their reflex roles remain uncertain (West and Van Vliet, 1992; Wang et al., in press).

While the existence of a separate group of oxygen-sensitive chemoreceptors affecting the cardiovascular system may explain the responses to anaemia, other, not mutually exclusive, explanations are possible. Several compounds have been suggested to regulate local blood flow in an $[O_2]$- or [Hb]-

dependent manner. These compounds include the release of ATP (Ellsworth et al., 1995) and arachidonic acid metabolites (Harder et al., 1996) from red blood cells. Haemoglobin is an effective scavenger of nitric oxide, and reduced [Hb] might result in more nitric oxide available for local vasodilation (Stamler et al., 1997). Anaemia and associated reductions in tissue P_{O_2} may, therefore, have induced some vasodilation in the systemic circulation that, in turn, would induce barostatic responses where increased heart rate and cardiac output act to maintain blood pressure. Furthermore, a reduction in haematocrit from 20% to 11%, as achieved with our anaemia protocol, would be expected to reduce viscosity by approximately 40% (Hillman et al., 1985), which would contribute to an apparent reduction in systemic vascular resistance. However, anaemia primarily affected $\dot{Q}_{pc}$ and f_H, so a classic barostatic response does not seem to explain the observations. This is further substantiated by the fact that *Bufo marinus* does not exhibit barostatic responses to reduced blood pressure (West and Van Vliet, 1992).

In conclusion, the cardiovascular and ventilatory responses to oxygen shortage of toads seem to differ with respect to the specific oxygen stimulus. Hypoxia elicited large changes in both ventilatory and cardiovascular parameters, whereas anaemia only had effects on cardiovascular parameters. Future investigations are needed to elucidate which receptor groups are responsible for this difference in oxygen modality.

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