The Journal of Experimental Biology 202, 965–975 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB1782

CO_2 EXCRETION AND POSTCAPILLARY pH EQUILIBRATION IN BLOOD-PERFUSED TURTLE LUNGS

ERICH K. STABENAU[1,2,*] AND THOMAS A. HEMING[2]

[1]*Department of Biology, Bradley University, Peoria, IL 61625, USA* and [2]*Departments of Internal Medicine and Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77555, USA*

*e-mail: eks@bumail.bradley.edu

Accepted 12 January; published on WWW 22 March 1999

Summary

Turtles possess a significant postcapillary CO_2 partial pressure (P_{CO_2}) disequilibrium between arterial blood and alveolar gas. There are several possible explanations for this blood disequilibrium including a slow rate of erythrocyte physiological anion shift (Cl^-/HCO_3^- exchange) or inaccessibility of plasma HCO_3^- to red blood cell or pulmonary carbonic anhydrase. The present study characterized the contribution of erythrocyte anion exchange and pulmonary and erythrocyte carbonic anhydrase to CO_2 excretion and, hence, to postcapillary CO_2–HCO_3^-–H^+ equilibration in blood-perfused turtle (*Pseudemys scripta*) lungs. Turtle lungs perfused *in situ* with red cell suspensions containing inhibitors of erythrocyte anion exchange and/or pulmonary and red cell carbonic anhydrase produced significant postcapillary blood P_{CO_2} and pH disequilibria, while no disequilibria were measured when lungs were perfused with control red cell suspensions. Erythrocyte anion exchange and pulmonary intravascular carbonic anhydrase contributed 11 % and 9 %, respectively, to CO_2 excretion during single-pass perfusion, whereas red cell and pulmonary carbonic anhydrase contributed 32 % to the measured CO_2 excretion. The lack of a measurable P_{CO_2} disequilibrium during perfusion with control erythrocyte suspensions in this study suggests that alternative mechanisms may be responsible for the arterial–lung P_{CO_2} disequilibrium measured during breathing or diving episodes in turtles.

Key words: turtle, *Pseudemys scripta*, lung, blood, P_{CO_2}, pH, post-capillary disequilibrium, carbonic anhydrase.

Introduction

The primary determinants of pulmonary CO_2 excretion rate ($\dot{V}_{CO_2}$) and postcapillary CO_2–HCO_3^-–H^+ equilibration are the rate of CO_2 diffusion across the blood–gas interface, the HCO_3^-, H^+ and CO_2 concentrations in the plasma and red blood cells, the activity of pulmonary and erythrocyte carbonic anhydrase, and the rate of erythrocyte HCO_3^-/Cl^- exchange (Bidani and Crandall, 1987; Bidani, 1991). Additional factors include the erythrocyte and plasma non-bicarbonate buffer capacities, the quantity of Bohr protons (H^+) released during hemoglobin oxygenation in the red blood cell, and the consumption of H^+ during carbamate dissociation (Bidani and Crandall, 1987; Bidani, 1991). Several investigators have demonstrated that red blood cell anion exchange and erythrocyte carbonic anhydrase contribute approximately 15–30 % and 60–80 %, respectively, to pulmonary CO_2 excretion (Crandall et al., 1981; Crandall and Bidani, 1981; Bidani et al., 1992; Swenson et al., 1993), whereas the *in vivo* contribution of vascular pulmonary carbonic anhydrase to $\dot{V}_{CO_2}$ ranges from approximately 2 % to 8 % (Bidani, 1991; Swenson et al., 1993). In the light of the nominal contribution of pulmonary carbonic anhydrase to $\dot{V}_{CO_2}$, the primary function attributed to intravascular carbonic anhydrase is to minimize postcapillary pH and P_{CO_2} disequilibria (Bidani et al., 1983; Heming et al., 1993, 1994).

Most of the data on pH and P_{CO_2} disequilibria have been collected in mammals, which are generally not thought to possess limitations to pulmonary CO_2 excretion. However, there are animals that possess significant *in vivo* P_{CO_2} disequilibria. Burggren and Shelton (1979) and Robin et al. (1981) measured a 10–15 mmHg (1.3–2.0 kPa) postcapillary arterial–alveolar P_{CO_2} disequilibrium ($\Delta Pa \rightarrow A_{CO_2}$, where the subscripts a and A denote arterial and alveolar, respectively) *in vivo* in unrestrained, undisturbed turtles (*Pseudemys scripta*). Intracardiac shunting, ventilation/perfusion mismatch and the failure of venous blood to equilibrate with alveolar gas during capillary transit could contribute to the arterial–alveolar P_{CO_2} disequilibrium (Burggren and Shelton, 1979). Completion of the chemical reactions in the red blood cell and the plasma during capillary transit under the influence of pulmonary and erythrocyte carbonic anhydrase and erythrocyte anion exchange would also influence CO_2 equilibration. Turtles possess pulmonary vascular carbonic anhydrase (Stabenau et al., 1996) and an erythrocyte anion-exchange mechanism (Stabenau et al., 1991). Hall and Schraer (1979) reported that turtle erythrocytes possess carbonic anhydrase isoenzymes that

are comparable in activity to those described in mammals. Previously, no study has examined the role of pulmonary and erythrocyte carbonic anhydrase and erythrocyte anion exchange in pulmonary CO_2 excretion. Nevertheless, the persistent *in vivo* postcapillary disequilibrium measured in turtles suggests that the turtle may serve as an animal model of impaired capillary CO_2 excretion and CO_2–HCO_3^-–H^+ equilibration in patients with lung disease treated with inhibitory pharmacological agents.

In this study, the contributions of erythrocyte anion exchange, and pulmonary and erythrocyte carbonic anhydrase, to CO_2 excretion and postcapillary CO_2–HCO_3^-–H^+ equilibration were examined in blood-perfused turtle (*Pseudemys scripta*) lungs. Inhibition of red blood cell anion exchange, erythrocyte carbonic anhydrase and/or pulmonary carbonic anhydrase produced a significant reduction in $\dot{V}_{CO_2}$ and, hence, postcapillary pH and P_{CO_2} disequilibria. In contrast, no pH disequilibria were measured during perfusion with control blood suspensions. The results indicate that impairing erythrocyte anion exchange, or decreasing red blood cell and pulmonary carbonic anhydrase activity, limits H^+ equilibration between the red blood cell and the plasma during capillary transit. These blood pH and P_{CO_2} disequilibria persist *in vivo* in the absence of ventilatory or cardiac adjustments.

Materials and methods

Blood collection and preparation

For each perfusion experiment, approximately 70 ml of blood was collected into heparinized syringes following ventricular transection in two or three turtles (*Pseudemys scripta* Gray). The hematocrit of the pooled sample was measured in duplicate. The blood was suspended to a hematocrit of 13–15 % in a saline solution containing (in mmol l^{-1}): 100, NaCl; 3, KCl; 2, $CaCl_2$; 2, $MgCl_2$; 10, Hepes; 5, D-glucose; 35, $NaHCO_3$ (pH approximately 7.5, 22 °C). The 100–140 ml suspended blood sample was then equilibrated with 5 % CO_2 and 5 % O_2 (balance N_2) in a round-bottomed 500 ml tonometer. To minimize foaming and erythrocyte hemolysis associated with direct gassing of the blood suspension, the inflowing gas line was placed at the surface of the blood suspension. The tonometer was gently rocked using a pendulum motion to create a thin layer of red blood cell suspension. This method ensured rapid equilibration with inflowing gases.

Blood perfusion

Turtles (*Pseudemys scripta* Gray; mean body mass 1122 g, range 726–1600 g, N=47) were anesthetized with sodium pentobarbital (200 mg kg^{-1}, intraperitoneally), intubated and mechanically ventilated with room air at a frequency of 20 breaths min^{-1} and a tidal volume of 7.5 ml. Initially, turtle lungs were perfused *in situ* through the common pulmonary artery cannula with a saline solution at 20 ml min^{-1}. The composition of the saline perfusate was identical to the solution used to suspend the blood samples, with the exceptions that 24 g l^{-1} dextran (mean molecular mass 74 kDa) was added to prevent pulmonary edema and that 1.8± 0.2 mmol l^{-1} (mean ± S.E.M.) isoproterenol was added to prevent the significant elevation in pulmonary arterial pressure (P_{ap}) that would otherwise occur during perfusion of turtle lungs with red cell suspensions (Stabenau, 1994). The saline solution was equilibrated with 5 % CO_2 and 20 % O_2 (balance N_2). After a steady P_{ap} and mean fractional excretion of CO_2 ($F_{E_{CO_2}}$) had been attained, the perfusate was switched to a control red blood cell suspension. Postcapillary CO_2–HCO_3^-–H^+ equilibria or disequilibria of the perfusate were monitored using a stop-flow pH apparatus (Stabenau et al., 1996). In brief, postcapillary perfusate was withdrawn at a rate of 15 ml min^{-1} across the pH and reference electrodes placed in an inverted T configuration in the pulmonary vein cannula. Perfusate flow across the electrodes was then terminated, and changes in perfusate pH and temperature were recorded. Stabenau et al. (1996) reported that the transit time from the pulmonary capillaries to the pH electrode during perfusion of turtle lungs was approximately 2–5 s. Precapillary (inflowing or venous) and postcapillary (outflowing or arterial) blood samples were then collected for analysis of perfusate pH, P_{CO_2} and P_{O_2} (Radiometer acid–base analyzer, model PHM 72), hematocrit and plasma hemoglobin levels approximately 3–5 min after initiating perfusion. To determine plasma hemoglobin levels, 1 ml of the blood sample was centrifuged at 14 000 ***g*** for 3 min. The supernatant was removed and stored at −41 °C prior to spectrophotometric analysis of the tetrameric hemoglobin concentration (Sigma, kit 527). All perfusion experiments were conducted at room temperature (21–25 °C).

After perfusing the turtle lungs with a control erythrocyte suspension, the perfusate was switched to the saline solution. Concurrently, the entire volume of the postcapillary red cell perfusate was centrifuged at 1000 ***g*** for 10 min. The supernatant was discarded, and the erythrocytes were resuspended to a hematocrit of 13–15 % in a saline solution that contained inhibitors of pulmonary and erythrocyte carbonic anhydrase and/or red blood cell anion exchange (see below). The erythrocyte suspension was then returned to the perfusate vessel and re-equilibrated with the inflowing gases for 15–25 min. The perfusate was switched from the saline solution to the red blood cell suspension containing inhibitors, and precapillary and postcapillary blood samples were collected and analyzed as described above. This procedure was repeated with a third red cell suspension containing combinations of inhibitors of red blood cell and lung carbonic anhydrase and of red cell anion exchange.

Four series of lung perfusion experiments were conducted using red blood cell suspensions to examine the effects of erythrocyte anion exchange, and pulmonary and red blood cell carbonic anhydrase, on $\dot{V}_{CO_2}$ and postcapillary pH equilibration. Each experiment was designed specifically to inhibit one or more of the components of pulmonary CO_2 excretion.

Series A

The contribution of erythrocyte HCO_3^-/Cl^- exchange to $\dot{V}_{CO_2}$ was examined by inhibiting red blood cell anion exchange with 0.1 mmol l^{-1} 4-4-diisothiocyano-2-2-disulphonic stilbene (DIDS). The simultaneous effects of inhibiting erythrocyte anion exchange and intravascular pulmonary carbonic anhydrase were then examined by perfusing turtle lungs with red blood cell suspensions treated with 0.1 mmol l^{-1} DIDS and 5 mmol l^{-1} quaternary ammonium sulfanilamide (QAS). QAS is a membrane-impermeant carbonic anhydrase inhibitor (Henry, 1987), which ensured that erythrocyte carbonic anhydrase was not inhibited during lung perfusion with QAS.

Series B

The contribution of intravascular pulmonary carbonic anhydrase to $\dot{V}_{CO_2}$ was examined during perfusion with red blood cell suspensions containing 5 mmol l^{-1} QAS. The effects of inhibiting red blood cell anion exchange and pulmonary and erythrocyte carbonic anhydrase were then examined by perfusing the lungs with blood suspensions containing 0.1 mmol l^{-1} DIDS and 0.1 mmol l^{-1} acetazolamide (ACTZ). In contrast to QAS, the membrane-permeating properties of ACTZ permit access both to erythrocyte carbonic anhydrase and extravascular pulmonary carbonic anhydrase.

Series C

The inhibitory effects of 0.1 mmol l^{-1} ACTZ on $\dot{V}_{CO_2}$ were examined without influencing erythrocyte anion exchange. The synergistic effects of inhibiting pulmonary carbonic anhydrase (intravascular and extravascular), erythrocyte carbonic anhydrase and red blood cell anion exchange on $\dot{V}_{CO_2}$ were then examined by perfusing lungs with red blood cell suspensions containing 0.1 mmol l^{-1} DIDS, 0.1 mmol l^{-1} ACTZ and 5 mmol l^{-1} QAS.

Series D

The contribution of erythrocyte and pulmonary carbonic anhydrase to $\dot{V}_{CO_2}$ was examined by perfusing the lungs with red blood cell suspensions containing ethoxzolamide (ETHX). ETHX is a rapidly permeating carbonic anhydrase inhibitor, which would be expected to increase accessibility to intracellular compartments. To ensure that erythrocyte carbonic anhydrase was maximally inhibited during the experiment, a red blood cell suspension was incubated overnight at 4 °C with 0.4 mmol l^{-1} ETHX. On the day of the experiment, the ETHX-containing erythrocyte suspension was centrifuged at 1000 ***g*** for 10 min. The supernatant was discarded, and the erythrocytes were resuspended to a hematocrit of 13–15 % in freshly prepared saline solution containing 0.4 mmol l^{-1} ETHX. Following perfusion with a control red blood cell suspension, the resuspended blood perfusate was added to the tonometer for equilibration with inflowing gases. After 15–25 min of equilibration, the perfusate was switched to the erythrocyte suspension containing ETHX-treated red blood cells.

Endogenous plasma inhibitors of pulmonary carbonic anhydrase

Potential inhibition of intravascular carbonic anhydrase by endogenous plasma proteins was evaluated utilizing two methods. First, turtle lungs were perfused with red blood cell suspensions, with and without plasma. The lungs were initially perfused with control red blood suspensions containing plasma at a hematocrit of 13–15 %. Precapillary and postcapillary blood samples were collected and analyzed as described above. The postcapillary red blood cell suspension was then centrifuged at 1000 ***g*** for 10 min. The supernatant was discarded, and the erythrocytes were resuspended (without plasma) to a hematocrit of 13–15 % in saline solution. The lungs were perfused with the washed red blood cell suspension following equilibration for 15–25 min with inflowing gases (5 % CO_2, 5 % O_2, balance N_2). Blood samples were collected and analyzed as described above.

The sensitivity of microsomal carbonic anhydrase to inhibition by plasma proteins was analyzed using a fluorescence spectrophotometric assay (Stabenau, 1994). Briefly, the fluorescent dye 2,7-bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorescein (BCECF) was used to measure the rate of CO_2 hydration when equal volumes of a CO_2-equilibrated water solution and a buffer solution containing 20 mmol l^{-1} imidazole and 5 mmol l^{-1} Tris (pH 7.4, 22 °C) were mixed in a cuvette at 4 °C in the presence and absence of pulmonary carbonic anhydrase and plasma. At the beginning of each experiment, the fluorescence of 1.5 ml of the imidazole/Tris solution was monitored for approximately 15 s. Then 1.5 ml of CO_2-equilibrated water (99.9 % CO_2) was added to the reaction vessel, and the change in fluorescence was monitored. In separate experiments, the rates of uncatalyzed and catalyzed CO_2 hydration were measured by adding 45 µl of distilled water or resuspended lung microsomal fraction, respectively, to 1.5 ml of the buffer solution. The lung microsomal fraction was prepared by differential centrifugation as described by Stabenau (1994). The fluorescence experiments were repeated in the presence of plasma separated from 1 ml whole blood samples by centrifugation at 1000 ***g*** for 10 min. In addition, a tangential flow system (Filtron, model OSO30C75) was used to concentrate turtle plasma proteins of molecular mass greater than 30 kDa to maximize potential inhibition of lung microsomal fractions by high-molecular-mass proteins. The molecular mass of the carbonic anhydrase inhibitor ranges from 10–30 kDa in piscine plasma (Haswell et al., 1983) to 79 kDa in porcine plasma (Roush and Fierke, 1992).

Red blood cell hemolysis

The estimated intracellular catalysis factor (A_i; catalyzed rate/uncatalyzed rate) of human red blood cells is approximately 6500 (Bidani and Crandall, 1978). Thus, erythrocytic hemolysis during the lung perfusion experiments would enhance perfusate HCO_3^- dehydration. To quantify the rate of hemolysis, the rate of CO_2 hydration catalyzed by turtle erythrocyte carbonic anhydrase was measured as a function of

plasma hemoglobin concentration. To obtain erythrocyte carbonic anhydrase, a blood sample was collected and centrifuged at 14 000 ***g*** for 3 min. The supernatant was discarded, and 1 ml of distilled water was added to lyse the red blood cells. Erythrocyte carbonic anhydrase activity and the plasma hemoglobin concentration were measured following dilution of the stock lysate with 300 mmol l^{-1} sucrose and 10 mmol l^{-1} Hepes. Red blood cell carbonic anhydrase activity was monitored using the fluorescence carbonic anhydrase assay described above, and plasma hemoglobin was determined spectrophotometrically.

Data analyses

Methods for calculating $\dot{V}_{CO_2}$, transpleural loss of CO_2 (ϕ_{CO_2}) and alveolar P_{CO_2} (P_{ACO_2}) were as described previously (Stabenau et al., 1996). In brief, arterial P_{CO_2} (P_{aCO_2}) is approximately equal to P_{ACO_2} during CO_2–HCO_3^-–H^+ equilibration (Heming and Bidani, 1990). During turtle lung perfusion experiments with carbonic anhydrase inhibitors, $P_{ACO_2} \neq P_{aCO_2}$, and thus, P_{ACO_2} was calculated from a rearrangement of the equation:

$$\dot{V}_{CO_2} = [f(V_T - V_D)P_{ACO_2}]/(P_B - P_{H_2O}),$$

where f is breathing frequency, V_T is tidal volume, V_D is dead space, P_B is barometric pressure, and P_{H_2O} is water vapor pressure.

All data are presented as arithmetic means ± S.E.M. Where appropriate, the data were analyzed statistically using paired and unpaired Student's *t*-tests. Differences associated with a probability of $P \leqslant 0.05$ were regarded as significant.

Results

Blood perfusion

The pulmonary arterial pressure averaged 15.4±1.1 mmHg (2.1±0.1 kPa) (N=47) during the blood perfusion experiments. This value was approximately twice the P_{ap} measured for saline perfused turtle lungs in this study and previously by Stabenau et al. (1996). Nevertheless, this slight increase in P_{ap} had no discernible effects on $\dot{V}_{CO_2}$ during blood perfusion.

Plasma inhibitors of pulmonary carbonic anhydrase

$\dot{V}_{CO_2}$ averaged 4.1±0.1 ml min^{-1} in lungs perfused with plasma-containing erythrocyte suspensions, and 3.9±0.1 ml min^{-1} (N=4) in lungs perfused with washed red blood cell suspensions (P>0.05). No postcapillary pH or P_{CO_2} disequilibria were measured during perfusion with plasma-containing blood suspensions (data not shown). Lung microsomal carbonic anhydrase was also insensitive to the addition of plasma proteins (P>0.05). The carbonic anhydrase activity of control microsomal fractions averaged 17.9±0.9 nmol s^{-1} (N=3), while the CO_2 hydration activity of the microsomal fractions in the presence of 25 and 75 µl of concentrated plasma averaged 16.4±1.6 nmol s^{-1} and 17.3±1.3 nmol s^{-1}, respectively.

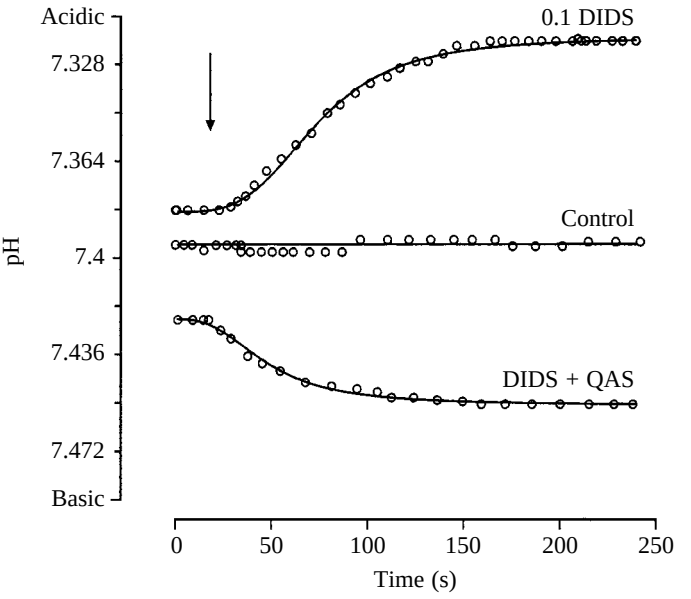

Fig. 1. Representative changes in postcapillary pH during perfusion of turtle lungs with a control red blood cell suspension, a red blood cell suspension containing 0.1 mmol l^{-1} 4-4-diisothiocyano-2-2-disulfonic stilbene (DIDS), and a red blood cell suspension containing 0.1 mmol l^{-1} DIDS and 5 mmol l^{-1} quaternary ammonium sulfanilamide (QAS). The symbols indicate digitized points from the original traces. The arrow represents termination of perfusate flow through the pH apparatus.

Pulmonary CO_2 excretion

Series A

An approximately 11 % reduction in pulmonary CO_2 excretion and an increase in $\Delta P_a \rightarrow A_{CO_2}$ were measured following inhibition of red blood cell HCO_3^-/Cl^- exchange with DIDS (Table 1). Fig. 1 shows a representative recording of the postcapillary acidification during perfusion with DIDS-treated erythrocytes. The measured half-time ($t_{1/2}$) of the pH disequilibrium was 25±2 s. No change in pH (ΔpH) was detected during perfusion with control red blood cell suspensions (Fig. 1). Perfusion with red blood cell suspensions containing 0.1 mmol l^{-1} DIDS and 5 mmol l^{-1} QAS produced significant changes in $\dot{V}_{CO_2}$, alveolar P_{CO_2} (P_{ACO_2}) and $\Delta P_a \rightarrow A_{CO_2}$ (Table 1). $\dot{V}_{CO_2}$ decreased by approximately 25 % during inhibition of erythrocyte anion exchange and pulmonary carbonic anhydrase. Fig. 1 shows a representative recording of ΔpH measured during perfusion with blood suspensions containing 0.1 mmol l^{-1} DIDS and 5 mmol l^{-1} QAS. Inhibition of red blood cell anion exchange and pulmonary carbonic anhydrase produced a mean postcapillary increase in blood pH of 0.035±0.000 ($t_{1/2}$=14.5±0.1 s), whereas a postcapillary decrease in pH of 0.067±0.010 was observed during perfusion of DIDS alone.

Series B

Perfusion with red blood cell suspensions containing 5 mmol l^{-1} QAS produced significant changes in arterial P_{CO_2} (P_{aCO_2}), P_{ACO_2}, $\dot{V}_{CO_2}$ and $\Delta P_a \rightarrow A_{CO_2}$ (Table 2). $\dot{V}_{CO_2}$

Table 1. *Effects of perfusing turtle lungs with control blood suspensions (perfusate A), blood suspensions containing 0.1 mmol l^{-1} DIDS (perfusate B) and blood suspensions containing 0.1 mmol l^{-1} DIDS and 5 mmol l^{-1} QAS (perfusate C) at 22.7±0.2 °C*

	pH_a	$P_{a_{O_2}}$ (mmHg)	$P_{a_{CO_2}}$ (mmHg)	$P_{A_{CO_2}}$ (mmHg)	$\Delta P_{a\rightarrow A_{CO_2}}$ (mmHg)	$\dot{V}_{CO_2}$ (ml min^{-1})	ΔpH
Perfusate A (control)	7.72±0.03	114±3	19.1±0.9	19.1±0.9	0	3.9±0.1	0
Perfusate B	7.71±0.01	116±4	19.4±1.0	17.0±1.0*	2.4±1.9	3.5±0.1*	–0.067±0.01*
Perfusate C	7.66±0.01	109±9	21.5±0.9	14.4±1.2*	7.2±2.1*	2.9±0.1*	+0.035±0*

Values are means ± S.E.M. (N=4).

P_{O_2} partial pressure of O_2; P_{CO_2}, partial pressure of CO_2; $\Delta P_{a\rightarrow A_{CO_2}}$, postcapillary P_{CO_2} disequilibrium; $\dot{V}_{CO_2}$, rate of CO_2 excretion; subscripts a and A denote arterial and alveolar, respectively.

For perfusate A, equilibrated venous values were pH = 7.50±0.03, P_{O_2} = 29±8.5 mmHg and P_{CO_2} = 31.8±0.8 mmHg; for perfusate B, equilibrated venous values were pH = 7.50±0.02, P_{O_2} = 39±6 mmHg and P_{CO_2} = 31.4±0.4 mmHg; for perfusate C, equilibrated venous values were pH = 7.51±0.01, P_{O_2} = 44±6 mmHg and P_{CO_2} = 31.0±0.3 mmHg.

*Significantly different from the control blood suspension (P<0.05); for ΔpH, negative or positive values indicate acidic or alkaline postcapillary ΔpH, respectively.

1 mmHg = 0.1333 kPa.

Table 2. *Effects of perfusing turtle lungs with control blood suspensions (perfusate A), blood suspensions containing 5 mmol l^{-1} QAS (perfusate B) and blood suspensions containing 0.1 mmol l^{-1} DIDS and 0.1 mmol l^{-1} ACTZ (perfusate C) at 23.2±0.4 °C*

	pH_a	$P_{a_{O_2}}$ (mmHg)	$P_{a_{CO_2}}$ (mmHg)	$P_{A_{CO_2}}$ (mmHg)	$\Delta P_{a\rightarrow A_{CO_2}}$ (mmHg)	$\dot{V}_{CO_2}$ (ml min^{-1})	ΔpH
Perfusate A (control)	7.73±0.03	99±10	20.3±1.1	20.3±1.1	0	3.7±0.1	0
Perfusate B	7.69±0.02	123±6	22.2±1.6*	18.5±1.0*	3.7±0.7*	3.4±0.1*	+0.078±0.007*
Perfusate C	7.64±0.03	117±5	24.3±1.5*	12.8±0.4*	11.5±1.1*	2.4±0.1*	+0.060±0.007*

Values are means ± S.E.M. (N=4).

For perfusate A, equilibrated venous values were pH = 7.52±0.02, P_{O_2} = 28±4 mmHg and P_{CO_2} = 32.3±1.5 mmHg; for perfusate B, equilibrated venous values were pH = 7.53±0.02, P_{O_2} = 52±2 mmHg and P_{CO_2} = 32.4±1.2 mmHg; for perfusate C, equilibrated venous values were pH = 7.55±0.01, P_{O_2} = 56±1 mmHg and P_{CO_2} = 31.6±1.2 mmHg.

*Significantly different from the control blood suspension (P<0.05).

Abbreviations are as in Table 1.

1 mmHg = 0.1333 kPa.

decreased by approximately 9 % during inhibition of intravascular pulmonary carbonic anhydrase with QAS. An alkaline postcapillary pH shift was measured during perfusion of lungs with blood suspensions containing QAS. The magnitude of the pH disequilibrium (0.078±0.007; Table 2) was approximately twice the ΔpH measured when erythrocyte anion exchange and pulmonary carbonic anhydrase were inhibited by DIDS and QAS, respectively (0.035±0; Table 1). The measured $t_{1/2}$ of the pH disequilibrium was 25±2 s. Perfusion of the lungs with blood suspensions containing 0.1 mmol l^{-1} DIDS and 0.1 mmol l^{-1} ACTZ produced an approximately 36 % reduction in $\dot{V}_{CO_2}$ and significant changes in $P_{a_{CO_2}}$, $P_{A_{CO_2}}$ and $\Delta P_{a\rightarrow A_{CO_2}}$. ΔpH averaged 0.060±0.007 with a $t_{1/2}$ of 20±1 s during inhibition of erythrocyte anion exchange and erythrocyte and pulmonary carbonic anhydrase with 0.1 mmol l^{-1} DIDS and 0.1 mmol l^{-1} ACTZ.

Series C

$\dot{V}_{CO_2}$ decreased by approximately 21 % from the control value during perfusion with blood suspensions containing 0.1 mmol l^{-1} ACTZ. ACTZ also produced significant changes in $P_{a_{CO_2}}$, $P_{A_{CO_2}}$ and $\Delta P_{a\rightarrow A_{CO_2}}$ (Table 3). The magnitude of the decrease in $\dot{V}_{CO_2}$ during ACTZ perfusion was significantly greater than that measured during QAS perfusion (Table 2). These results suggest that ACTZ inhibited pulmonary and erythrocyte carbonic anhydrase. However, the postcapillary increase in pH of 0.057±0.006 ($t_{1/2}$=33±1 s) during ACTZ perfusion was comparable (P=0.1598) with the ΔpH measured during QAS inhibition of intravascular carbonic anhydrase (Table 2). Perfusion of turtle lungs with red blood cell suspensions containing 0.1 mmol l^{-1} DIDS, 0.1 mmol l^{-1} ACTZ and 5 mmol l^{-1} QAS produced significant changes in $\dot{V}_{CO_2}$, $P_{a_{CO_2}}$, $P_{A_{CO_2}}$ and $\Delta P_{a\rightarrow A_{CO_2}}$ (Table 3). An alkaline postcapillary pH change (0.041±0.003) was measured during perfusion with DIDS, ACTZ and QAS. The measured $t_{1/2}$ of the pH disequilibrium was 20±1 s. A 30 % reduction in $\dot{V}_{CO_2}$ from the control value was measured during inhibition of erythrocyte anion exchange and pulmonary and erythrocyte carbonic anhydrase. This additional reduction in $\dot{V}_{CO_2}$ above that measured with ACTZ alone reflected the contribution of red blood cell HCO_3^-/Cl^- exchange to pulmonary CO_2 excretion.

Table 3. *Effects of perfusing turtle lungs with control blood suspensions (perfusate A), blood suspensions containing 0.1 mmol l^{-1} ACTZ (perfusate B) and blood suspensions containing 0.1 mmol l^{-1} ACTZ, 0.1 mmol l^{-1} DIDS, and 5 mmol l^{-1} QAS (perfusate C) at 22.4±0.1 °C*

	pH_a	P_{aO_2} (mmHg)	P_{aCO_2} (mmHg)	P_{ACO_2} (mmHg)	$\Delta P_{a\rightarrow ACO_2}$ (mmHg)	$\dot{V}_{CO_2}$ (ml min^{-1})	ΔpH
Perfusate A (control)	7.77±0.02	125±8	19.3±0.1	19.3±0.1	0	3.7±0.1	0
Perfusate B	7.66±0.01	136±4	23.7±0.3*	15.2±0.5*	8.4±0.5*	2.9±0.1*	+0.057±0.006*
Perfusate C	7.65±0	137±4	23.6±0.4*	13.7±0.1*	9.8±0.5*	2.6±0.1*	+0.041±0.003*

Values are means ± S.E.M. (*N*=4).

For perfusate A, equilibrated venous values were pH = 7.51±0.02, P_{O_2} = 33±4 mmHg and P_{CO_2} = 31.6±0.4 mmHg; for perfusate B, equilibrated venous values were pH = 7.54±0.01, P_{O_2} = 51±2 mmHg and P_{CO_2} = 31.2±0.5 mmHg; for perfusate C, equilibrated venous values were pH = 7.53±0.01, P_{O_2} = 51±4 mmHg and P_{CO_2} = 30.6±0.5 mmHg.

*Significantly different from the control blood suspension (P<0.05).

Abbreviations are as in Table 1.

1 mmHg = 0.1333 kPa.

Series D

Perfusing turtle lungs with ETHX-treated red cell suspensions produced an increase in postcapillary pH of 0.042±0.001 ($t_{1/2}$=22±2 s) (Table 4) which was comparable in direction and magnitude to that measured during perfusion with red cell suspensions containing ACTZ (Table 3). However, the disequilibrium measured during perfusion of lungs with ETHX was significantly smaller in magnitude than that measured during perfusion with erythrocyte cell suspensions containing QAS (Table 2). ETHX also produced significant changes in P_{aCO_2}, P_{ACO_2}, $\Delta P_{a\rightarrow ACO_2}$ and $\dot{V}_{CO_2}$ (Table 4). The approximately 32 % reduction in $\dot{V}_{CO_2}$ during lung perfusion with ETHX-treated cells was significantly greater than during perfusion with the slowly membrane-permeating carbonic anhydrase inhibitor ACTZ (Table 3). These results suggest that ETHX had greater access to erythrocyte carbonic anhydrase than did 0.1 mmol l^{-1} ACTZ.

O_2 transport, hematocrit and plasma hemoglobin

Despite equilibrating red blood cell suspensions with 5 % O_2 for 15–25 min, the venous P_{O_2} was variable throughout the blood perfusion experiments. Precapillary P_{O_2} averaged 37±2 mmHg (4.9±0.3 kPa; *N*=44). However, single-pass perfusion of turtle lungs with blood suspensions containing inhibitors of erythrocyte anion exchange and red blood cell and pulmonary carbonic anhydrase did not affect the venous to arterial P_{O_2} difference (Tables 1–4). Postcapillary arterial P_{O_2} averaged 115±3 mmHg (15.3±0.4 kPa; *N*=44).

Bidani and Crandall (1982) reported that $\dot{V}_{CO_2}$ was sensitive to changes in hematocrit. Therefore, every effort was made to minimize hematocrit changes during the lung perfusion experiments. The venous and arterial hematocrits of the red blood cell suspension averaged 13.4±0.2 % and 13.3±0.1 % (*N*=44), respectively. The similarity between the precapillary and postcapillary hematocrit suggests that there was no plasma skimming of the red blood cell suspensions during capillary transit. The hematocrit of the whole blood samples collected during the surgical procedure ranged from 20 to 27 %. Thus, the red blood cell suspensions utilized in the perfusion experiments were diluted by approximately 35–52 %.

Fig. 2 shows the linear relationship between erythrocyte carbonic anhydrase activity and plasma hemoglobin concentration. This relationship (carbonic anhydrase activity=0.063 [hemoglobin]–0.840; r^2=0.998) was used to estimate CO_2 hydration caused by erythrocyte hemolysis during perfusion of turtle lungs with blood suspensions. The lung perfusate venous and arterial plasma hemoglobin concentrations averaged 14.6±1.3 and 15.5±1.2 μmol l^{-1}

Table 4. *Effects of perfusing turtle lungs with control blood suspensions (perfusate A) and blood suspensions containing 0.4 mmol l^{-1} ETHX (perfusate B) at 22.1±0.1 °C*

	pH_a	P_{aO_2} (mmHg)	P_{aCO_2} (mmHg)	P_{ACO_2} (mmHg)	$\Delta P_{a\rightarrow ACO_2}$ (mmHg)	$\dot{V}_{CO_2}$ (ml min^{-1})	ΔpH
Perfusate A (control)	7.68±0.01	88±15	19.7±0.7	19.7±0.7	0	4.0±0.1	0
Perfusate B	7.58±0.02	99±9	25.9±0.8*	13.5±1.0*	12.4±1.3*	2.7±0.2*	+0.042±0.001*

Values are means ± S.E.M. (*N*=4).

For perfusate A, equilibrated venous values were pH = 7.43±0.02, P_{O_2} = 22±8 mmHg and P_{CO_2} = 35.4±1.4 mmHg; for perfusate B, equilibrated venous values were pH = 7.47±0.02, P_{O_2} = 34±2 mmHg and P_{CO_2} = 32.8±0.8 mmHg.

*Significantly different from the control blood suspension (P<0.05).

Abbreviations are as in Table 1.

1 mmHg = 0.1333 kPa.

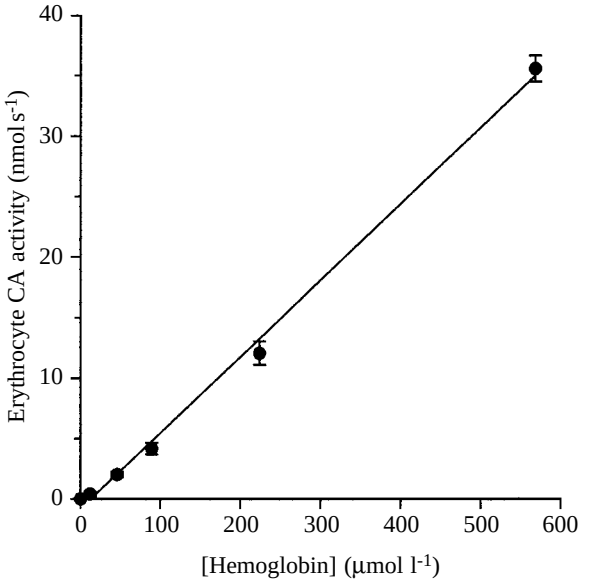

Fig. 2. Relationship between erythrocyte carbonic anhydrase (CA) activity and plasma hemoglobin concentration. Values are means ± S.E.M. (N=4). The line is the least-squares regression fitted to the data (r=0.998, P<0.05, y=0.063x–0.840).

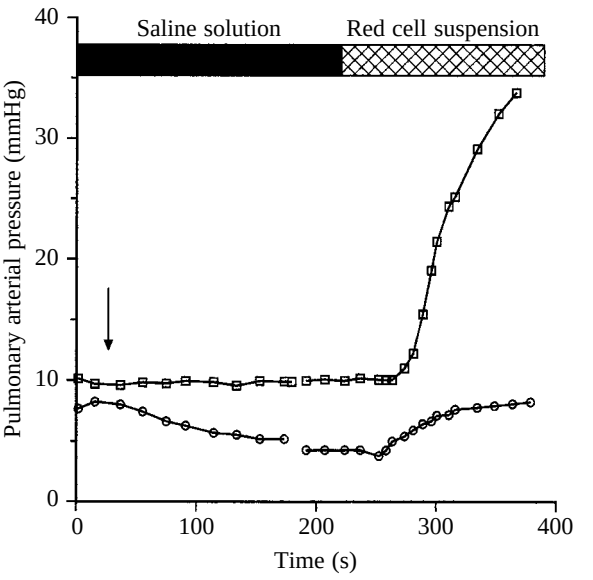

Fig. 3. Representative changes in the pulmonary arterial pressure in the lungs of a turtle perfused with saline solution, followed by a red blood cell suspension, in the absence (□) and presence (○) of 1.8 mmol l^{-1} isoproterenol. Isoproterenol was added to the saline solution at the arrow (1 mmHg=0.1333 kPa).

(N=44), respectively. Thus, the carbonic anhydrase activity resulting from red blood cell hemolysis would catalyze CO_2 hydration at a rate of approximately 0.10 nmol s^{-1}. For comparison, the hemoglobin concentration in whole blood is approximately 6 mmol l^{-1}, and the rate of CO_2 hydration catalyzed by carbonic anhydrase in 1 ml of whole blood is approximately 0.4 nmol s^{-1}. These results suggest that minimal hemolysis occurred during single-pass lung perfusion with the red blood cell suspensions and that the erythrocyte carbonic anhydrase released had little effect on the results.

Discussion

Blood perfusion

A substantial pulmonary hypertension and pulmonary edema were associated with switching the turtle lung perfusate from a saline solution to a red blood cell suspension, despite diluting the hematocrit of the red blood cell suspensions (Fig. 3). This hypertension was relieved by adding isoproterenol to the saline solutions prior to perfusion of turtle lungs with red blood cell suspensions. Isoproterenol is an endothelium-independent, cyclic-AMP-dependent, β-adrenergic agonist used in the treatment of hypoxia-induced pulmonary hypertension (Onodera, 1992). It is plausible that isoproterenol reduced vasoconstriction of the muscularized pulmonary artery or vasoconstriction of the muscle surrounding the lung in the turtle. Nevertheless, P_{ap} during lung perfusion with blood suspensions was approximately twice that measured during perfusion with saline solutions. As a result of the possible effects of isoproterenol on erythrocyte transport mechanisms, isoproterenol was not added directly to the red blood cell suspensions. The present study was not designed to examine pulmonary vasoconstriction in the turtle; however, the mechanisms responsible for the development and alleviation of the pulmonary hypertension observed during perfusion with blood suspensions warrant further investigation.

Plasma inhibitors of carbonic anhydrase

Inhibition of intravascular carbonic anhydrase by endogenous plasma proteins provides a plausible explanation for the measured *in vivo* 10–15 mmHg (1.3–2.0 kPa) postcapillary P_{CO_2} disequilibrium in the turtle (Burggren and Shelton, 1979). However, no postcapillary P_{CO_2} or pH disequilibria were measured during perfusion of turtle lungs with plasma-containing blood suspensions. Moreover, turtle lung microsomal carbonic anhydrase was not inhibited by plasma. Booth (1938) originally described carbonic anhydrase inhibitors in the blood of mammals, including rats, oxen and horses, whereas inhibitors were not found in human or avian blood. More recently, carbonic anhydrase inhibitors have been characterized in piscine (Haswell et al., 1983), porcine (Roush and Fierke, 1992) and canine (Hill, 1986) plasma. It is not known whether turtle plasma inhibits red blood cell carbonic anhydrase activity as it does in other species (Roush and Fierke, 1992), and the possibility that turtle plasma contains inhibitors of the erythrocyte carbonic anhydrase isoenzymes carbonic anhydrase I and II cannot be excluded. The present study, however, demonstrates that plasma proteins had no measurable effect on intravascular carbonic anhydrase activity (i.e. carbonic anhydrase IV activity) in the turtle lung.

CO_2 excretion and CO_2–HCO_3^-–H^+ equilibria

Erythrocyte anion exchange and intravascular carbonic anhydrase contributed 11 % and 9 %, respectively, to CO_2 excretion during single-pass lung perfusion. These data are comparable to the contribution of erythrocyte anion exchange (15 %) and pulmonary carbonic anhydrase (8 %) to $\dot{V}_{CO_2}$ in anesthetized, mechanically ventilated dogs (Swenson et al., 1993). However, the 32 % reduction in $\dot{V}_{CO_2}$ measured during inhibition of turtle erythrocyte and pulmonary carbonic anhydrase with ETHX is considerably less than the 60–80 % reduction in $\dot{V}_{CO_2}$ during inhibition of carbonic anhydrase in endothermic vertebrates (Bidani et al., 1992; Swenson et al., 1993). This discrepancy in the contribution of carbonic anhydrase to $\dot{V}_{CO_2}$ results from differences in experimental protocols. The primary driving force for pulmonary capillary CO_2 excretion is the P_{CO_2} gradient between the inflowing venous blood in the capillary and the alveolar gas ($P\text{v}\rightarrow_{A}CO_2$) (Bidani, 1991; Heming et al., 1994). During the single-pass lung perfusion experiments in the turtle, blood flow ($\dot{Q}$) and ventilation rate (f) were maintained at 20 ml min^{-1} and 20 breaths min^{-1}, respectively. The $P\text{v}\rightarrow_{A}CO_2$ gradient during perfusion with control blood suspensions was approximately 14 mmHg (1.9 kPa), whereas perfusion with erythrocyte suspensions containing ETHX increased the $P\text{v}\rightarrow_{A}CO_2$ gradient to approximately 20 mmHg (2.7 kPa) by reducing CO_2 excretion and preventing mobilization of HCO_3^- to CO_2 during pulmonary capillary transit. Thus, increasing the $P\text{v}\rightarrow_{A}CO_2$ gradient during ETHX perfusion augmented the driving force for uncatalyzed CO_2 excretion. In the whole-animal experiments conducted by Swenson et al. (1993), reductions in pulmonary CO_2 excretion were monitored during the initial pass of the blood through the capillary while the $P\text{v}\rightarrow_{A}CO_2$ gradient was relatively constant. These analyses were restricted to a single ventilatory cycle to prevent *in vivo* compensatory mechanisms from confounding the results. Bidani (1991) reported that steady-state CO_2 excretion could be maintained in humans during complete inhibition of carbonic anhydrase by compensatory increases in alveolar ventilation and cardiac output. It is noteworthy that Cain and Otis (1961) measured only a 40 % reduction in $\dot{V}_{CO_2}$ during complete carbonic anhydrase inhibition in anesthetized dogs following intrinsic changes in the $P\text{v}\rightarrow_{A}CO_2$ gradient. These data suggest that a greater reduction in CO_2 excretion would have been observed during the turtle lung perfusion experiments if the $P\text{v}\rightarrow_{A}CO_2$ gradient had been held constant.

The mean postcapillary pH acidification during perfusion of turtle lungs with DIDS-treated erythrocytes (–0.067) was comparable in magnitude to the postcapillary pH acidification (–0.057) measured during perfusion of rat lungs with DIDS-treated red blood cell suspensions (Crandall et al., 1981). However, DIDS-treated erythrocyte suspensions produced the only detectable postcapillary pH acidification in the turtle lung perfusion experiments. Alkaline pH changes were measured during perfusion of lungs with red blood cells suspensions containing QAS, ACTZ or ETHX, with and without DIDS (Tables 1–4; Fig. 1). In contrast, Crandall et al. (1981) reported that perfusion of rat lungs with blood suspensions containing ACTZ, or ACTZ and DIDS, produced biphasic postcapillary pH changes, in which a small postcapillary pH alkalization (<0.02 pH units) was followed by a large (>0.06 pH units) pH decrease. Crandall and Bidani (1981) reported that changes in red blood cell HCO_3^- permeability, in the presence or absence of vascular carbonic anhydrase activity, influence plasma pH changes during and after capillary transit. For example, decreasing erythrocyte HCO_3^- permeability from 10^{-4} cm s^{-1} to 10^{-6} cm s^{-1} produces biphasic postcapillary pH changes comparable in magnitude to the ΔpH measured during perfusion of rat lungs with ACTZ-treated red blood cell suspensions. Moreover, a biphasic postcapillary ΔpH is predicted in endothermic vertebrates if the extracellular catalytic carbonic anhydrase activity (A_o) is varied (i.e. A_o is changed from 1 to 1000), while maintaining the erythrocyte HCO_3^- permeability at 10^{-4} cm s^{-1} (Bidani and Crandall, 1988). Comparable data on the interaction between red blood cell HCO_3^- permeability and vascular carbonic anhydrase activity are not available for ectothermic vertebrates. These data suggest, however, that the direction and magnitude of the postcapillary pH changes in blood-perfused turtle lungs were determined by a synergism of factors that influenced $\dot{V}_{CO_2}$ and blood CO_2–HCO_3^-–H^+ reactions.

No pH disequilibria were measured during perfusion of turtle lungs with control blood suspensions. As the venous blood suspension enters the pulmonary capillary, CO_2 diffuses across the red blood cell membrane and the blood–gas interface. Diffusion of CO_2 and rapid dehydration of HCO_3^- in the erythrocyte prompt entry of plasma HCO_3^- down its concentration gradient into the red cell in exchange for intracellular Cl^- (Fig. 4). The release of Bohr protons during oxygenation of hemoglobin and the approximately 10-fold difference in the erythrocyte and plasma nonbicarbonate buffer capacities ensure that intracellular carbonic anhydrase-catalyzed HCO_3^- dehydration proceeds to a greater extent than extracellular carbonic anhydrase-catalyzed HCO_3^- dehydration (Bidani and Crandall, 1988). These reactions permit CO_2 equilibration during capillary transit (Fig. 4). Nevertheless, a small H^+ disequilibrium should exist between the plasma and the erythrocyte at the end of pulmonary capillary transit (Crandall et al., 1981), such that extracellular $[H^+]$ is reduced relative to erythrocyte $[H^+]$. The absence of a measurable ΔpH, however, indicates that H^+ is rapidly equilibrated between the erythrocyte and the plasma *via* the Jacobs–Stewart cycle (Jacobs and Stewart, 1942). The net result of the Jacobs–Stewart cycle is rapid postcapillary movement of HCl from the erythrocyte into the plasma and equilibration of H^+ (Fig. 4). Under these circumstances, the CO_2 partial pressure and pH values of postcapillary blood (i.e. $P_{A}CO_2$ and pHa) reflect the gas tension and pH of end-capillary blood (i.e. $P_{C}CO_2$ and pHc).

A postcapillary pH acidification was measured when turtle lungs were perfused with DIDS-treated erythrocytes. During capillary transit, carbonic anhydrase-catalyzed HCO_3^-

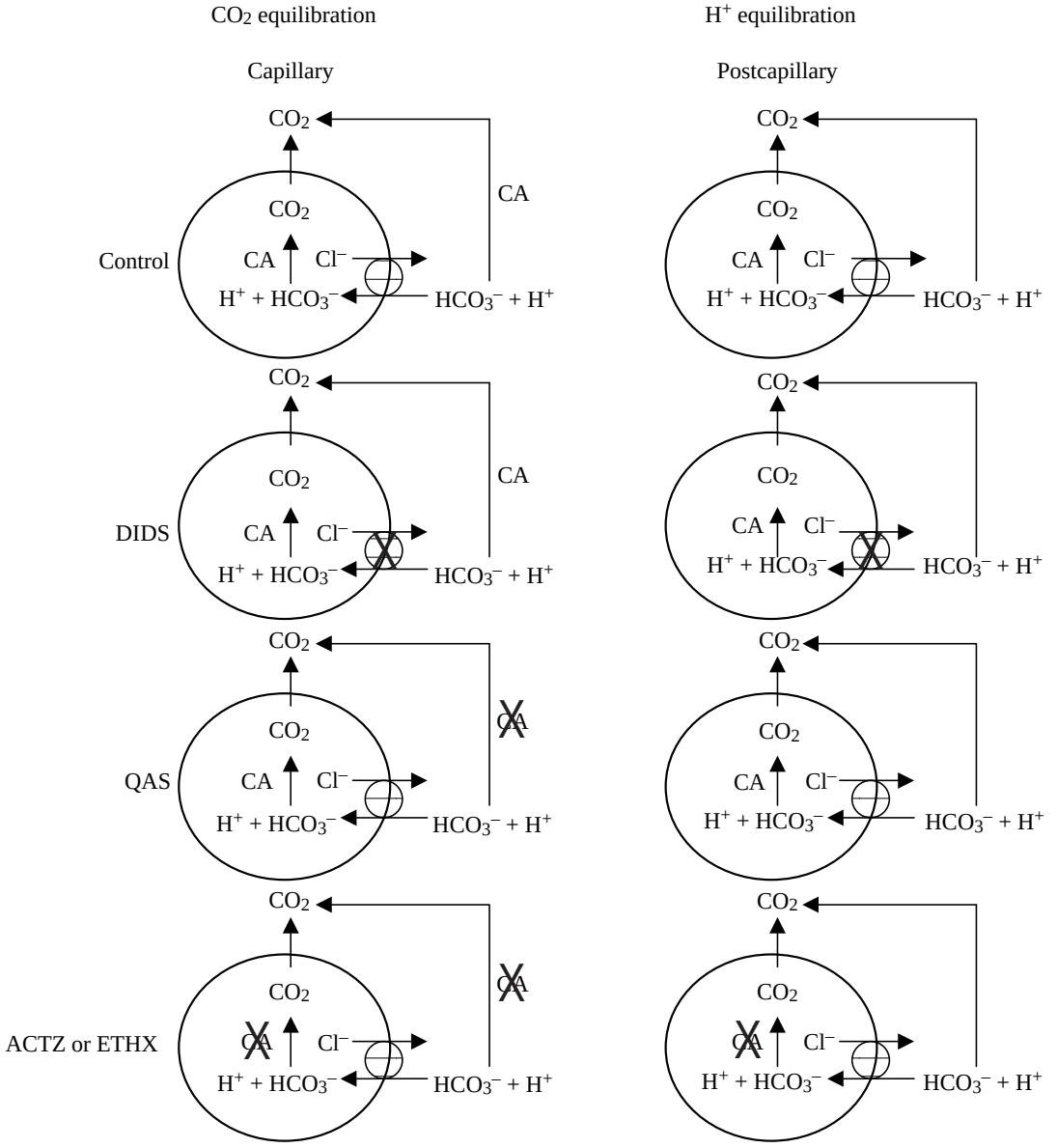

Fig. 4. Simplified scheme of capillary CO_2 equilibration and postcapillary H^+ equilibration across the red blood cell during perfusion of turtle lungs with control blood suspensions, red blood cell suspensions containing 0.1 mmol l^{-1} 4-4-diisothiocyano-2-2-disulphonic stilbene (DIDS) to inhibit anion-exchange protein band 3, blood suspensions containing 5 mmol l^{-1} quaternary ammonium sulfanilamide (QAS) to inhibit intravascular carbonic anhydrase (CA) and red blood cell suspensions containing 0.1 mmol l^{-1} acetazolamide (ACTZ) or 0.4 mmol l^{-1} ethoxzolamide (ETHX) to inhibit pulmonary and erythrocyte carbonic anhydrase. Perfusion of turtle lungs with blood suspensions involves two systems: (1) an open system which involves CO_2 diffusion from the plasma and the erythrocyte across the blood–gas interface during capillary transit; and (2) a closed system which involves postcapillary equilibration of the CO_2–HCO_3^-–H^+ system between the plasma and the erythrocyte. Inhibition of erythrocyte band 3, or erythrocyte and intravascular carbonic anhydrase, is denoted by an X.

dehydration proceeds in the plasma and the erythrocyte as described above for the control blood suspensions. However, entry of plasma HCO_3^- into the red blood cell *via* band-3-mediated anion exchange is inhibited by DIDS (Fig. 4). The inaccessibility of the plasma HCO_3^- pool to the red blood cell produces a small reduction in pulmonary CO_2 excretion and prevents CO_2–HCO_3^-–H^+ equilibration during capillary transit. In addition, inhibition of band 3 results in a reduction in extracellular $[H^+]$ relative to intracellular $[H^+]$ at the end of capillary transit. Unlike the control blood suspensions, rapid postcapillary H^+ equilibration is prevented by inhibition of erythrocyte anion exchange. The large postcapillary pH acidification is, therefore, produced by slow equilibration of H^+ from the erythrocyte into the plasma *via* the Jacobs–Stewart cycle (Fig. 4). Under these conditions, the end-capillary P_{CO_2} is lower than the postcapillary P_{CO_2} (i.e. $Pc_{CO_2} \cong P_{ACO_2} < Pa_{CO_2}$), and the end-capillary pH is more alkaline than the postcapillary pH (i.e. pHc>pHa).

Perfusion of turtle lungs with QAS produced a 9 % decrement in $\dot{V}_{CO_2}$ by inhibiting carbonic anhydrase-catalyzed extracellular HCO_3^- dehydration. Inhibition of intravascular carbonic anhydrase produces a reactive CO_2–HCO_3^-–H^+ disequilibrium during pulmonary capillary transit. The combination of carbonic anhydrase-catalyzed dehydration and consumption of Bohr protons in the red blood cell ensures that erythrocyte [H^+] is reduced relative to plasma [H^+] at the end of capillary transit. The pH change measured during perfusion with QAS-treated blood suspensions, therefore, resulted from postcapillary H^+ equilibration *via* the Jacobs–Stewart cycle (Fig. 4). The net result of the Jacobs–Stewart cycle is movement of HCl from the plasma into the erythrocyte and alkalization of the postcapillary perfusate. Under these circumstances, $Pc_{CO_2} \cong P_{ACO_2} \ll Pa_{CO_2}$, and pHc≪pHa.

Perfusion of turtle lungs with blood suspensions containing ACTZ or ETHX produced a 21 % and a 32 % decrement in $\dot{V}_{CO_2}$, respectively, during inhibition of plasma and erythrocyte carbonic anhydrase-catalyzed HCO_3^- dehydration. Postcapillary P_{CO_2} and pH disequilibria were measured during perfusion with ACTZ- or ETHX-treated red blood cell suspensions, which is consistent with a disequilibrium of the CO_2–HCO_3^-–H^+ system during capillary transit (Fig. 4). Under these circumstances, $Pc_{CO_2} \cong P_{ACO_2} \ll Pa_{CO_2}$, and pHc≪pHa. Therefore, erythrocyte [H^+] is reduced relative to plasma [H^+]. The measured alkaline postcapillary pH change resulted from net movement of HCl from the plasma into the red blood cell *via* the Jacobs–Stewart cycle (Fig. 4).

Inhibition of intravascular carbonic anhydrase or the band 3 anion-exchange protein during single-pass blood perfusion produced significant P_{CO_2} postcapillary disequilibria. Yet the $\Delta Pa \rightarrow_{ACO_2}$ values of 3.7 mmHg (0.5 kPa) and 2.4 mmHg (0.32 kPa), respectively, were substantially lower than the 10–15 mmHg (1.3–2.0 kPa) P_{CO_2} disequilibrium measured *in vivo* by Burggren and Shelton (1979) and Robin et al. (1981).

On the basis of the rate coefficient of the physiological anion shift in turtle erythrocytes, the times required for 90 % and 99 % completion of the physiological anion shift are 1.3 s and 2.6 s, respectively (Stabenau et al., 1991). These data suggest that erythrocyte anion transfer may limit the rate of capillary CO_2 exchange and, hence, produce significant postcapillary pH and P_{CO_2} disequilibria during perfusion of turtle lungs with control blood suspensions. There are at least two possible explanations for the absence of a measurable disequilibrium during the control blood perfusion experiments. First, the 2–5 s transit time from the pulmonary capillaries to the postcapillary pH electrodes permitted chemical equilibration of the blood prior to its arrival at the pH stop-flow apparatus. Second, the capillary transit time of the blood entering the lung may have exceeded the time required for completion of the physiological anion shift. The flow rate ($\dot{Q}$) during the lung perfusion experiments was 20 ml min^{-1}. The combined capillary blood volume (V_c) of both lungs in a 1 kg *Pseudemys scripta* is 2.75 ml (Perry, 1978). Therefore, the capillary transit time ($V_c/\dot{Q}$) was approximately 8 s, or approximately three times longer than the time required for 99 % completion of the physiological anion shift. The actual time of exposure of the inflowing venous blood to alveolar gas during capillary transit may be considerably shorter *in vivo*. It is noteworthy that a pulmonary blood flow of 84.5 ml min^{-1} has been measured in *Pseudemys scripta* (White and Bickler, 1987). Thus, capillary CO_2 exchange and equilibration of pulmonary CO_2 reactions may be limited by noncompletion of the physiological anion transfer during capillary transit (approximately 2 s), thereby producing postcapillary pH and P_{CO_2} disequilibria. These data suggest that erythrocyte anion exchange may limit the rate of pulmonary CO_2 excretion under certain conditions.

The results presented here were part of the PhD dissertation submitted by E.K.S. to the University of Texas Medical Branch. Grateful appreciation is expressed to Dr Akhil Bidani for his guidance during these studies.

References

Bidani, A. (1991). Analysis of abnormalities of capillary CO_2 exchange *in vivo*. *J. Appl. Physiol*. **70**, 1686–1699.

Bidani, A. and Crandall, E. D. (1978). Slow postcapillary pH changes in blood in anesthetized animals. *J. Appl. Physiol*. **45**, 674–680.

Bidani, A. and Crandall, E. D. (1982). Analysis of the effects of hematocrit on pulmonary CO_2 transfer. *J. Appl. Physiol*. **53**, 413–418.

Bidani, A. and Crandall, E. D. (1987). Disequilibrium phenomena in CO_2 transport and exchange. *Appl. Cardiopulm. Pathophysiol*. **2**, 251–262.

Bidani, A. and Crandall, E. D. (1988). Velocity of CO_2 exchanges in the lung. *Annu. Rev. Physiol*. **50**, 639–652.

Bidani, A., Mathew, S. J. and Crandall, E. D. (1983). Pulmonary carbonic anhydrase activity. *J. Appl. Physiol*. **55**, 75–83.

Bidani, A., Smith, R., Cardenas, V. and Heming, T. (1992). Modelling analysis of pulmonary capillary gas exchange. In *Proceedings of the 1992 International Simulation Technology Conference and 1992 Workshop on Neural Networks*, pp. 395–401. San Diego: Soc. Computer Simul.

Booth, V. H. (1938). The carbonic anhydrase inhibitor in serum. *J. Physiol., Lond.* **91**, 474–489.

Burggren, W. W. and Shelton, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. Exp. Biol*. **82**, 75–92.

Cain, S. M. and Otis, A. B. (1961). Carbon dioxide transport in anesthetized dogs during carbonic anhydrase inhibition. *J. Appl. Physiol*. **16**, 1023–1028.

Crandall, E. D. and Bidani, A. (1981). Effects of red blood cell HCO_3^-/Cl^- exchange kinetic on lung CO_2 transfer: theory. *J. Appl. Physiol*. **50**, 265–271.

Crandall, E. D., Mathew, S. J., Fleischer, R. S., Winter, H. I. and Bidani, A. (1981). Effects of inhibition of RBC HCO_3^-/Cl^- exchange on CO_2 excretion and downstream pH disequilibrium in isolated rat lungs. *J. Clin. Invest*. **68**, 853–862.

Hall, G. E. and Schraer, R. (1979). Purification and partial characterization of high and low activity carbonic anhydrase

isozymes from *Malaclemys terrapin centrata*. *Comp. Biochem. Physiol*. **63**B, 561–567.

Haswell, M. S., Raffin, J. and LeRay, C. (1983). An investigation of the carbonic anhydrase inhibitor in eel plasma. *Comp. Biochem. Physiol*. **74**A, 175–177.

Heming, T. A. and Bidani, A. (1990). *In situ* characterization of carbonic anhydrase activity in isolated rat lungs. *J. Appl. Physiol*. **69**, 2155–2162.

Heming, T. A., Stabenau, E. K., Vanoye, C. G., Moghadasi, H. and Bidani, A. (1994). Roles of intra- and extracellular carbonic anhydrase in alveolar–capillary CO_2 equilibration. *J. Appl. Physiol*. **77**, 697–705.

Heming, T. A., Vanoye, C. G., Stabenau, E. K., Roush, E. D., Fierke, C. A. and Bidani, A. (1993). Inhibitor sensitivity of pulmonary vascular carbonic anhydrase. *J. Appl. Physiol*. **75**, 1642–1649.

Henry, R. P. (1987). Quaternary ammonium sulfanilamide: a membrane-impermeant carbonic anhydrase inhibitor. *Am. J. Physiol*. **252**, 959–965.

Hill, E. (1986). Inhibition of carbonic anhydrase by plasma of dogs and rabbits. *J. Appl. Physiol*. **60**, 191–197.

Jacobs, M. H. and Stewart, D. R. (1942). The role of carbonic anhydrase in certain ionic exchanges involving the erythrocyte. *J. Gen. Physiol*. **25**, 539–552.

Onodera, S. (1992). Pulmonary vasoconstrictor responses. *Nippon Kyobu Shikkan Gakkai Zasshi*. **30**, 15–25.

Perry, S. F. (1978). Quantitative anatomy of the lungs of the red-eared turtle, *Pseudemys scripta elegans*. *Respir. Physiol*. **35**, 245–262.

Robin, E. D., Robin, D. A., Ackerman, R., Lewiston, N., Hance, A. J., Caligiuri, M. and Theodore, J. (1981). Prolonged diving and recovery in the freshwater turtle, *Pseudemys scripta*. I. Lung and blood gases, pH, lactate concentrations and 'cation' gap. *Comp. Biochem. Physiol*. **70**A, 359–364.

Roush, E. D. and Fierke, C. A. (1992). Purification and characterization of a carbonic anhydrase II inhibitor from porcine plasma. *Biochemistry* **31**, 12536–12542.

Stabenau, E. K. (1994). Pulmonary carbon dioxide excretion and postcapillary carbon dioxide–bicarbonate–proton equilibration in the turtle. PhD dissertation, University of Texas Medical Branch. 137pp.

Stabenau, E. K., Bidani, A. and Heming, T. A. (1996). Physiological characterization of pulmonary carbonic anhydrase in the turtle. *Respir. Physiol*. **104**, 187–196.

Stabenau, E. K., Vanoye, C. G. and Heming, T. A. (1991). Characteristics of the anion transport system in sea turtle erythrocytes. *Am. J. Physiol*. **261**, 1218–1225.

Swenson, E. R., Gronlund, J., Ohlsson, J. and Hlastala, M. P. (1993). *In vivo* quantitation of carbonic anhydrase and band 3 protein contributions to pulmonary gas exchange. *J. Appl. Physiol.* **74**, 838–848.

White, F. N. and Bickler, P. E. (1987). Cardiopulmonary gas exchange in the turtle: a model analysis. *Am. Zool*. **27**, 31–40.