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Acetazolamide prevents hypoxic pulmonary vasoconstriction in conscious dogs

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Höhne, Claudia, Martin O. Krebs, Manuela Seiferheld, Willehad Boemke, Gabriele Kaczmarczyk, and Erik R. Swenson. Acetazolamide prevents hypoxic pulmonary vasoconstriction in conscious dogs. *J Appl Physiol* 97: 515–521, 2004; 10.1152/japplphysiol.01217.2003.—Acute hypoxia increases pulmonary arterial pressure and vascular resistance. Previous studies in isolated smooth muscle and perfused lungs have shown that carbonic anhydrase (CA) inhibition reduces the speed and magnitude of hypoxic pulmonary vasoconstriction (HPV). We studied whether CA inhibition by acetazolamide (Acz) is able to prevent HPV in the unanesthetized animal. Ten chronically tracheotomized, conscious dogs were investigated in three protocols. In all protocols, the dogs breathed 21% O_2 for the first hour and then 8 or 10% O_2 for the next 4 h spontaneously via a ventilator circuit. The protocols were as follows: *protocol 1*: controls given no Acz, inspired O_2 fraction ($F_{I_{O_2}}$) = 0.10; *protocol 2*: Acz infused intravenously (250-mg bolus, followed by 167 $\mu g \cdot kg^{-1} \cdot min^{-1}$ continuously), $F_{I_{O_2}}$ = 0.10; *protocol 3*: Acz given as above, but with $F_{I_{O_2}}$ reduced to 0.08 to match the arterial P_{O_2} (Pa_{O_2}) observed during hypoxia in controls. Pa_{O_2} was 37 Torr during hypoxia in controls, mean pulmonary arterial pressure increased from 17 ± 1 to 23 ± 1 mmHg, and pulmonary vascular resistance increased from 464 ± 26 to 679 ± 40 $dyn \cdot s^{-1} \cdot cm^{-5}$ ($P < 0.05$). In both Acz groups, mean pulmonary arterial pressure was 15 ± 1 mmHg, and pulmonary vascular resistance ranged between 420 and 440 $dyn \cdot s^{-1} \cdot cm^{-5}$. These values did not change during hypoxia. In dogs given Acz at 10% O_2, the arterial Pa_{O_2} was 50 Torr owing to hyperventilation, whereas in those breathing 8% O_2 the Pa_{O_2} was 37 Torr, equivalent to controls. In conclusion, Acz prevents HPV in conscious spontaneously breathing dogs. The effect is not due to Acz-induced hyperventilation and higher alveolar P_{O_2}, nor to changes in plasma endothelin-1, angiotensin-II, or potassium, and HPV suppression occurs despite the systemic acidosis with CA inhibition.

hypoxia; high altitude; carbonic anhydrase; endothelin; angiotensin II

PREVENTION AND TREATMENT OF acute mountain sickness (AMS) are the main and best-defined indications for oral carbonic anhydrase (CA) inhibitors (3, 36). Acetazolamide (Acz; 250–500 mg in adults) started before ascent to high altitude reduces the incidence of AMS by 60% (28). The second leading cause of acute high-altitude intolerance is high-altitude pulmonary edema (HAPE). The pathogenesis of HAPE is excessive hypoxic pulmonary vasoconstriction (HPV) leading to edema formation when pressure and flow stresses exceed the functional and structural integrity of the alveolar-capillary barrier (21, 39). Prevention and treatment of HAPE include drugs that reduce HPV, a response of both precapillary resistance arterioles and postcapillary venules (31).

CA inhibitors such as Acz are used commonly to augment ventilation for high-altitude acclimatization. Acz causes a mild metabolic acidosis by blocking kidney CA (inhibition of urinary acidification and tubular bicarbonate reabsorption), induces CO_2 retention in tissues by inhibition of endothelial and red cell CA, and increases ventilation by acting on peripheral and central chemoreceptors (36, 37). There has been little study, however, of their effects on the pulmonary circulation. Emery et al. (8) were the first to observe a reduction in HPV with Acz in isolated perfused lungs. In the only other study, we have shown that Acz not only blunts the magnitude but also slows the onset of HPV in isolated perfused rabbit lungs (6). Inhibition of HPV was not associated with an attenuation of the normal decrease in exhaled NO excretion with lowered inspired O_2, suggesting that a change in NO metabolism and production does not contribute to the response (6).

The impact of CA inhibition on HPV over longer periods of time and its effects in living organisms have received no attention. To close this gap and to examine the potential utility of Acz in the prevention and treatment of HAPE, we investigated the effect of Acz on HPV during 4 h of hypoxia in conscious spontaneously breathing dogs, in whom all systemic humoral and neural regulatory mechanisms remain intact and CA inhibition leads to certain acid-base and fluid balance changes, which are not accounted for in the isolated perfused lung studies (6, 8). We made a number of ventilatory and gas-exchange measurements in conjunction with the pulmonary vascular and systemic hemodynamic data. In addition, we made a number of renal and humoral measurements because CA inhibitors cause diuresis and mild extracellular volume depletion, which evoke changes in circulating salt and water-regulating hormones, such as renin, angiotensin II, and endothelin-1, that are known to have vasoactive effects in the lung.

MATERIALS AND METHODS

Animals, maintenance, and diets. A total of 30 experiments were performed on 10 purebred female beagle dogs (body wt 12.6 ± 0.9 kg). The dogs were obtained from the Central Animal Facilities of the Humboldt-University in Berlin. They were tested for their social behavior, tolerance to urinary bladder catheterization, and intravascular cannulas. The permission to perform the experiments was obtained from the Governmental Animal Protection Committee (AZ 4-5855/17-114/95).

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The dogs were kept under highly standardized conditions: air-conditioned animal room during the day and large individual kennels (5 m^2) during the night (21°C, 55% humidity). General status, body temperature, and body weight were checked daily. A permanent tracheotomy was performed 4–5 wk before the experiments (for details see Ref. 14). Thereafter, the dogs were trained to lie quietly on their right side on a padded animal table for at least 5 h. During the entire experimental period, the dogs were attended by a technician who knew the animals well and could provide a calming influence by voice and petting.

Beginning 5 days before the experiments, the dogs were fed a standardized diet. The diet contained 91 ml of water, 2.5 mmol sodium, and 3.5 mmol potassium (all values given per kg body wt and day). The calories supplied with this diet (277 kJ·kg body wt^{-1}·day^{-1}) were sufficient to keep the dogs' body weight constant. The food mash was offered once a day at 2 PM. Eight days before an experiment, 100 ml of the dog's own blood were collected via puncture of a foreleg vein and stored in a blood bag at 4°C (Biopack, Biotrans, Dreieich, Germany). The blood served to replace the blood withdrawn for analysis during the experiments.

After completion of the studies, the tracheotomy was surgically closed, and the dogs were adopted by private persons with the assistance of our university veterinarians.

Procedures during the experiments. Preparation of the dogs started at 7:30 AM. Body weight and temperature were recorded. The urinary bladder was catheterized with a self-retaining Foley catheter. After local anesthesia was administered (lidocaine 1%, Braun, Melsungen, Germany), an arterial line (20 G, no. 4235-8, Ohmeda, Erlangen, Germany) was advanced into the abdominal aorta via the femoral artery and a pulmonary artery catheter (5 F, no. 132F5, Baxter, Unterschleissheim, Germany) was inserted via the right external jugular vein. The catheters were used for continuous systemic and pulmonary blood pressure monitoring, cardiac output measurements, and blood sampling. After catheter insertion, the dogs were placed on a padded animal table and positioned on their right side. The pressure transducers were adjusted to the level of the right atrium. The distance between transducer and table was recorded and also used for the next experiment on this individual dog. Finally, a cuffed tracheal tube (8 mm ID) was inserted, inflated, and connected to a ventilator set to a continuous positive airway pressure mode (4 mmHg), which permitted spontaneous breathing through a low-resistance circuit. Thereafter, the conscious dogs were given 60 min to adjust to the experimental situation.

The 10 dogs were studied three times in randomized order: *1*) control (no Acz) with an inspired O_2 fraction ($F_{I_{O_2}}$) of 0.10, *2*) Acz with a $F_{I_{O_2}}$ of 0.10, and *3*) Acz with a $F_{I_{O_2}}$ of 0.08 to match the arterial P_{O_2} (Pa_{O_2}) of the control dogs. The interval between experiments on the same dog was at least 14 days to permit complete washout of Acz and resolution of any hypoxia exposure and diuretic effects.

In control experiments, the dogs breathed room air (21% O_2-79% N_2; normoxia) for 1 h, followed by breathing a gas mixture containing 10% O_2 and 90% N_2 for 4 h (hypoxia). In the Acz experiments, the dogs received a bolus injection of Acz (Diamox, Lederle, Wolfratshausen, Germany) (250 mg iv) before the start of the experiment, a dose chosen to completely inhibit CA (40). This was followed by a continuous infusion of 167 $\mu g \cdot kg^{-1} \cdot min^{-1}$ during 1 h of normoxia and 4 h of hypoxia to maintain high drug levels. The dogs were studied at the two levels of inspired hypoxia, because Acz-induced hyperventilation in the dogs breathing 10% O_2 causes the Pa_{O_2} to rise above the values observed in the controls at the same $F_{I_{O_2}}$. By reducing the $F_{I_{O_2}}$ to 0.08, it was possible then to match the alveolar P_{O_2} and Pa_{O_2} of the controls at a $F_{I_{O_2}}$ of 0.10, and thus maintain equal the two major stimuli to HPV.

Mean arterial blood pressure, heart rate, central venous pressure, mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure, and minute ventilation (via the flow transducer in the ventilator) were measured continuously, and the data were stored on a computer. Cardiac output was measured by the thermodilution technique (5-ml injection volume at 5–10°C; Vigilance, Baxter Edwards Critical Care, Unterschleissheim, Germany). Five consecutive measurements were performed. The highest and lowest values were rejected. The mean cardiac output was calculated from the remaining three determinations and taken for calculation of systemic and pulmonary vascular resistance by standard formulas.

At the end of each experimental hour, blood samples were drawn to determine arterial blood gases, actual bicarbonate, base excess, and plasma electrolytes. Plasma renin activity (PRA) and plasma angiotensin II concentration (ANG II) were measured at the end of the normoxia period and after 3 and 4 h of hypoxia. Plasma endothelin-1 concentration was determined after normoxia and after 4 h of hypoxia. The blood withdrawn was immediately replaced with an equal amount of the dog's own stored blood.

At hourly intervals, urine flow and urinary concentrations of sodium, potassium, bicarbonate, and creatinine were determined after complete bladder evacuation. Exogenous creatinine clearance (priming dose 1.4 g over 30 min before the start of experiments, maintenance infusion 4.7 mg/min) was calculated by the standard formula to assess glomerular filtration rate (GFR).

Measurement of urinary and plasma values. Urinary sodium and potassium concentrations were measured by flame photometry (Photometer Eppendorf, Hamburg, Germany). Creatinine was determined with a creatinine analyzer (modified Jaffé reaction; Beckmann Instruments, Brea, CA). Blood-gas analysis, plasma sodium, and potassium measurements were performed at hourly intervals (ABL 505, Radiometer, Copenhagen, Denmark). Blood samples for plasma hormone analysis were cooled and centrifuged at 4°C and stored at −20°C until analysis by commercial kits: PRA (New England Nuclear, North Billerica, MA), ANG II (Eurodiagnostika, Arnhem, The Netherlands), and plasma endothelin-1 concentration (Biomedica, Vienna, Austria).

Statistical analysis. Values are given as means ± SE ($n = 10$). For intragroup comparisons (treatment and time), a general linear model of analysis of variance for repeated measures was applied (SPSS 9.0, Chicago, IL). Post hoc testing of means was performed with Student's *t*-test. Level of significance for error of the first order was adjusted according to Holm's procedure (30). Statistical significance was assumed at $P < 0.05$.

RESULTS

Arterial blood gases, pH, and ventilation. During hypoxia, Pa_{O_2} decreased from 94 to 37–38 Torr in the control dogs, from 105 to 37–41 Torr with Acz at 8% O_2, and from 105 only to 47–51 Torr in dogs with Acz at 10% O_2 ($P < 0.05$; Table 1). At the same time, mixed venous P_{O_2} decreased during hypoxia from 46 ± 1 to 26–27 Torr in the control dogs and to 29 ± 1 Torr with Acz at 8% O_2. With Acz at 10% O_2, mixed venous P_{O_2} decreased from 44 ± 1 Torr during normoxia to 34–35 Torr during hypoxia ($P < 0.05$; Table 1). The arterial CO_2 tension decreased from 34 ± 1 Torr during normoxia to 26–28 Torr during hypoxia in the control dogs, and from 34 ± 1 to 29–31 Torr in both Acz groups ($P < 0.05$; Table 1). The mixed venous CO_2 tension was ~39–40 Torr in all groups, decreased during hypoxia to 34–35 Torr in the Acz groups, but fell to 30 ± 1 Torr in the control group ($P < 0.05$; Table 1).

Plasma pH increased in the control dogs during hypoxia but did not change in the Acz groups ($P < 0.05$; Table 2). Bicarbonate concentration (21–22 mmol/l) did not change during hypoxia in the controls but decreased equally from 20 to 16 mmol/l in the Acz-treated dogs ($P < 0.05$, Table 2). Base

Table 1. *Respiratory blood gases and minute ventilation in control dogs at a $F_{I_{O_2}}$ of 0.10 and in dogs given acetazolamide at a $F_{I_{O_2}}$ of 0.10 (Acz) or 0.08 (Acz8) during hypoxia*

	Normoxia 1st h	Hypoxia 2nd h	Hypoxia 3rd h	Hypoxia 4th h	Hypoxia 5th h
Pa_{O_2}, Torr					
Controls	94±1	37±1*	38±2*	38±2*	37±2*
Acz	105±1†	47±2†	51±1*†	50±1*†	50±2*†
Acz8	105±1†	39±1*‡	37±2*‡	41±1*‡	41±1*‡
Pa_{CO_2}, Torr					
Controls	34±1	28±1*	26±1*	27±1*	26±1*
Acz	34±1	31±1*	30±1*	30±1*	29±1*
Acz8	35±1	31±1*	31±1*	30±1*	29±1*
$P\bar{v}_{O_2}$, Torr					
Controls	46±1	27±1*	27±1*	26±1*	27±1*
Acz	44±2	34±1*†	35±1*†	34±1*†	35±1*†
Acz8	46±1	29±1*‡	29±1*‡	29±1*‡	29±1*‡
$P\bar{v}_{CO_2}$, Torr					
Controls	39±1	31±1*	30±1*	31±1*	30±1*
Acz	39±1	34±1*†	34±1*†	32±1*†	33±1*†
Acz8	40±1	35±1*†	35±1*†	34±1*†	34±1*†
$\dot{V}_E$, l/min					
Controls	3.0±0.2	4.1±0.3*	4.4±0.5*	4.8±0.5*	4.6±0.5*
Acz	4.5±0.2†	6.1±0.2*†	6.8±0.4*†	7.4±0.5*†	8.4±0.6*†
Acz8	4.7±0.2†	6.4±0.3*†	6.9±0.4*†	8.0±0.6*†	8.7±0.6*†

Values are means ± SE, $n = 10$. Pa_{O_2}, arterial oxygen tension; Pa_{CO_2}, arterial carbon dioxide tension; $P\bar{v}_{O_2}$, mixed venous oxygen tension; $P\bar{v}_{CO_2}$, mixed venous carbon dioxide tension; $\dot{V}_E$, minute ventilation. Values were measured during 1 h of normoxia and 4 h of hypoxia. *$P < 0.05$ vs. normoxia, †$P < 0.05$ vs. controls; ‡$P < 0.05$ vs. Acz.

excess was negative (−4.5 to −6.5 mmol/l) during normoxia in all protocols. During hypoxia, base excess did not change in controls but decreased to −10 mmol/l in both Acz groups ($P < 0.05$; Table 2).

Minute ventilation rose from 3.0 ± 0.2 to 4.6 ± 0.5 l/min ($P < 0.05$; Table 1) in the control dogs when the $F_{I_{O_2}}$ was lowered to 0.10. Acz increased normoxic ventilation from 3.0 ± 0.2 to 4.6 ± 0.2 l/min ($P < 0.05$), and with both $F_{I_{O_2}}$ values (0.10 and 0.08), minute ventilation rose equally from 4.6 ± 0.2 to 8.5 ± 0.6 l/min ($P < 0.05$).

Pulmonary and systemic hemodynamics. During hypoxia, heart rate, cardiac output, and mean systemic arterial pressure increased to a similar extent in all three protocols ($P < 0.05$, Table 3). In the control dogs, MPAP and pulmonary vascular resistance increased during hypoxia, but these remained in the range of normoxic values in the Acz-treated dogs at both $F_{I_{O_2}}$ values ($P < 0.05$, Fig. 1). Central venous pressure (1–3 cmH_2O), pulmonary capillary wedge pressure (4–5 cmH_2O), and systemic vascular resistance (3,371–3,940 $dyn \cdot s^{-1} \cdot cm^{-5}$) were similar in all protocols and did not change during hypoxia or with drug treatment (Table 3).

Table 3. *Hemodynamic parameters in control dogs at a $F_{I_{O_2}}$ of 0.10 during hypoxia and in dogs given acetazolamide at a $F_{I_{O_2}}$ of 0.10 or 0.08 during hypoxia*

	Normoxia 1st h	Hypoxia 2nd h	Hypoxia 3rd h	Hypoxia 4th h	Hypoxia 5th h
HR, beats/min					
Controls	75±4	91±5*	94±4*	95±4*	93±5*
Acz	81±3	86±5	88±5	94±6*	93±6*
Acz8	82±3	95±4*	98±4*	94±5*	99±4*
MAP, mmHg					
Controls	97±2	109±3*	110±3*	107±2*	110±2*
Acz	97±2	105±3*	105±3*	106±5*	110±4*
Acz8	95±3	104±3*	107±4*	108±4*	109±4*
CO, l/min					
Controls	2.1±0.1	2.3±0.1*	2.4±0.1*	2.2±0.1	2.2±0.1
Acz	2.1±0.1	2.3±0.1*	2.3±0.1*	2.3±0.1*	2.3±0.1*
Acz8	2.0±0.1	2.4±0.1*	2.3±0.1*	2.3±0.1*	2.4±0.1*
PCWP, cmH_2O					
Controls	4.2±1.8	5.0±1.5	4.9±1.6	4.5±1.7	4.8±1.3
Acz	4.0±1.3	4.2±1.7	4.0±1.2	4.0±1.2	4.1±1.7
Acz8	4.0±1.8	4.1±1.8	4.5±2.0	3.8±1.2	3.9±0.9

Values are means ± SE; $n = 10$. HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; PCWP, pulmonary capillary wedge pressure. Values were measured during 1 h of normoxia and 4 h of hypoxia. *$P < 0.05$ vs. normoxia.

Plasma hormones, electrolytes, and renal function. PRA decreased during hypoxia in the controls ($P < 0.05$, Fig. 2), whereas it increased equivalently with Acz at both $F_{I_{O_2}}$ values ($P < 0.05$, Fig. 2). The same applied to plasma ANG II (Fig. 2). Plasma concentrations of endothelin-1 (1.3–1.5 pg/ml) were similar during normoxia in all protocols and increased slightly during hypoxia (1.7–2.1 pg/ml) irrespective of drug treatment ($P < 0.05$, Table 4). Plasma sodium concentration varied between 142 and 146 mmol/l in all protocols and did not change during the experiments. Plasma potassium concentra-

Table 2. *Arterial pH, bicarbonate concentration, and base excess in control dogs at a $F_{I_{O_2}}$ of 0.10 and in dogs given acetazolamide at a $F_{I_{O_2}}$ of 0.10 or 0.08 during hypoxia*

	Normoxia 1st h	Hypoxia 2nd h	Hypoxia 3rd h	Hypoxia 4th h	Hypoxia 5th h
pH_a					
Controls	7.38±0.01	7.46±0.01*	7.46±0.02*	7.48±0.01*	7.49±0.01*
Acz	7.35±0.01	7.35±0.01†	7.34±0.01†	7.33±0.01†	7.32±0.01†
Acz8	7.35±0.01	7.37±0.01†	7.34±0.01†	7.32±0.01†	7.32±0.01†
HCO_{3a}, mM					
Controls	20.9±0.6	21.3±0.4	21.4±0.7	22.3±0.4	22±0.4
Acz	19.5±0.6	18.1±0.5†	17.4±0.3†	16.9±0.2*†	16.3±0.4*†
Acz8	20.1±0.4	18.6±0.4	17.7±0.5*†	16.5±0.3*†	16.2±0.5*†
BE_a, mM					
Controls	−4.5±0.8	−4.3±0.6	−4.1±0.6	−3.3±0.5	−3.5±0.5
Acz	−6.5±0.8	−8.1±0.7*	−9±0.4*†	−9.6±0.4*†	−10.3±0.5*†
Acz8	−5.5±0.5	−7.1±0.5*	−8.8±0.4*†	−9.6±0.3*†	−10.5±0.6*†

Values are means ± SE; $n = 10$. pH_a, arterial pH; HCO_{3a}, arterial bicarbonate concentration; BE_a, arterial base excess. Values were measured during 1 h of normoxia and 4 h of hypoxia. *$P < 0.05$ vs. normoxia; †$P < 0.05$ vs. controls.

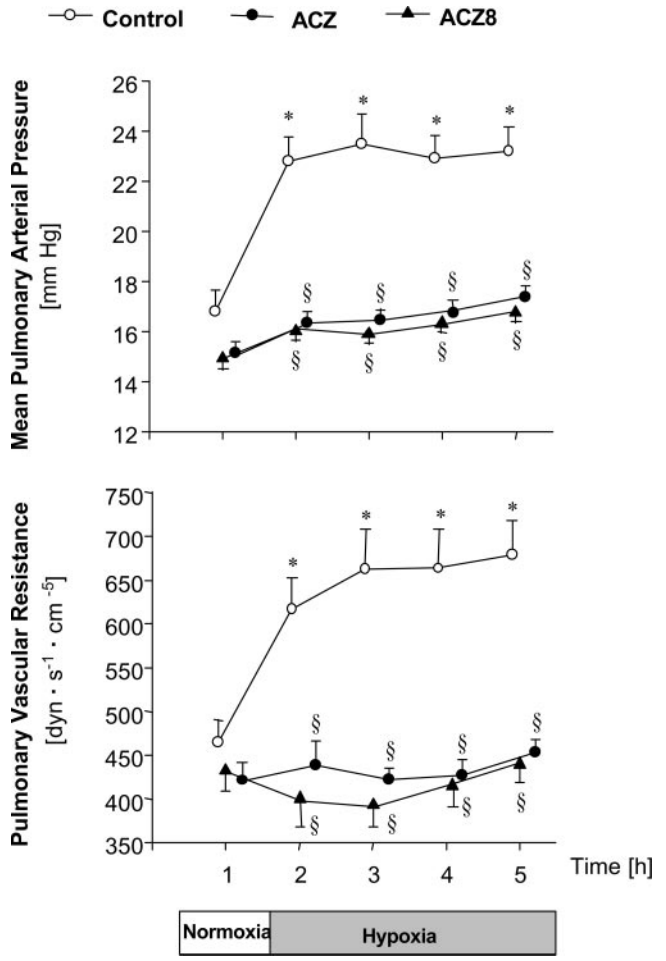

Fig. 1. Mean pulmonary arterial pressure and pulmonary vascular resistance after 1 h of normoxia and 4 h of hypoxia [inspired O_2 fraction ($F_{I_{O_2}}$) = 0.10] in control dogs and in dogs that received acetazolamide (Acz) at a $F_{I_{O_2}}$ of 0.10 during hypoxia and at a $F_{I_{O_2}}$ = 0.08 (Acz8) to keep alveolar P_{O_2} equivalent to that of the controls with hypoxia. Values are means ± SE (n = 10). *P < 0.05 vs. normoxia, §P < 0.05 vs. control.

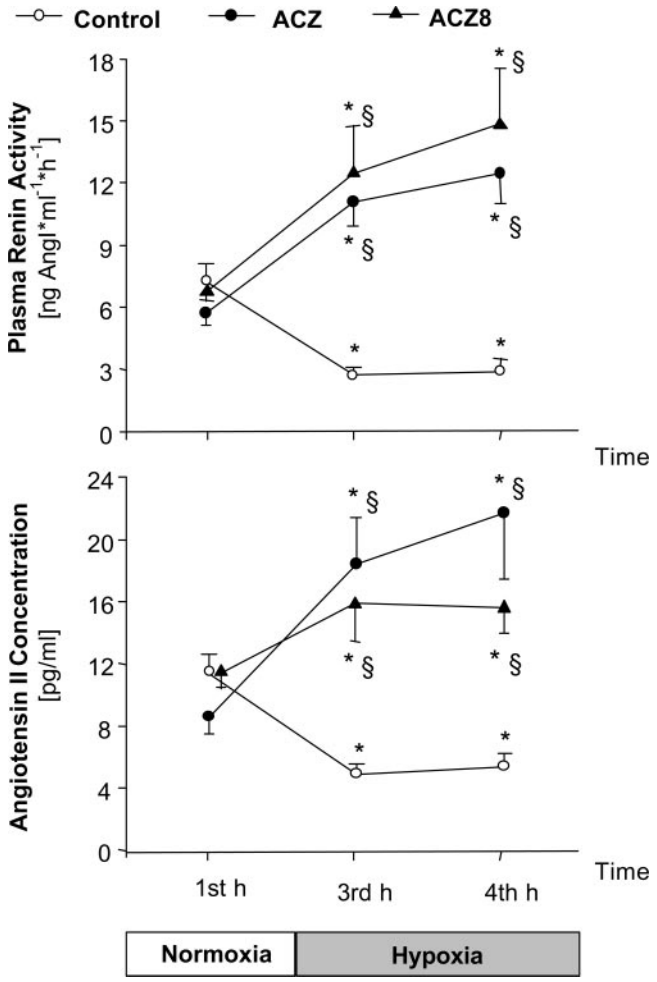

Fig. 2. Plasma renin activity and angiotensin II concentration after 1 h of normoxia and after 3 and 4 h of hypoxia ($F_{I_{O_2}}$ = 0.10) in control dogs and in dogs given Acz at a $F_{I_{O_2}}$ of 0.10 and at a $F_{I_{O_2}}$ = 0.08 (Acz8) to keep alveolar P_{O_2} equivalent to that of the controls with hypoxia. Values are means ± SE (n = 10). *P < 0.05 vs. normoxia, §P < 0.05 vs. control.

tion decreased slightly from 3.8 ± 0.1 to 3.4 ± 0.1 mmol/l during hypoxia in controls (P < 0.05). In normoxia, Acz caused a decrease in plasma potassium from 3.8 to 3.3. mmol/l (P < 0.05). With Acz at either $F_{I_{O_2}}$, plasma potassium was always slightly lower than in controls (P < 0.05; Table 4). Plasma potassium fell by a lesser magnitude with hypoxia during CA inhibition compared with the controls, despite the greater and sustained urinary potassium excretion (Table 5).

In controls, hypoxia caused a fourfold increase in urine output and increased sodium, potassium, and bicarbonate excretion 7-, 3-, and 20-fold, respectively. Acz in normoxia increased urine output roughly fivefold and that of sodium, potassium and bicarbonate excretion by 30-, 8-, and 400-fold, respectively. Hypoxia slightly enhanced the magnitude of urinary volume and electrolyte excretion with CA inhibition, with changes of 20–30% (Table 5). Hypoxia in controls did not alter GFR, although there was a trend toward an increase of roughly 25%. Acz did not reduce GFR, although a trend toward a decrease of 10–15% was evident. These two statistically insignificant but oppositely directed trends resulted in a statistically significant reduction of GFR with hypoxia and CA inhibition compared with the controls.

DISCUSSION

The aim of the present study was to determine whether CA inhibition by Acz is able to reduce or prevent hypoxic pulmonary vasoconstriction during acute hypoxia of 4 h duration.

Table 4. *Plasma endothelin concentration and plasma potassium concentration in control dogs at a $F_{I_{O_2}}$ of 0.10 during hypoxia and in dogs given acetazolamide at a $F_{I_{O_2}}$ of 0.10 or 0.08 during hypoxia*

	Normoxia 1st h	Hypoxia 2nd h	Hypoxia 3rd h	Hypoxia 4th h	Hypoxia 5th h
Endothelin-1, pg/ml					
Controls	1.4±0.1				1.9±0.1*
Acz	1.3±0.1				1.7±0.1*
Acz8	1.5±0.1				2.1±0.1*
P_K, mmol/l					
Controls	3.8±0.1	3.6±0.1	3.4±0.1*	3.4±0.1*	3.3±0.1*
Acz	3.3±0.1†	3.1±0.1†	3.2±0.1†	3.3±0.1	3.1±0.1
Acz8	3.3±0.1†	3.1±0.1†	3.1±0.1†	3.1±0.1†	3.2±0.1

Values are means ± SE, n = 10. P_K, plasma potassium concentration. Values were measured during 1 h of normoxia and 4 h of hypoxia. *P < 0.05 vs. normoxia; †P < 0.05 vs. controls.

Table 5. *Renal excretion parameters in control dogs at a $F_{I_{O_2}}$ of 0.10 and in dogs given acetazolamide at a $F_{I_{O_2}}$ of 0.10 or 0.08 during hypoxia*

	Normoxia 1st h	Hypoxia 2nd h	Hypoxia 3rd h	Hypoxia 4th h	Hypoxia 5th h
UV, $\mu l \cdot min^{-1} \cdot kg^{-1}$					
Controls	22±0.5	65±11*	83±16*	76±15*	87±11*
Acz	101±10†	123±23†	148±8*†	120±12	93±10
Acz8	112±10†	125±13†	118±9†	112±11	134±21
$U_{Na}V$, $\mu mol \cdot min^{-1} \cdot kg^{-1}$					
Controls	0.2±0.04	0.3±0.07	0.4±0.1	0.5±0.1*	1.4±0.4*
Acz	6.6±1†	10.5±1*†	10.7±0.9*†	9.5±0.6*†	8±0.4†
Acz8	6.5±0.9†	10.4±1.6*†	11±1.2*†	8.5±0.7†	8.3±0.8†
U_KV, $\mu mol \cdot min^{-1} \cdot kg^{-1}$					
Controls	0.3±0.06	0.6±0.1	0.7±0.08*	0.9±0.2*	1±0.2*
Acz	7.7±0.8†	8.1±0.7†	7.4±0.4†	7±0.3†	7.1±0.5†
Acz8	8.3±0.6†	7.7±0.6†	8.2±0.8†	7.1±0.7†	8±0.6†
$U_{HCO_3}V$, $\mu mol \cdot min^{-1} \cdot kg^{-1}$					
Controls	0.03±0.01	0.1±0.01*	0.18±0.03*	0.39±0.1*	0.57±0.2*
Acz	11.9±2†	15.7±1.6*†	17.1±1.3*†	15.9±1.4†	11.3±0.9†
Acz8	14±0.9†	16.5±1.4†	15.9±0.6*†	12.4±0.4†	11.7±0.5†
GFR, $ml \cdot min^{-1} \cdot kg^{-1}$					
Controls	3.2±0.1	4±0.2	3.8±0.2	4±0.2	4.2±0.2
Acz	3.6±0.3	3.1±0.2†	3.2±0.2†	3.2±0.1†	3.2±0.1†
Acz8	3.5±0.2	3±0.2†	3.3±0.2†	3.2±0.2†	3.2±0.2†

Values are means ± SE, $n = 10$. UV, urine volume; $U_{Na}V$, urinary sodium excretion; U_KV, urinary potassium excretion; GFR, glomerular filtration rate; U_{HCO_3}, urinary bicarbonate excretion. Values were measured during 1 h of normoxia and 4 h of hypoxia. *$P < 0.05$ vs. normoxia; †$P < 0.05$ vs. controls.

Data were obtained in trained, conscious beagle dogs, each studied under three conditions of hypoxia: control with 10% hypoxia, Acz with 10% hypoxia, and Acz with 8% hypoxia to yield Pa_{O_2} values similar to those in the control animals with 10% hypoxia. The results demonstrate that CA inhibition with Acz (with and without changes in $F_{I_{O_2}}$ to control for hyperventilation-related increases in alveolar P_{O_2}) prevents the increase in pulmonary arterial pressure and pulmonary vascular resistance with inspired hypoxia. The mediation of HPV inhibition by Acz is not related to changes in plasma renin, angiotensin-II, endothelin-1, or hypokalemia, and it occurs despite the mild drug-induced systemic acidosis.

HPV. In control dogs, MPAP increased with hypoxia more by a rise in pulmonary vascular resistance than the small rise in cardiac output (Fig. 1, Table 3). This is consistent with earlier results from our laboratory (12, 14) and many studies in humans and animals (7, 10, 41). The stimulus for HPV is the decrease in O_2 tension of the pulmonary arterial vascular smooth muscle cells (and, to a smaller degree, venular vascular smooth muscle) determined by O_2 tensions primarily in the alveolar gas, but also to a lesser extent by the P_{O_2} of mixed venous blood, and bronchial arterial blood that supplies the vasa vasorum of pulmonary vasculature (7).

In the conscious dog, we show that Acz at full CA-inhibiting concentrations (20 mg/kg) fully abolishes HPV. This is in contrast to our earlier work in the isolated perfused lung of the rabbit, in which we found only a 50% inhibition of HPV in 10 min, and also the study of Emery et al. (8). The lack of complete suppression of HPV in our rabbit studies (6) either is a species difference or more likely is because only a 10-min period of hypoxia was studied rather than 4 h as in the present experiments. Additionally, attributes of the live animal including the acid-base and renal-fluid balance changes that occur with CA inhibition and systemic hypoxia are not present or cannot be mimicked in an isolated perfused lung preparation, such as the lower mixed venous and bronchial arterial P_{O_2} values. The complete inhibition of HPV with Acz with a $F_{I_{O_2}}$ of 0.10 could have been the consequence of a higher alveolar P_{O_2} arising from the well known drug-induced stimulation of ventilation (Table 1) and thus a smaller stimulus for HPV. Greater ventilation would also yield a higher bronchial Pa_{O_2} and mixed venous P_{O_2} and thus reductions in these other stimuli to HPV. This was not the case, however, because reducing the $F_{I_{O_2}}$ to 0.08 to generate equivalent arterial and alveolar P_{O_2} values (Table 1) yielded the same degree of HPV suppression. Although we did not control for a possible direct effect of the hyperventilation (increased tidal volume and rate) induced by Acz in spontaneously breathing animals, which might have altered hypoxic vasoreactivity, Lindenberg et al. (16) found that normocapnic hyperventilation of even greater magnitude than that caused by Acz in our studies did not reduce HPV in lambs.

Hypoxia has multiple actions on pulmonary vascular smooth muscle that lead to vasoconstriction. These include direct changes in membrane potassium channel activity, membrane calcium channels, and internal calcium storage and release (2, 7, 22, 26, 29, 42). It is known that hypoxia causes intracellular alkalinization of isolated distal pulmonary artery smooth muscle cells (19, 20) and that this is, in part, dependent on membrane-bound sodium-dependent chloride-bicarbonate exchange, in which extracellular bicarbonate is exchanged for intracellular chloride. In other studies, nonhypoxic intracellular alkalinization of pulmonary vascular smooth muscle also causes vasoconstriction (13, 17, 27, 35). Investigations examining how intracellular pH changes separately and in combination with changes in extracellular pH have not always been consistent (1, 5). These studies reveal a very complex interplay between the size of vessel studied, methods of inducing pH changes, and species differences. How these pH changes affect potassium and calcium channel activity, however, is not well characterized.

Owing to the complexity of ion and acid-base regulation in pulmonary vascular smooth muscle and multiple mechanisms of HPV, it is not obvious how CA inhibition depresses or abolishes HPV. A direct effect of CA inhibition on the lung vascular endothelium and smooth muscle is likely because the enzyme is present in both the vascular endothelium and alveolar epithelium, and probably in vascular smooth muscle cells (25, 36, 37). Thus the enzyme, which is both membrane bound and cytosolic, ensures that CA activity is active in mediating intracellular as well as extracellular local perivascular pH changes.

Although CA inhibitors cause a state of systemic acidosis by blocking renal reabsorption of bicarbonate and causing tissue CO_2 retention (36, 37), these consequences would alone likely enhance HPV rather than depress it, because acidosis is well known to strengthen HPV (15, 18). The drug-induced hyperventilation, however, and resultant lower alveolar P_{CO_2} may lead to a slight tissue alkalosis in the lung, which might dampen HPV. Although this possibility cannot be completely excluded, a local alkalosis need not be invoked because Deem et al. (6) showed HPV inhibition by Acz in the isolated perfused lung in which ventilation and acid-base parameters were fixed.

Studies by Pickkers et al. (24) in isolated systemic mesenteric arteries show that Acz and other CA-inhibiting sulfonamides increase intracellular pH and relax these vessels when they are preconstricted with norepinephrine. They have further shown that CA inhibitors and/or the intracellular alkalinization appear to activate a calcium-dependent potassium channel, resulting in hyperpolarization of the vascular smooth muscle cell, reduction of voltage-dependent calcium channel activity, a decrease in intracellular calcium, and vasorelaxation (25). It remains unstudied whether this mechanism is operant in the smooth muscle cells of the pulmonary vasculature, which with respect to CO_2 and O_2 have opposite responses to systemic vessels.

Hypoxia, ventilation, and acid-base balance. In the control experiments, acute hypoxia increased minute ventilation by 1.8 l/min inducing respiratory alkalosis (Tables 1 and 2) (11, 12, 14). With Acz, the dose is fully inhibiting for all CA activity and produces a metabolic acidosis by renal CA inhibition and respiratory acidosis by red cell and tissue CA inhibition (38, 40). These are the main contributors to the increase in minute ventilation during normoxia (Table 1). During hypoxia, an equivalent increase in minute ventilation was found with Acz at both $F_{I_{O_2}}$ values (Table 1) owing to a combined stimulation of ventilation by CA inhibition and hypoxia (36). It is interesting that there was no difference in the magnitude of increased ventilation with hypoxia during CA inhibition between the two levels of $F_{I_{O_2}}$. This may be explained, in part, by the known suppressive effects of CA inhibition on hypoxic peripheral chemoreceptor responsiveness (36).

Plasma hormones and potassium. PRA and ANG II decreased during hypoxia in controls but increased in both Acz experiments (Fig. 2). The reasons for the decrease in PRA in controls are multiple and may include the mild hypertensive effect of hypoxia (33) (Table 3) and hypoxia-mediated adenosine (12), endothelin (23, 32), and NO release (34). The likeliest explanation for the rise in PRA with hypoxia during CA inhibition is the large diuretic effect of Acz (Table 5) and reduction in extracellular volume (36) that dominates any hypoxic-mediated depression of PRA.

Hypoxia led to roughly a 20–25% increase in plasma endothelin-1 concentration, which was not affected by CA inhibition (Table 4). Thus it is unlikely that Acz abolishes HPV by suppressing the rise in endothelin-1 with hypoxia. Because the membrane polarization and smooth muscle tone are sensitive to changes in extracellular potassium (9), it would be predicted that the smaller fall in plasma potassium with hypoxia in the Acz-treated dogs should have acted against a reduction in HPV, which was not the case. Therefore, it is improbable that Acz reduces HPV by any action on plasma potassium concentration, despite the increased urinary loss of potassium (Table 5). It is not clear why, despite the significantly greater kaliuresis with Acz, plasma potassium fell less than in the controls with hypoxia, but a Acz-induced acidosis shift of potassium from the intracellular space into the extracellular space may have occurred to counter the increased urinary potassium excretion that had already occurred in the first hour of normoxia.

Implications for CA inhibitors in high-altitude acclimatization. CA inhibitors such as Acz are commonly used at high altitude to prevent and treat AMS. Their effectiveness in AMS is achieved at a dosing of 2.5–5.0 mg/kg, roughly 12–25% of that used in this study. These lower doses fully inhibit renal CA (36, 40) and generate a mild metabolic acidosis, which is thought to be the primary cause of the ventilatory stimulation and better oxygenation observed in all studies of Acz (36). It is likely that HPV may be blunted at these clinical doses simply by the higher alveolar P_{O_2} resulting from hyperventilation. Whether at these doses Acz also directly inhibits HPV as observed in this study remains for further investigation. Because any medication or intervention that improves alveolar oxygenation directly, or blunts HPV directly, is effective in preventing and treating HAPE (31), it may be that Acz is protective against HAPE. No retrospective or prospective trials, however, have addressed this possibility. We have recently found that rats given Acz (20 mg/kg) have less alveolar protein leak and hemorrhage (HAPE-like pathology) than controls when subjected to a 24-h 18,000-feet exposure in a hypobaric chamber (4). Further studies will need to address two questions in animals and then humans: *1*) can lower dose Acz, as used clinically, directly inhibit HPV independently of ventilatory stimulation and the secondary increase in alveolar P_{O_2}, or are fully inhibiting doses necessary? and *2*) does Acz blunt the greater pulmonary arterial pressures with hypoxic exercise, such as mountaineering?

In summary, we have shown that high concentrations of Acz fully inhibit hypoxic pulmonary vasoconstriction in the live animal. Although the effect may be, in part, due to higher alveolar oxygenation with hyperventilation, the effect is just as strong when alveolar P_{O_2} is kept constant. HPV inhibition by Acz is furthermore not related to changes in acid-base status, endothelin-1, PRA, ANG II, or plasma potassium concentration. These data are consistent with a direct action of CA inhibition in pulmonary vascular smooth muscle to oppose HPV.

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