The effect of low-dose acetazolamide on the ventilatory CO_2 response curve in the anaesthetized cat

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1. The effect of 4 mg kg^{-1} acetazolamide (I.V.) on the slope (S) and intercept on the P_{a,CO_2} axis (B) of the ventilatory CO_2 response curve of anaesthetized cats with intact or denervated carotid bodies was studied using the technique of dynamic end-tidal forcing.

2. This dose did not induce an arterial-to-end-tidal P_{CO_2} ($P_{(a-ET),CO_2}$) gradient, indicating that erythrocytic carbonic anhydrase was not completely inhibited. Within the first 2 h after administration, this small dose caused only a slight decrease in mean standard bicarbonate of 1·8 and 1·7 mmol l^{-1} in intact ($n = 7$) and denervated animals ($n = 7$), respectively. Doses of acetazolamide larger than 4 mg kg^{-1} (up to 32 mg kg^{-1}) caused a significant increase in the $P_{(a-ET),CO_2}$ gradient.

3. In carotid body-denervated cats, 4 mg kg^{-1} acetazolamide caused a decrease in the CO_2 sensitivity of the central chemoreflex loop (S_c) from 1·52 ± 0·42 to 0·96 ± 0·32 l min^{-1} kPa^{-1} (mean ± S.D.) while the intercept on the P_{a,CO_2} axis (B) decreased from 4·5 ± 0·5 to 4·2 ± 0·7 kPa.

4. In carotid body-intact animals, 4 mg kg^{-1} acetazolamide caused a decrease in the CO_2 sensitivity of the peripheral chemoreflex loop (S_p) from 0·28 ± 0·18 to 0·19 ± 0·12 l min^{-1} kPa^{-1}. S_c and B decreased from 1·52 ± 0·55 to 0·84 ± 0·21 l min^{-1} kPa^{-1}, and from 4·0 ± 0·5 to 3·0 ± 0·6 kPa, respectively, not significantly different from the changes encountered in the denervated animals.

5. It is argued that the effect of acetazolamide on the CO_2 sensitivity of the peripheral chemoreflex loop in intact cats may be caused by a direct effect on the carotid bodies. Both in intact and in denervated animals the effects of the drug on S_c and B may not be due to a direct action on the central nervous system, but rather to an effect on cerebral vessels resulting in an altered relationship between brain blood flow and brain tissue P_{CO_2}.

Carbonic anhydrase is present in several tissues directly or indirectly involved in the control of breathing, for example in renal tubular cells, erythrocytes, lung and brain capillary endothelium, in peripheral and possibly also central chemoreceptors (Giacobini, 1962; Lee & Mattenheimer, 1964; Maren, 1967; Effros, Chang & Silverman, 1978; Ridderstråle & Hanson, 1985; Torrance, 1993). The most widely used inhibitor of the enzyme is acetazolamide (Maren, 1967). At doses sufficient to cause more than 99% inhibition of erythrocytic carbonic anhydrase, this drug effects a large gradient between arterial P_{CO_2} (determined from *in vitro* samples) and end-tidal P_{CO_2}, and an increase in ventilation (e.g. Carter & Clark, 1958; Travis, Wiley & Maren, 1966; Teppema, Rochette & Demedts, 1992).

Clinically, acetazolamide is mostly used in too small a dose to inhibit erythrocytic carbonic anhydrase completely. Usually, however, ventilation increases at these low doses, which is probably caused by an ensuing metabolic acidosis (e.g. Lerche, Katsaros, Lerche & Loeschcke, 1960; Chiesa, Stretton, Massoud & Howell, 1969; Bashir, Kann & Stradling, 1990; Swenson & Hughes, 1993).

Several authors have studied the effect of acetazolamide on the ventilatory response curve to CO_2 by applying the Read rebreathing technique (e.g. Chiesa *et al.* 1969; Bashir *et al.* 1990). It has been shown, however, that applying this rebreathing technique in conditions of metabolic acidosis may result in a considerable overestimation of the CO_2 response slope (Linton, Poole-Wilson, Davies & Cameron,

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1973; Berkenbosch, Bovill, Dahan, DeGoede & Olievier, 1989). Furthermore, neither the Read technique nor conventional steady-state techniques are able to assess possible effects of drugs on the sensitivities of the peripheral and central chemoreflex loops separately from each other.

In this study in anaesthetized cats we investigated the effect on the peripheral and central chemoreflex loop of a low dose of acetazolamide (4 mg kg^{-1}) not causing complete inhibition of erythrocytic carbonic anhydrase, indicated by the absence of an arterial-to-end-tidal P_{CO_2} ($P_{(a-ET),CO_2}$) gradient. To this aim, we applied the dynamic end-tidal forcing (DEF) technique in animals with intact carotid bodies, to assess the CO_2 sensitivity of the peripheral and central chemoreflex loops, as well as the intercept on the P_{a,CO_2} axis of the CO_2 response curve (DeGoede, Berkenbosch, Ward, Bellville & Olievier, 1985). In this study we also determined the effect of acetazolamide on the CO_2 response curve in carotid body-denervated cats. The aim was to see whether applying the data thus obtained on a steady-state CO_2 mass balance of the brain (Read & Leigh, 1967; Berkenbosch *et al.* 1989) could give us more insight into a possible mechanism of action of this drug on the central chemoreflex loop.

METHODS

Animals and surgery

Fourteen adult cats (weight 4·0–5·6 kg) were sedated with 15 mg kg^{-1} ketamine hydrochloride (I.M.). Atropine sulphate (0·5 mg S.C.) was given. The animals were anaesthetized by inhalation of a gas mixture containing 0·5–1 % halothane and 30 % O_2 in N_2 while the femoral arteries and veins were cannulated. Subsequently a dose of 20 mg kg^{-1} α-chloralose and 100 mg kg^{-1} urethane was slowly infused intravenously and the addition of halothane to the inspirate was discontinued. Anaesthesia was maintained with a continuous infusion of 1 mg kg^{-1} h^{-1} α-chloralose and 5 mg kg^{-1} h^{-1} urethane. Rectal temperature was monitored with a thermistor and maintained within 0·5 °C in the range from 36·3 to 39·3 °C by a heating blanket and an infrared lamp.

The trachea was cannulated at midcervical level and connected to a respiratory circuit. In seven cats both carotid sinus nerves were identified at their junctions with the glossopharyngeal nerves and were cut. To check the effectiveness of carotid nerve section, these animals were exposed to a short hypoxic challenge. All seven cats responded with a decrease in ventilation, indicating that the peripheral chemoreceptors were functionally eliminated.

The animals were connected to an extracorporeal circuit (ECC) for continuous blood gas measurement. Using the ECC, blood from the left femoral artery was pumped back via the right femoral vein with a flow of 6 ml min^{-1}.

Measurements

Respiratory airflow was measured with a Fleisch No. 0 flow transducer (Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Statham PM197, Los Angeles, CA, USA), and was electrically integrated to yield tidal volume. The CO_2 and O_2 concentrations in the tracheal gas were measured with an infrared analyser (Gould Godard MK2 Capnograph, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger O_2-test, Würzburg, Germany), respectively. The inspiratory gas concentrations were made with mass flow controllers (type AFC 260, Advanced Semiconductor Materials, De Bilt, The Netherlands).

Arterial pH and P_{CO_2} in the blood passing the extracorporeal circuit were measured continuously with a pH electrode (Radiometer E-5037-0, Copenhagen, Denmark) calibrated with phosphate buffers, and a CO_2 electrode (General Electric A312AB, Milwaukee, WI, USA) calibrated with water equilibrated with CO_2–O_2–N_2 gas mixtures delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). The transport delay from the lungs to this CO_2 electrode was approximately 50 s. The CO_2 electrode was recalibrated about every 2 h and corrections were made for drift when necessary. Arterial blood pressure was measured using a Statham pressure transducer (P23ac).

All signals were recorded on polygraphs, digitized (sample frequency 100 Hz), processed by a PDP 11/23 computer (Digital Equipment Corp., Maynard, MA, USA) and stored on disc. Steady-state values of ventilation, blood pressure, end-tidal and arterial blood gas tensions were averaged over twenty breaths.

Experimental protocol

Each DEF run started with a steady-state period of ventilation of about 2 min. Thereafter the P_{ET,CO_2} was elevated by about 1–1·5 kPa within one or two breaths, maintained at a constant level for a period of about 7 min and then lowered stepwise to the previous value and kept constant for a further 7 min (see Fig. 3). The P_{ET,O_2} was held constant at about 15 kPa throughout all runs. In this way three to five control DEF runs were performed in each cat. Subsequently in ten animals (five of seven intact and five of seven carotid body-denervated cats) the effects on the $P_{(a-ET),CO_2}$ gradient of low cumulative doses of acetazolamide (Diamox, Lederle, The Netherlands) up to 4 mg kg^{-1} (I.V.) were determined. The drug was dissolved in saline. The doses, which were infused at constant end-tidal P_{CO_2}, were 0·5, 0·5, 1 and 2 mg kg^{-1} (i.e. 4 mg kg^{-1} in total). There was at least a 20 min pause after each dose in order to let all parameters stabilize. Four animals (two of seven intact cats and two of seven carotid body-denervated cats) received a bolus infusion of 4 mg kg^{-1}. About 45–60 min after the animals had received 4 mg kg^{-1} of the agent, another three to five DEF runs (acetazolamide runs) were performed. Thereafter, to three of the (seven) carotid body-denervated animals a single subsequent intravenous dose of, respectively, 8, 17 and 34 mg of bovine carbonic anhydrase C (Sigma, dialysed and lyophilized from bovine erythrocytes, approximately 5500 Wilbur-Anderson units per mg) in saline was administered and again three DEF runs were performed. After completion of the acetazolamide DEF runs the respiratory effects of additional doses (2, 2, 8, 16 and 16 mg kg^{-1}) acetazolamide were studied. In five intact cats and in three animals with denervated carotid bodies we determined minute ventilation at three different steady-state levels of end-tidal P_{CO_2} after they had received a total dose of 32 mg kg^{-1}.

Data analysis

The steady-state relation of ventilation ($\dot{V}_I$) to P_{ET,CO_2} at constant P_{ET,O_2} in the cat is linear down to the P_{ET,CO_2} axis and well described by DeGoede, Berkenbosch, Olievier & Quanjer (1981):

$$\dot{V}_I = (S_p + S_c)(P_{ET,CO_2} - B). \quad (1)$$

The parameters S_c and S_p are the central and peripheral ventilatory CO_2 sensitivities and the offset B represents the apnoeic threshold or extrapolated P_{ET,CO_2} of the steady-state ventilatory response to CO_2 at zero ventilation.

For the analysis of the dynamic response of the ventilation we used a two-compartment model (DeGoede *et al.* 1985), viz.:

$$\tau_c \frac{d\dot{V}_c}{dt} + \dot{V}_c = S_c(P_{ET,CO_2}(t - T_c) - B_c), \quad (2)$$

$$\tau_p \frac{d\dot{V}_p}{dt} + \dot{V}_p = S_p(P_{ET,CO_2}(t - T_p) - B_p), \quad (3)$$

$$\tau_c = \tau_{on} x + (1 - x)\tau_{off}, \quad (4)$$

$$\dot{V}_I = \dot{V}_c + \dot{V}_p + Ct. \quad (5)$$

In the equations, $\dot{V}_c$ and $\dot{V}_p$ denote the contributions of the central and peripheral chemoreceptors to the ventilation $\dot{V}_I$. B_c and B_p are the offsets of the central and peripheral ventilatory response. The time constants of the central and peripheral ventilatory responses are denoted by τ_c and τ_p. T_c and T_p are the delay times needed to transport the CO_2 disturbance from the lungs to the central and peripheral chemoreceptors, respectively. To model the central time constant of the ventilatory on-transient to be different from that of the off-transient, τ_c is written according to eqn (4) in which $x = 1$ when P_{ET,CO_2} is high and $x = 0$ when P_{ET,CO_2} is low. In some experiments a small drift in ventilation was present. Therefore we included a drift term Ct in the model (eqn (5)). However, the trend was usually small and in multiple DEF studies in the same cat it was positive as well as negative.

We emphasize that the DEF technique can only separate the change in ventilation following a change in end-tidal CO_2 into parts belonging to the central and peripheral chemoreflex loops. This is reflected in the fact that the offset parameters B_c and B_p in eqns (2) and (3), respectively, cannot be estimated individually since they are not identifiable. We therefore reduce the number of parameters in the model. To this end it is customary to choose the same offset parameter for both loops, viz. $B_c = B_p = B$ (Berkenbosch, DeGoede, Olievier & Ward, 1986). This offset B is then equal to the extrapolated P_{ET,CO_2} of the steady-state ventilatory response curve to zero ventilation (apnoeic threshold). As a consequence when a drug causes a change in apnoeic threshold it cannot be determined, using the DEF technique, whether the change has a central or peripheral origin. Although it is not correct to call $\dot{V}_c$ and $\dot{V}_p$ the central and peripheral part of the ventilation due to the arbitrary choice of $B_c = B_p$, we usually do so for the sake of simplicity of the presentation. For the steady state the two compartment model reduces to eqn (1) as it should.

All the parameters of the model were estimated simultaneously using the actual P_{ET,CO_2} as input and by fitting the data of each DEF study with a least-squares method. To obtain optimal time delays a 'grid search' was applied and all combinations of T_c and T_p with increments of 1 s and with $T_c \geqslant T_p$ were tried until a minimum in the residual sum of squares was found. The minimal time delays were somewhat arbitrarily chosen to be 1 s and τ_p was constrained to be at least 0·3 s (DeGoede *et al.* 1985). For the analysis of the response of the carotid body-denervated cats S_p and τ_p were set to zero, since no fast component was present.

Statistical analysis

To compare the values obtained from the analysis of the DEF runs in the control situation with those after acetazolamide infusion, a two-way analysis of variance was performed, using a fixed model. The level of significance was set at 0·05. Results are given as means ± S.D.

The design of this study and the use of cats were approved by the Ethical Committee for Animal Experiments of Leiden University.

RESULTS

In Fig. 1 cumulative dose–response curves of acetazolamide are shown for intact as well as denervated animals. It appeared that, in a (total) dose smaller than or equal to 4 mg kg^{-1}, the drug did not induce a systematic $P_{(a-ET),CO_2}$ gradient, indicating incomplete inhibition of erythrocytic carbonic anhydrase (Maren, 1967). Maximal widening of the arterial-to-end-tidal P_{CO_2} difference (approximately 2·5–3 kPa) was reached at a total dose of approximately 30 mg kg^{-1} in intact cats, similar to the gradient reached after a bolus infusion of 50 mg kg^{-1} (Teppema *et al.* 1992).

Figure 2 shows a recording of the respiratory effects of 4 mg kg^{-1} acetazolamide in one of the two carotid body-intact animals to which a bolus infusion of this dose was given. The infusion was performed at constant end-tidal P_{CO_2}. It shows that no arterial $P_{(a-ET),CO_2}$ gradient was

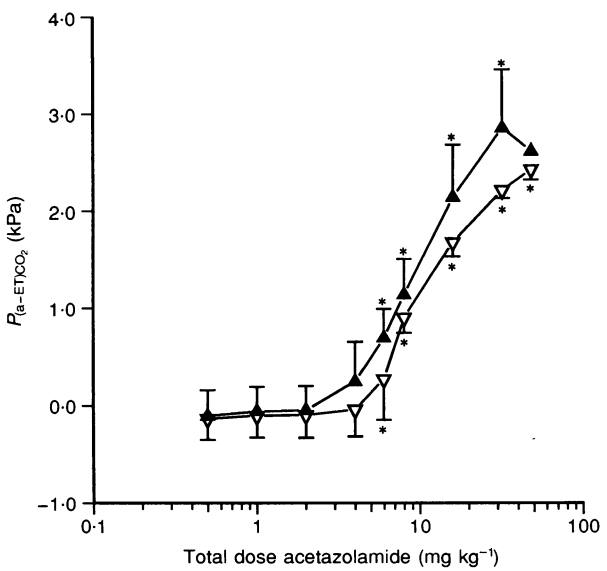

Figure 1. Dose–response curve of acetazolamide
The effect of acetazolamide on the arterial-to-end-tidal P_{CO_2} difference ($P_{(a-ET),CO_2}$). Mean values ± S.D. ▲, intact cats; $n = 5$ at 0·5, 1, 2, 4, 6, 8, 16 and 32 mg kg^{-1}; $n = 1$ at 48 mg kg^{-1}. ▽, carotid body-denervated cats; $n = 5$ at 0·5, 1, 2 and 4 mg kg^{-1}; $n = 3$ at 6, 8, 16, 32 and 48 mg kg^{-1}. * Significantly different from control.

induced since arterial P_{CO_2} also remained virtually constant. Shortly after the injection ventilation decreased and then slowly increased to a level above that existing before drug administration. The second intact animal showed a similar response. However, the two denervated animals receiving a bolus of 4 mg kg^{-1} did not show the slow ventilatory decrease.

In Fig. 3 two examples of a computer analysis of DEF runs from an intact animal are shown, performed before and after infusion of 4 mg kg^{-1} acetazolamide. It shows that after acetazolamide administration S_p, S_c and B were decreased. A total of fifty-one DEF runs (31 control and 20 acetazolamide runs) were analysed. In Table 1 the effects of 4 mg kg^{-1} acetazolamide on the DEF parameters in intact animals, together with those on standard bicarbonate and on the $P_{(a-ET),CO_2}$ gradient are summarized. The decrease in S_c, S_p and B were highly significant. A small but significant effect on T_c and τ_{off} was found. The significant but slight decrease in standard bicarbonate indicates that the acute renal effect of acetazolamide was mild. An arterial-to-end-tidal P_{CO_2} difference was not detectable, indicating incomplete inhibition of erythrocytic carbonic anhydrase at this dose (Maren, 1967).

In Table 2 the mean data obtained from twenty-six control and twenty-four acetazolamide runs of 4 mg kg^{-1} acetazolamide in the seven carotid body-denervated animals are summarized. In these animals we also found a decrease in S_c and B. As in the intact animals, no arterial-to-end-tidal P_{CO_2} gradient was found, and only a slight (insignificant) metabolic acidosis. The change in mean B in the denervated animals was not significantly different from the mean change found in those with intact carotid bodies (two-tailed t test for independent samples, $P = 0{\cdot}06$). The same was true for the effect of acetazolamide on mean S_c in both groups ($P = 0{\cdot}5$).

Infusion of respectively 8, 17 and 34 mg bovine carbonic anhydrase C (approximately 5500 Wilbur-Anderson units

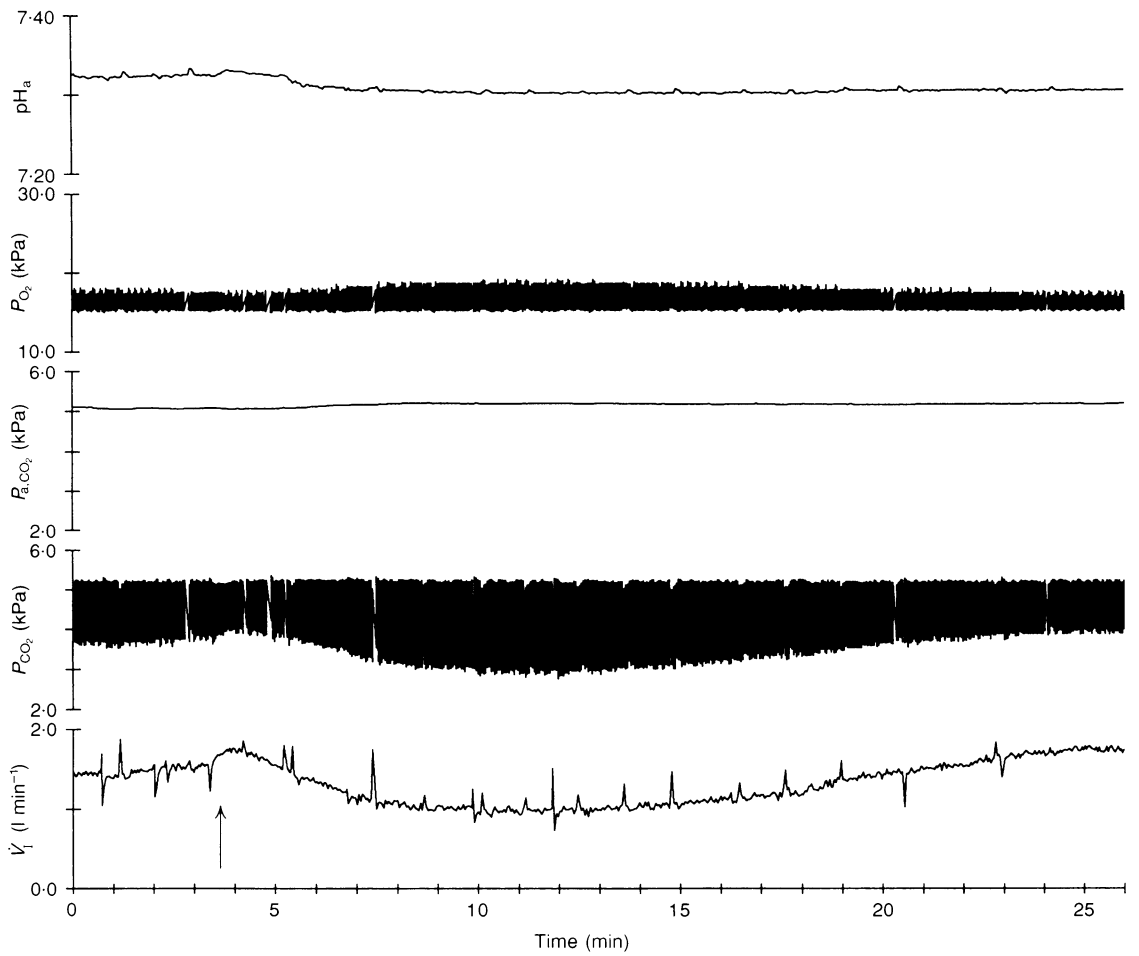

Figure 2. Respiratory effects of 4 mg kg^{-1} acetazolamide (I.V.)

Intravenous infusion of 4 mg kg^{-1} acetazolamide (arrow) at constant end-tidal P_{CO_2} in a cat with intact carotid bodies results in a rapid initial increase in ventilation $\dot{V}_I$. This initial increase is followed by a slow decrease, for which an effect of the drug on the peripheral chemoreceptors may be responsible, and by a secondary gradual increase in ventilation to a level above control. Note that the arterial P_{CO_2} (P_{a,CO_2}) remains virtually constant indicating incomplete inhibition of erythrocytic carbonic anhydrase. P_{O_2} and P_{CO_2} denote gas tensions in tracheal gas; pH_a is pH in arterial blood.

Table 1. Effects of 4 mg kg^{-1} acetazolamide in cats with intact carotid bodies

	S_c (l min^{-1} kPa^{-1})	S_p (l min^{-1} kPa^{-1})	B (kPa)	$P_{(a-ET),CO_2}$ (kPa)	Standard bicarbonate (mmol l^{-1})	T_p (s)	τ_p (s)	T_c (s)	τ_{on} (s)	τ_{off} (s)
Control	1·52 ± 0·55	0·28 ± 0·18	4·0 ± 0·5	0·04 ± 0·31	20·6 ± 0·9	5 ± 2	4 ± 3	8 ± 3	89 ± 11	141 ± 31
Acet.	0·84 ± 0·21	0·19 ± 0·12	3·0 ± 0·6	0·17 ± 0·56	18·8 ± 0·6	5 ± 3	6 ± 4	10 ± 4	107 ± 28	115 ± 20
P	0·0001	0·0001	0·0001	0·45	0·0003	0·31	0·11	0·03	0·24	0·01

S_p and S_c are the CO_2 sensitivities of the peripheral and central chemoreflex loops, with delay times T_p and T_c. τ_p is the peripheral time constant and τ_{on} and τ_{off} are the central on- and off-transient time constants. B is the intercept on the P_{a,CO_2} axis of the CO_2 ventilatory response curve. Acet., acetazolimide. Values are presented as mean of the means per cat ± S.D. P values are obtained from the ANOVA on the individual data.

per mg) in three denervated animals did not change the values of S_c and B obtained after 4 mg kg^{-1} acetazolamide. From data reported by Travis *et al.* (1966) and Maren (1967) we assume that about 2 h after an intravenous infusion of 4 mg kg^{-1} acetazolamide the concentration of free, unbound, acetazolamide in plasma will be too low to completely inhibit these large quantities of infused carbonic anhydrase.

Finally, in five of seven intact cats and in three of seven carotid body-denervated animals we determined ventilatory CO_2 sensitivity after a total dose of 32 mg kg^{-1} of the inhibitor. In the denervated animals, the slope of the $\dot{V}_I$–P_{a,CO_2} response curve decreased further to a mean value of 20 ± 7 % of the control value, i.e. that existing before any acetazolamide infusion. In the intact animals the slope decreased to a mean value of 32 ± 7 % of the control value.

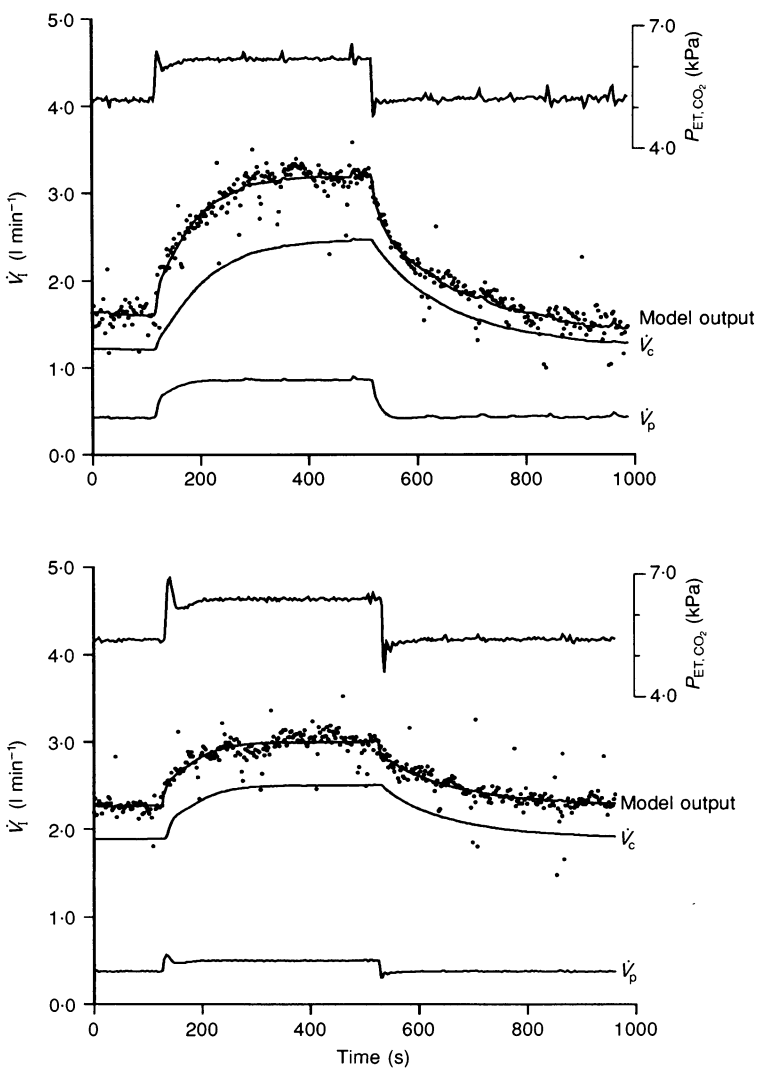

Figure 3. DEF runs before and after 4 mg kg^{-1} acetazolamide

Examples of two representative DEF runs in a carotid body-intact animal before (upper panel) and after (lower panel) acetazolamide administration. The upper part of each panel shows the P_{ET,CO_2}. The dots represent breath-to-breath ventilation. The curve through these data points is the model fit to the ventilation data, using the actual breath by breath P_{ET,CO_2} data as input. The model fit is the sum of the central component $\dot{V}_c$, the peripheral component $\dot{V}_p$ and the drift (not shown separately). The values in the control run of the intercept on the P_{ET,CO_2} axis (B), the sensitivity of the central and peripheral chemoreflex loops (S_c and S_p) are 4·21 kPa and 1·24 and 0·43 l min^{-1} kPa^{-1}, respectively. After acetazolamide these values decreased to levels of 2·27 kPa and 0·61 and 0·12 l min^{-1} kPa^{-1} for B, S_c and S_p, respectively.

Table 2. Effects of 4 mg kg^{-1} acetazolamide in carotid body-denervated cats

	S_c (l min^{-1} kPa^{-1})	B (kPa)	$P_{(a-ET),CO_2}$ (kPa)	Standard bicarbonate (mmol l^{-1})	T_c (s)	τ_{on} (s)	τ_{off} (s)
Control	1·52 ± 0·42	4·5 ± 0·5	0·02 ± 0·20	19·9 ± 2·1	4 ± 1	85 ± 25	132 ± 31
Acetazolamide	0·96 ± 0·32	4·2 ± 0·7	−0·02 ± 0·18	18·2 ± 2·5	6 ± 3	116 ± 83	105 ± 21
P	0·0001	0·0021	0·60	0·18	0·06	0·03	0·005

S_c is the sensitivity of the central chemoreflex loop, with time constants τ_{on} and τ_{off} and delay time T_c. B is the intercept on the P_{a,CO_2} axis of the ventilatory CO_2 response curve. Values are presented as mean of the means per cat ± S.D. P values are obtained from the ANOVA on the individual data.

In all animals a large decrease in B was seen, corresponding to that encountered during complete inhibition of erythrocytic carbonic anhydrase induced by an infusion of 70 mg kg^{-1} benzolamide (Teppema, Berkenbosch DeGoede & Olievier, 1995).

DISCUSSION

This study shows that acetazolamide, at doses of 4 mg kg^{-1} and below, did not induce a significant $P_{(a-ET),CO_2}$ gradient, indicating absence of effective inhibition of erythrocytic carbonic anhydrase. In dogs, such a dose causes an appreciable gradient (Travis, Wiley, Nechay & Maren, 1964), and the maximal widening of the $P_{(a-ET),CO_2}$ difference is reached at 20 mg kg^{-1} (Maren, 1967). We found that the maximal effect in the intact cat is reached at about 30 mg kg^{-1}. Red cell enzyme activity in cat is about three times higher than in dog (Dodgson & Forster, 1983). This may explain the higher doses needed to achieve inhibition in the cat.

The main findings of this study are that in carotid body-intact cats S_p and S_c as well as B decreased after 4 mg kg^{-1} acetazolamide. In carotid body-denervated cats S_c and B were decreased to about the same extent.

For the interpretation of these results we start with the denervated cats in which we consider ventilation a function of brain tissue P_{CO_2} (P_{t,CO_2}). Since in these animals 4 mg kg^{-1} acetazolamide induced neither a significant decrease in standard bicarbonate nor a $P_{(a-ET),CO_2}$ gradient (Table 2), we conclude that the observed changes in the slope and intercept of the CO_2 response curve (relating ventilation to P_{a,CO_2}) were not due to renal or erythrocytic carbonic anhydrase inhibition of the drug. The infused low dose of acetazolamide, if evenly distributed, would yield a brain concentration of $1·8 \times 10^{-5}$ M, which is insufficient to give full inhibition of local carbonic anhydrase (Maren, 1967). Furthermore and importantly, acetazolamide is not evenly distributed at all, and is relatively excluded from the brain, even when administered in large doses (Roth, Schoolar &

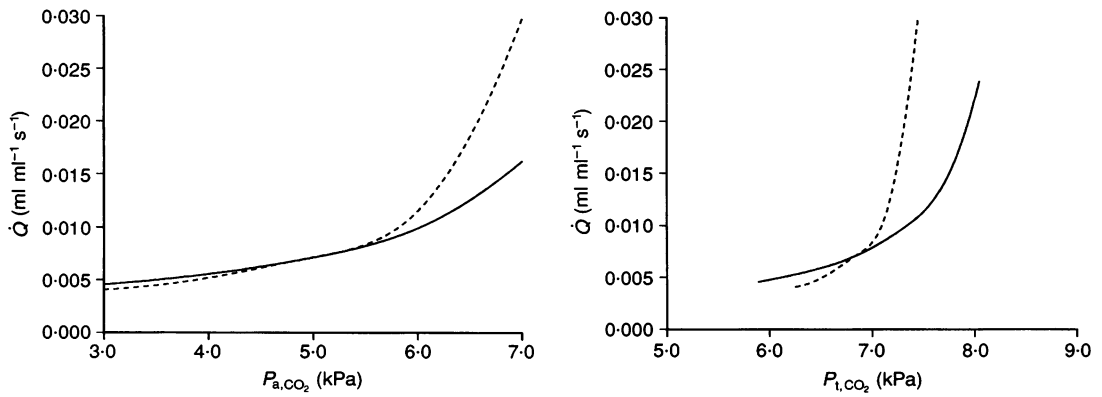

Figure 4. Cerebral blood flow density ($\dot{Q}$) as a function of arterial or brain tissue P_{CO_2}

Cerebral blood flow density ($\dot{Q}$) is calculated from the hyperbolic relation:

$$\dot{Q} = \frac{a}{b - P_{CO_2}},$$

in which either P_{a,CO_2} (left panel) or P_{t,CO_2} (right panel) can be taken as the independent variable. The continuous curves represent the control situation; the dashed curves that after 4 mg kg^{-1} acetazolamide. For calculation of parameters a and b in both conditions see Appendix.

Barlow, 1959; Maren, 1967; see also Hanson, Nye & Torrance, 1981). Consequently the brain concentration reached 1–2 h after infusion of 4 mg kg^{-1} will be very much smaller than that needed to achieve effective inhibition of CNS carbonic anhydrase. We therefore reason that the observed decreases in S_c and B were not due to a direct effect on central chemoreceptors or other CNS nerve cells and ascribe these effects to a change in the relation between brain tissue P_{CO_2} and arterial P_{CO_2}. To express this relationship we have previously used a steady-state mass balance equation for CO_2 for a brain compartment (Teppema *et al.* 1995) which was originally proposed by Read & Leigh (1967) and modified by Berkenbosch *et al.* (1989) (see eqn (A1)). Our model yields a linear relationship between P_{t,CO_2} and P_{a,CO_2} (see also Fig. 5). The slope and intercept of this relationship depend, among other factors, on brain metabolism, on the slope of the blood CO_2 dissociation curve and on the relationship between cerebral blood flow density ($\dot{Q}$) and P_{t,CO_2} (see eqn (A3)). As reasoned above brain metabolism will not be changed. Apparently the slope of the blood CO_2 dissociation curve also remained constant, since the dose of 4 mg kg^{-1} did not result in a $P_{(a-ET),CO_2}$ gradient and since subsequent intravenous infusion of carbonic anhydrase did not further influence the effects of acetazolamide on the CO_2 response curve. The effects of the drug on S and B should thus be caused by an alteration of the relationship between $\dot{Q}$ and P_{t,CO_2}, resulting in a change in the P_{t,CO_2}–P_{a,CO_2} relationship.

Figure 4 shows the relationship between brain blood flow density and arterial and brain tissue P_{CO_2}; the dashed curves show the calculated course of both hyperbolas after acetazolamide infusion if the effects of the agent on the CO_2 response curve were entirely due to an action on cerebral vessels. We calculate from the slope ratio of the CO_2 response curve after and before drug infusion (0·63) that a decrease of about 50% in the 'shape factor' of the hyperbola (parameter a in eqns (A2) and (A3)) accounts for the observed decrease in S. The change in B would result from this decrease in a, combined with a left shift of the asymptotes of the hyperbolas (parameter b in the equations in the Appendix). This effect of acetazolamide on cerebral blood flow leads to a change in the P_{t,CO_2}–P_{a,CO_2} relationship shown in Fig. 5.

Figure 4 implies that the effects of acetazolamide on $\dot{Q}$, in a dose which does not completely inhibit the erythrocytic enzyme, depends on the P_{a,CO_2} at which it is given. In many studies a considerable increase in $\dot{Q}$ was reported, but in most of these, large doses were used (cf. Laux & Raichle, 1978; Vorstrup, Henriksen & Paulson, 1984; Ringelstein, VanEyk & Mertens, 1992). This appreciable effect on $\dot{Q}$ is probably mediated via an increase in brain tissue P_{CO_2} (see also Vorstrup *et al.* 1984) resulting from a decrease in slope of the blood CO_2 dissociation curve (Teppema *et al.* 1995). However, at the usual clinical dose, when given at normocapnic or slightly hypocapnic P_{a,CO_2} values, acetazolamide does not increase cerebral blood flow in men (cf. Huang *et al.* 1988). As illustrated in Fig. 4, our calculations show that in the normocapnic P_{a,CO_2} range, a low dose of acetazolamide will have an effect on $\dot{Q}$ which is hard to detect, thus explaining the findings of Huang *et al.* (1988). The effect of a low dose of acetazolamide on the $\dot{Q}$–P_{a,CO_2} and $\dot{Q}$–P_{t,CO_2} relationship may be due to a direct action on cerebral vessels. There are indeed experimental data indicating that vascular carbonic anhydrase may play an important role in the control of vascular tone by endothelial cells (for references see Farcau & Farcau, 1994). We also mention that acetazolamide has been shown to induce vasoconstriction in an *in vitro* choroid plexus preparation from the rat (Macri, Politoff, Rubin, Dixon & Rall, 1966). We suggest that acetazolamide may act on a carbonic anhydrase isoenzyme located intracellularly in brain arteriolar and/or capillary endothelial cells, to which, due to its physical and chemical properties, benzolamide has no access (Holder & Hayes, 1965). These properties also deny benzolamide access to the brain (Travis *et al.* 1966; Maren, 1967).

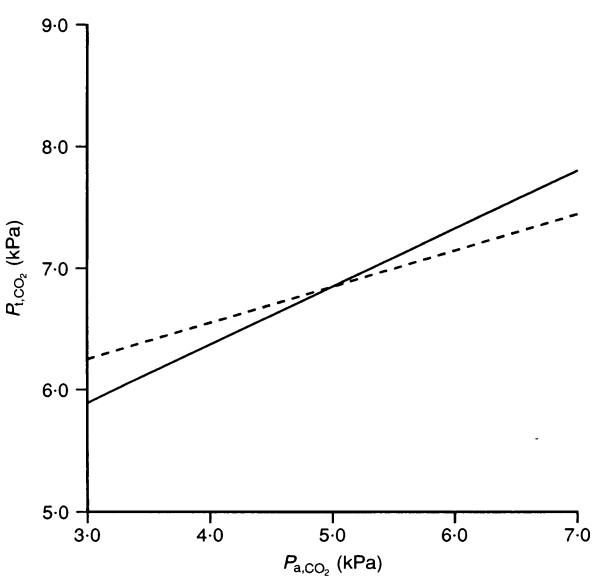

Figure 5. Relation between P_{t,CO_2} and P_{a,CO_2}
Applying the mass balance equation for CO_2 for a brain compartment yields a linear relationship between P_{t,CO_2} and P_{a,CO_2} (eqn (A3)). The continuous line represents the control situation and the dashed line that calculated after administration of 4 mg kg^{-1} acetazolamide. The difference in slope and intercept between both conditions is caused by an effect on the coupling between brain blood flow density and brain tissue P_{CO_2}.

An alternative explanation for the observed effects of acetazolamide on S_c and B might be that despite incomplete red cell inhibition the slope of the *in vivo* CO_2 dissociation curve was reduced by inhibition of a membrane-bound carbonic anhydrase at the luminal side of brain capillaries (cf. Hanson *et al.* 1981). Ridderstråle & Hanson (1985) showed that the structure of the cat brain which was most intensely stained for carbonic anhydrase is the capillary endothelium. It is reasonable to assume that this easily accessible luminal carbonic anhydrase will be inhibited by a low dose of acetazolamide. This may tend to affect the CO_2 transport capacity of the capillary blood while perfusing the brain, albeit to a much lesser degree than during complete red cell inhibition. However, under these circumstances, assuming an unchanged tissue P_{CO_2}, one would expect an increase in the contribution of carboxyhaemoglobin to total CO_2 transport (cf. Cain & Otis, 1961), thus compensating for the lesser contribution of rapidly formed bicarbonate. This would tend to restore the slope of the *in vivo* CO_2 dissociation curve of the capillary blood towards normal, thus masking a physiological effect of inhibition of the luminal carbonic anhydrase. In our opinion, the facts that after infusion of acetazolamide a $P_{(a-ET),CO_2}$ gradient was absent and that large quantities of intravenously infused carbonic anhydrase (probably sufficient to restore the normal contribution of rapidly formed bicarbonate) failed to reverse the decreases in S_c and B, indicate that this indeed might have been the case. This led us to suggest the effect of acetazolamide on the $\dot{Q}$–P_{a,CO_2} and $\dot{Q}$–P_{t,CO_2} relationships as a mechanism by which acetazolamide, at a low dose, may change slope and intercept of the CO_2 response curve. It is obvious, however, that our explanation awaits further experimental verification.

Our model predicts that if the only additional effect of a supplemental dose of about 30 mg kg^{-1} acetazolamide is to decrease the slope of the *in vivo* blood CO_2 dissociation curve to the same value as with 70 mg kg^{-1} benzolamide (Teppema *et al.* 1995), the slope of the ventilatory CO_2 response curve should decrease to 23% of the control value (see Appendix). This corresponds closely to the observed value of 20% in the four denervated animals in which the effect of 32 mg kg^{-1} acetazolamide was studied.

We cannot exclude the possibility that the effects of acetazolamide on S_c and B are mediated by an action on the central nervous system; however, for reasons mentioned above we consider this less likely. Furthermore, the decrease in S_c and B developed within 30–50 min, a period too short for this effect to be mediated centrally (see Roth *et al.* 1959). We are unaware of central actions of acetazolamide occurring independently of inhibition of carbonic anhydrase.

In carotid body-intact cats the decrease in S_c was of about the same magnitude as in denervated animals (45 *vs.* 38%) as expected. The decrease in mean B in the intact animals also did not differ significantly from that observed in the denervated animals, although the effect tended to be more pronounced in the former. Note that the numeric values for the parameters a and b after acetazolamide administration used in Fig. 4 were calculated for denervated animals only. In the intact animals these values could have been somewhat different, thus resulting in different values for B. Furthermore, in intact cats the intercept on the P_{a,CO_2} axis (B) of the CO_2 response curve is also dependent on the peripheral chemoreflex loop (DeGoede *et al.* 1981). Finally, as remarked earlier, the DEF technique is not able to separate the effect on B into a peripheral and central part but can only separate the change in ventilation following a change in end-tidal CO_2 into parts belonging to the peripheral and central chemoreflex loops (cf. DeGoede *et al.* 1981).

If the effect of acetazolamide is not mediated by a direct action on the central nervous system, the effect on the peripheral chemoreflex loop (decrease in S_p) in the intact animals is probably caused by a local action on the carotid bodies. Several studies have indeed reported a decrease in baseline carotid body activity and/or sensitivity to P_{a,CO_2} changes (e.g. Hayes, Maini & Torrance, 1976; Lahiri, Delaney & Fishman, 1976). In these studies, however, high inhibitor doses were used. We believe we have indirect evidence that carotid body output may be decreased by a dose of acetazolamide as small as 4 mg kg^{-1}. Figure 2 shows that, shortly after a bolus infusion of the drug, ventilation decreased and then underwent a secondary gradual increase. This was also found in the other cat (carotid body intact) receiving the bolus but not in the two denervated animals receiving the drug in this way. During hypoxia, when the contribution of the carotid bodies to ventilation is relatively large, we consistently observe a considerable initial decrease in ventilation (authors' unpublished observations). When larger doses are infused during hypoxia, an initial period of apnoea ensues (Teppema *et al.* 1992).

Acetazolamide may also act on respiratory muscles. The role of muscle carbonic anhydrase may be complicated, since isoenzymes have been identified at various muscular sites. We mention cytosolic CA III in muscle cells (mainly type I), cytosolic CA I and II in muscle cells as well as in capillary endothelium, and a membrane-bound form in sarcolemma, sarcoplasmatic reticulum and capillary endothelium (Geers & Gros, 1991). Scheid & Siffert (1985) showed that concentrations $>10^{-4}$ M acetazolamide were necessary to inhibit maximal isometric force of frog skeletal muscle by 50%. Barclay (1987) found that exposing mouse soleus muscle to 10^{-5} M acetazolamide for 25 min did not affect isometric tension. From these and other data no conclusions can be drawn as to a possible effect of low doses of acetazolamide on respiratory muscles *in vivo* in the anaesthetized cat. Given the low permeability of acetazolamide and the small dose that we used, we think, however, that the observed decreases in S and B are unlikely to be mediated at muscular level. Further studies are necessary to investigate this.

The present observation of a decrease in slope of the CO_2 response curve may seem to conflict with known studies in humans reporting either an increase (cf. Bashir *et al.* 1990;

Swenson & Hughes, 1993) or no change in slope, but only an upward shift of the response curve (cf. Lerche *et al.* 1960; Chiesa *et al.* 1969). Several of these studies used the Read rebreathing technique or a modification to determine CO_2 sensitivity. However, chronic use of acetazolamide (as applied in these studies) by humans usually leads to a considerable metabolic acidosis, an increase in ventilation and a substantial decrease in arterial P_{CO_2}. However, during metabolic acidosis, using conditions formulated by Read, the rebreathing technique results in a considerable overestimation of the response slope (see Linton *et al.* 1973 and Berkenbosch *et al.* 1989). In most studies in which a conventional steady-state technique was used, end-tidal P_{CO_2} was taken as an independent variable. However, in patients suffering from lung disease a relatively large $P_{(a-ET),CO_2}$ gradient may be present which, in addition, may be altered if lung carbonic anhydrase is inhibited (Swenson, Robertson & Hlastala, 1993). If sufficiently large oral doses of the drug are used to cause partial inhibition of erythrocytic carbonic anhydrase, a gradient may also be present in healthy subjects (cf. Bashir *et al.* 1990; Swenson & Hughes, 1993). One should therefore preferably use arterial P_{CO_2} as the independent variable; combined with end-tidal data it can then be judged if an unusual arterial-to-end-tidal gradient exists. It may be illustrative that in re-analysing the data of Lerche *et al.* (1960) by means of linear regression, using their arterial P_{CO_2} values (see their Table 2), we found a decrease of 30% in the slope of their CO_2 response curve by acetazolamide. Finally, Swenson & Hughes (1993) showed that chronic and acute treatments with the drug led to different effects on the CO_2 response curve. Although baseline ventilation was increased, they concluded that acute (intravenous) treatment has an inhibitory effect on the control of breathing. Obviously further studies are needed to document the effect of clinical doses of acetazolamide on the control of breathing in humans, taking into consideration these methodological problems.

In conclusion, the effects of low dose acetazolamide on S_c and B are probably due to an effect on cerebral vessels resulting in an altered relationship between cerebral blood flow and brain tissue P_{CO_2}. The effect on the peripheral chemoreflex loop may be caused by a direct action on the carotid bodies.

APPENDIX

The mass balance for CO_2 of a brain compartment in steady state can be written as (Read & Leigh, 1967; Berkenbosch *et al.* 1989):

$$P_{t,CO_2} = \frac{(1-\gamma)(\dot{M}-h)}{l\dot{Q}} + P_{a,CO_2}, \tag{A1}$$

where P_{a,CO_2} and P_{t,CO_2} denote the arterial P_{CO_2} and brain tissue P_{CO_2}, respectively. $\dot{Q}$ and $\dot{M}$ are the brain blood flow density and brain metabolism density, respectively, l the slope of the linearized blood CO_2 dissociation curve, h the Haldane parameter and γ a parameter which locates P_{t,CO_2} between P_{a,CO_2} and the cerebral venous P_{CO_2} (P_{v,CO_2}).

The cerebral blood flow density is assumed to be coupled to P_{t,CO_2} in a hyperbolic fashion (Teppema *et al.* 1995):

$$\dot{Q} = \frac{a}{b - P_{t,CO_2}}, \tag{A2}$$

with 'shape factor' a and P_{CO_2} asymptote b. Substituting eqn (A2) in eqn (A1) yields a linear relation between P_{t,CO_2} and P_{a,CO_2}:

$$P_{t,CO_2} = \frac{1}{1 + (1-\gamma)(\dot{M}-h)/al} P_{a,CO_2} + \frac{b}{1 + al/(1-\gamma)(\dot{M}-h)}. \tag{A3}$$

A linear relation between P_{t,CO_2} and P_{a,CO_2} was indeed found experimentally (Pontén & Siesjö, 1966) according to:

$$P_{t,CO_2} = \alpha P_{a,CO_2} + \beta, \tag{A4}$$

with slope α and intercept β. Using eqns (A3) and (A4) it follows that:

$$b = \frac{\beta}{1-\alpha}. \tag{A5}$$

We assume that in carotid body-denervated animals ventilation ($\dot{V}_I$) is linearly related to P_{t,CO_2} so that:

$$\dot{V}_I = S_t(P_{t,CO_2} - B_t), \tag{A6}$$

in which S_t is the CO_2 sensitivity at the site of the central chemoreceptors and B_t an offset. The ventilation as a function of the P_{a,CO_2} is:

$$\dot{V}_I = S(P_{a,CO_2} - B), \tag{A7}$$

with the slope S and the intercept B on the P_{a,CO_2} axis.

From eqns (A1), (A4) and (A6) it follows that the slope S is:

$$S = \alpha S_t = \frac{1}{1 + (1-\gamma)(\dot{M}-h)/al} S_t. \tag{A8}$$

Using eqns (A4), (A5), (A6), (A7) and (A8), the intercept on the P_{a,CO_2} axis can be written as:

$$B = \frac{B_t - b}{\alpha} + b. \tag{A9}$$

From eqn (A8) it follows that:

$$a = \frac{\alpha(1-\gamma)(\dot{M}-h)}{l(1-\alpha)}. \tag{A10}$$

In the control situation, with $\alpha = 0{\cdot}48$, $\gamma = 0{\cdot}5$, $\dot{M} = 8{\cdot}17 \times 10^{-4}$ ml^{-1} s^{-1}, $h = 1{\cdot}83 \times 10^{-4}$ and $l = 2{\cdot}4 \times 10^{-2}$ ml ml^{-1} kPa^{-1} (see Teppema *et al.* 1995), we calculate a value for a of $1{\cdot}23 \times 10^{-2}$ ml ml^{-1} s^{-1} kPa.

Since acetazolamide crosses the blood–brain barrier only very slowly we assume that under the present experimental conditions the parameters γ, $\dot{M}$, h, and S_t remained constant after administration of the drug. Since infusion of 4 mg kg^{-1} was not followed by a $P_{(a-ET),CO_2}$ gradient and since the decreases in S and B were not affected by a

subsequent intravenous infusion of carbonic anhydrase, we conclude that parameter l also remained constant. In this way the change in S by acetazolamide can be entirely attributed to a change in a (cf. eqn (A8)), i.e. to a change in the shape of the hyperbola relating $\dot{Q}$ to P_{t,CO_2} (eqn (A2)). Introducing the subscripts 'd' for the parameters after drug administration and 'n' for the control situation, the ratio a_d/a_n can be written as:

$$\frac{a_d}{a_n} = \frac{(S_d/S_n)(1-\alpha_n)}{1-(S_d/S_n)\alpha_n}, \quad \text{(A11)}$$

where we have used the relation:

$$\alpha_d = S_d\frac{\alpha_n}{S_n}. \quad \text{(A12)}$$

In the carotid body-denervated cats we found for the ratio S_d/S_n a value of 0·63. Taking a value of $1{\cdot}23 \times 10^{-2}$ ml^{-1} s^{-1} kPa for a_n (see above) it can now be calculated that $a_d = 0{\cdot}0057$ ml ml^{-1} s^{-1} kPa. It also follows from eqn (A8) that if $\alpha_n = 0{\cdot}48$, the value of α_d should be 0·30. Using eqns (A5) and (A9), taking a value of 4·45 for β_n (see Teppema *et al.* 1995) and assuming that B_t remained constant, it can be calculated that the observed change in the intercept on the P_{a,CO_2} axis (B) from 4·5 to 4·2 kPa can be explained by a shift in the value of b (i.e. the asymptote of the $\dot{Q}$–P_{t,CO_2} relation) from 8·6 to 7·6 kPa, together with the change in α from 0·48 to 0·30. It is thus possible to attribute the effect of 4 mg kg^{-1} acetazolamide on the CO_2 response curve entirely to an effect of this agent on cerebral vessels. Since acetazolamide is relatively excluded from the brain (Roth *et al.* 1959; Maren, 1967), even an additional dose of about 30 mg kg^{-1} will not result in effective inhibition of CNS carbonic anhydrase within a time span as short as 30 min (see also Hanson *et al.* 1981). We thus assume that the only additional short-term effect of this dose will consist of a decrease in the slope l of the *in vivo* blood CO_2 dissociation curve, due to inhibition of erythrocytic carbonic anhydrase. In a previous study (Teppema *et al.* 1995), we have calculated that complete inhibition of the red cell enzyme leads to a decrease in l from $2{\cdot}4 \times 10^{-2}$ to $6{\cdot}9 \times 10^{-3}$ ml ml^{-1} kPa^{-1}. Using eqn (A8) and taking a value for a of 0·0057 ml ml^{-1} s^{-1} kPa already existing after 4 mg kg^{-1} acetazolamide (see above), we calculate that infusion of this large dose of the drug should result in a 77% decrease in slope of the CO_2 response curve. This corresponds closely to the observed mean decrease of 80% in slope in the four carotid body-denervated animals tested.

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