The effect of low-dose acetazolamide on the ventilatory CO₂ 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 Pa,CO₂ axis (B) of the ventilatory CO₂ 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 Pₐ,CO₂ (P(a)-ET,CO₂) 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 1.78 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,CO₂ gradient.

3. In carotid body-denervated cats, 4 mg kg⁻¹ acetazolamide caused a decrease in the CO₂ sensitivity of the central chemoreflex loop (Sₚ) from 1.52 ± 0.42 to 0.96 ± 0.32 l min⁻¹ kPa⁻¹ (mean ± s.d.) while the intercept on the Pa,CO₂ 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 CO₂ sensitivity of the peripheral chemoreflex loop (Sₚ) from 0.28 ± 0.18 to 0.19 ± 0.12 l min⁻¹ kPa⁻¹. Sₚ and B decreased from 1.52 ± 0.65 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 CO₂ 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 Sₚ 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 Pₐ,CO₂.

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 Pₐ,CO₂ (determined from in vitro samples) and end-tidal Pₐ,CO₂, and an increase in ventilation (e.g. Carter & Clark, 1968; 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. Leeche, Katsaros, Leeche & Loescheke, 1960; Chiesa, Stradling & Stradling, 1990; Bashir, Kann & Stradling, 1990; Swenson & Hughes, 1963).

Several authors have studied the effect of acetazolamide on the ventilatory response curve to CO₂ 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 CO₂ response slope (Linton, Poole-Wilson, Davies & Cameron, 1993).
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 of acetazolamide (4 mg kg$^{-1}$) not causing complete inhibition of erythrocytic carbonic anhydrase, indicated by the absence of an arterial-to-end-tidal $P_{CO_2}$ ($P_{a-Co_2}$-ambient$P_{CO_2}$) 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$^{-1}$ 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$^{-1}$ 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 into a possible mechanism of action of this drug on the central chemoreflex loop.

The parameters $S_0$ and $S_1$ are the central and peripheral ventilatory $CO_2$ sensitivities and the offset $B$ represents the apnoeic threshold or extrapolated $P_{a-Co_2}$ of the steady-state ventilatory response to $CO_2$ at zero ventilation.

**Data analysis**

The steady-state relation of ventilation ($V_t$) to $P_{ET-Co_2}$ at constant $P_{ET-o_2}$ is linear to the $P_{ET-Co_2}$-axis and well described by DeGoede, Berkenbosch, Olievier & Quanjer (1981):

$$V_t = (S_0 + S_1)(P_{ET-Co_2} - B).$$  

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

\[ \tau C \frac{dV_C}{dt} + \dot{V}_C = S_c(P_{\text{ET},CO_2}(l - T_c) - B_C), \]  (2)

\[ \tau P \frac{dV_P}{dt} + \dot{V}_P = S_p(P_{\text{ET},CO_2}(l - T_p) - B_P), \]  (3)

\[ \tau_v = v_0 \times (1 - x) + \tau_{o, v}, \]  (4)

\[ \dot{V}_i = \dot{V}_C + \dot{V}_P + C t. \]  (5)

In the equations, \( \dot{V}_i \) and \( \dot{V}_p \) denote the contributions of the central and peripheral chemoreceptors to the ventilation \( V_i \). \( B_C \) 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 \( \tau_v \) and \( \tau_{p} \). \( T_c \) 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, \( \tau_v \) is written according to eqn (4) in which \( x = 1 \) when \( P_{\text{ET},CO_2} \) is high and \( x = 0 \) when \( P_{\text{ET},CO_2} \) is low. In some experiments a small drift in ventilation was present. Therefore we included a drift term \( C t \) 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. \( B_C = B_P = B \) (Berkensbosch, DeGoede, Olievier & Ward, 1986). This offset \( B \) is then equal to the extrapolated \( P_{\text{ET},CO_2} \) 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 \( P_{\text{ET},CO_2} \) 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 \( T_c \geq T_p \) 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 \( \tau_p \) 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 \( S_c \) and \( \tau_p \) 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 change in the arterial-to-end-tidal \( P_{CO_2} \) 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 change in the arterial-to-end-tidal \( P_{CO_2} \) gradient was
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 $S_p$, $S_c$ and $B$ were highly significant. A small but significant effect on $T_c$ and $\tau_{off}$ 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, $P = 0.06$). The same was true for the effect of acetazolamide on mean $S_c$ in both groups ($P = 0.5$).

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

![Figure 2](image-url)

**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 $V_t$. 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. $P_{CO_2}$ 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

<table>
<thead>
<tr>
<th></th>
<th>(S_p) (l min(^{-1}) kPa(^{-1}))</th>
<th>(S_c) (l min(^{-1}) kPa(^{-1}))</th>
<th>(B) (kPa)</th>
<th>(P_{\text{ET}, CO_2}) (kPa)</th>
<th>Standard bicarbonate (mmol l(^{-1}))</th>
<th>(\tau_p) (s)</th>
<th>(\tau_c) (s)</th>
<th>(\tau_{on}) (s)</th>
<th>(\tau_{off}) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.52 ± 0.55</td>
<td>0.28 ± 0.18</td>
<td>4.9 ± 0.5</td>
<td>0.94 ± 0.31</td>
<td>20.6 ± 0.9</td>
<td>5 ± 2</td>
<td>4 ± 3</td>
<td>8 ± 3</td>
<td>89 ± 11</td>
</tr>
<tr>
<td>Acet.</td>
<td>0.84 ± 0.21</td>
<td>0.19 ± 0.12</td>
<td>3.0 ± 0.6</td>
<td>0.17 ± 0.56</td>
<td>18.8 ± 0.6</td>
<td>5 ± 3</td>
<td>6 ± 4</td>
<td>10 ± 4</td>
<td>107 ± 28</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.045</td>
<td>0.0003</td>
<td>0.31</td>
<td>0.11</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(S_p\) and \(S_c\) are the CO\(_2\) sensitivities of the peripheral and central chemoreflex loops, with delay times \(\tau_p\) and \(\tau_c\). \(\tau_p\) is the peripheral time constant and \(\tau_{on}\) and \(\tau_{off}\) are the central on- and off-transient time constants. \(B\) is the intercept on the \(P_{\text{ET}, CO_2}\) axis of the CO\(_2\) ventilatory response curve. Acet., acetazolamide. 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 \(S_p\) and \(B\) obtained after 4 mg kg\(^{-1}\) acetazolamide. From data reported by Travis et al. (1965) 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 \(V_e\)-\(P_{\text{ET}, CO_2}\) 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.

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**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 \(P_{\text{ET}, CO_2}\). 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 \(P_{\text{ET}, CO_2}\) data as input. The model fit is the sum of the central component \(V_e\), the peripheral component \(V_p\) and the drift (not shown separately). The values in the control run of the intercept on the \(P_{\text{ET}, CO_2}\) axis (\(B\)), the sensitivity of the central and peripheral chemoreflex loops (\(S_c\) and \(S_p\)) are 4.21 kPa and 1.22 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

<table>
<thead>
<tr>
<th></th>
<th>( S_0 )</th>
<th>( B )</th>
<th>( P_{\text{ET}, \text{CO}_2} )</th>
<th>Standard bicarbonate</th>
<th>( \tau_c )</th>
<th>( \tau_{on} )</th>
<th>( \tau_{off} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1·52 ± 0·42</td>
<td>4·5 ± 0·5</td>
<td>0·92 ± 0·30</td>
<td>19·0 ± 2·1</td>
<td>4 ± 1</td>
<td>85 ± 26</td>
<td>132 ± 31</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>0·96 ± 0·32</td>
<td>4·2 ± 0·7</td>
<td>0·92 ± 0·18</td>
<td>18·2 ± 2·5</td>
<td>6 ± 3</td>
<td>116 ± 83</td>
<td>105 ± 21</td>
</tr>
<tr>
<td>( P )</td>
<td>0·0001</td>
<td>0·0021</td>
<td>0·60</td>
<td>0·18</td>
<td>0·06</td>
<td>0·03</td>
<td>0·005</td>
</tr>
</tbody>
</table>

\( S_0 \) is the sensitivity of the central chemoreflex loop, with time constants  \( \tau_{on} \) and  \( \tau_{off} \) and delay time  \( \tau_c \).  \( B \) is the intercept on the  \( P_{\text{CO}_2} \) axis of the ventilatory CO₂ 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 & Olivier, 1995).

DISCUSSION

This study shows that acetazolamide, at doses of 4 mg kg⁻¹ and below, did not induce a significant  \( P_{\text{ET}, \text{CO}_2} \) 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  \( P_{\text{ET}, \text{CO}_2} \) 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.

For the interpretation of these results we start with the denervated cats in which we consider ventilation a function of brain tissue  \( P_{\text{CO}_2} \) (Maren, 1967). We found that the maximal inhibition of the drug, if evenly distributed, would yield a brain concentration of 1·8 x 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 (Q) as a function of arterial or brain tissue P_C02](image)

Cerebral blood flow density (Q) is calculated from the hyperbolic relation:

\[
Q = \frac{a}{b - P_{\text{CO}_2}},
\]

in which either  \( P_{\text{ET}, \text{CO}_2} \) (left panel) or  \( P_{\text{CO}_2} \) (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 \(S\) 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 \(P_{\text{CO}_2}\) and arterial \(P_{\text{a, CO}_2}\). To express this relationship we have previously used a steady-state mass balance equation for \(\text{CO}_2\) 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 \(P_{\text{TCO}_2}\) and \(P_{\text{a, CO}_2}\) (see also Fig. 5). The slope and intercept of this relationship depend, among other factors, on brain metabolism, on the slope of the blood \(\text{CO}_2\) dissociation curve and on the relationship between cerebral blood flow density \((\dot{Q})\) and \(P_{\text{a, CO}_2}\) (see eqn (A3)). As reasoned above brain metabolism will not be changed. Apparently the slope of the blood \(\text{CO}_2\) dissociation curve also remained constant, since the dose of 4 mg kg\(^{-1}\) did not result in a \(P_{\text{a, CO}_2}\) gradient and since subsequent intravenous infusion of carbonic anhydrase did not further influence the effects of acetazolamide on the \(\text{CO}_2\) 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 \(P_{\text{a, CO}_2}\), resulting in a change in the \(P_{\text{TCO}_2} - P_{\text{a, CO}_2}\) relationship.

Figure 4 shows the relationship between brain blood flow density and arterial and brain tissue \(P_{\text{CO}_2}\); the dashed curves show the calculated course of both hyperbolas after acetazolamide infusion if the effects of the agent on the \(\text{CO}_2\) response curve were entirely due to an action on cerebral vessels. We calculate from the slope ratio of the \(\text{CO}_2\) dissociation curve \(a\), 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 \(P_{1, \text{CO}_2} - P_{\text{a, CO}_2}\) 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 \(P_{\text{a, CO}_2}\) 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 & Raisbehl, 1978; Vorstrup, Henriksen & Paulson, 1984; Ringelstein, VanBjyk & Mertens, 1992). This appreciable effect on \(\dot{Q}\) is probably mediated via an increase in brain tissue \(P_{\text{CO}_2}\) (see also Vorstrup et al. 1984) resulting from a decrease in slope of the blood \(\text{CO}_2\) dissociation curve (Teppema et al. 1995). However, at the usual clinical dose, when given at normocapnic or slightly hypocapnic \(P_{\text{a, CO}_2}\) 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 \(P_{\text{a, CO}_2}\) 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 \(\dot{Q} - P_{\text{a, CO}_2}\) and \(\dot{Q} - P_{1, \text{CO}_2}\) 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 Parcau & Parcau, 1994). We also mention that acetazolamide has been shown to induce vasoconstriction in an in vitro choroid plexus preparation from the rat (Maeri, Politoff, Rubin, Dixon & Rall, 1986). 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. 1986; Maren, 1967).

**Figure 5. Relation between \(P_{1, \text{CO}_2}\) and \(P_{\text{a, CO}_2}\)**

Applying the mass balance equation for \(\text{CO}_2\) for a brain compartment yields a linear relationship between \(P_{1, \text{CO}_2}\) and \(P_{\text{a, CO}_2}\) (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 \(P_{\text{CO}_2}\).
An alternative explanation for the observed effects of acetazolamide on \( S_i \) and \( B \) might be that despite incomplete red cell inhibition the slope of the \( \text{in vivo} \) \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( P_{\text{CO}_2} \) one would expect an increase in the contribution of carboxyhaemoglobin to total \( \text{CO}_2 \) transport (cf. Cain & Otis, 1961), thus compensating for the lesser contribution of rapidly formed bicarbonate. This would tend to restore the slope of the \( \text{in vivo} \) \( \text{CO}_2 \) 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 \( P_{\text{in-vivo} \text{CO}_2} \) 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 \( S_i \) and \( B \), indicate that this indeed might have been the case. This led us to suggest the effect of acetazolamide on the \( Q-P_{\text{e,CO}_2} \) and \( Q-P_{\text{a,CO}_2} \) relationships as a mechanism by which acetazolamide, at a low dose, may change slope and intercept of the \( \text{CO}_2 \) 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 \( \text{in vivo} \) blood \( \text{CO}_2 \) dissociation curve to the same value as with 70 mg kg\(^{-1}\) benzolamide (Teppema et al. 1985), the slope of the ventilatory \( \text{CO}_2 \) 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 \( S_i \) 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 \( S_i \) and \( B \) developed within 80–50 min, a period too short for this effect to be mediated centrally (see Roth et al. 1950). We are unaware of central actions of acetazolamide occurring independently of inhibition of carbonic anhydrase.

In carotid body-intact cats the decrease in \( S_i \) 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 \( P_{\text{e},\text{CO}_2} \) axis (\( B \)) of the \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( S_i \)) 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 \( P_{\text{e},\text{CO}_2} \) 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 sarcomlemma, 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%. Barley (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 \( \text{in vivo} \) 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 \( \text{CO}_2 \) response curve may seem to conflict with known studies in humans reporting either an increase (cf. Bashir et al. 1990;
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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 CO₂ 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 PₐₜCO₂. 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 PₐₜCO₂ was taken as an independent variable. However, in patients suffering from lung disease a relatively large P₀ₕ-ETCO₂ gradient may be present which, in addition, may be altered if lung carbonic anhydrase is inhibited (Swenson, Robertson & Hlasta, 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. Basbör et al. 1990; Swenson & Hughes, 1993). One should therefore preferably use arterial PₐₜCO₂ 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 PₐₜCO₂ values (see their Table 2), we found a decrease of 30% in the slope of their CO₂ response curve by acetazolamide. Finally, Swenson & Hughes (1993) showed that chronic and acute treatments with the drug led to different effects on the CO₂ 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 Sₛ and B are probably due to an effect on cerebral vessels resulting in an altered relationship between cerebral blood flow and brain tissue PₐₜCO₂. The effect on the peripheral chemoreflex loop may be caused by a direct action on the carotid bodies.

APPENDIX

The mass balance for CO₂ of a brain compartment in steady state can be written as (Read & Leigh, 1967; Berkenbosch et al. 1989):

$$P_{t,CO_2} = \frac{(1 - \gamma)(M - h)}{hQ} + P_{a,CO_2}$$  \hspace{1cm} (A1)

where $P_{a,CO_2}$ and $P_{t,CO_2}$ denote the arterial $P_{CO_2}$ and brain tissue $P_{CO_2}$ respectively. $Q$ and $M$ are the brain blood flow density and brain metabolism density respectively, $l$ the slope of the linearized blood CO₂ dissociation curve, $h$ the Haldane parameter and $\gamma$ 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):

$$Q = \frac{a}{b - P_{t,CO_2}}$$  \hspace{1cm} (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 $P_{a,CO_2}$:

$$P_{t,CO_2} = \frac{1}{l + (1 - \gamma)(M - h)/\alpha} P_{a,CO_2}$$

$$- \frac{b}{1 + \alpha/(1 - \gamma)(M - h)}$$  \hspace{1cm} (A3)

A linear relation between $P_{t,CO_2}$ and $P_{a,CO_2}$ was indeed found experimentally (Pontén & Siesjö, 1960) according to:

$$P_{t,CO_2} = aP_{a,CO_2} + \beta$$  \hspace{1cm} (A4)

with slope $a$ and intercept $\beta$. Using eqns (A3) and (A4) it follows that:

$$\frac{b}{1 - a}$$  \hspace{1cm} (A5)

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

$$V_i = S(P_{t,CO_2} - B_i)$$  \hspace{1cm} (A6)

in which $S_i$ is the CO₂ sensitivity at the site of the central chemoreceptors and $B_i$ an offset. The ventilation as a function of the $P_{a,CO_2}$ is:

$$V_i = S(P_{a,CO_2} - B)$$  \hspace{1cm} (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:

$$S = aS_i = \frac{1}{1 + (1 - \gamma)(M - h)/\alpha} S_i$$  \hspace{1cm} (A8)

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

$$\beta = \frac{B - \beta}{a} + b$$  \hspace{1cm} (A9)

From eqn (A8) it follows that:

$$a = \frac{\alpha(1 - \gamma)(M - h)}{l(1 - a)}$$  \hspace{1cm} (A10)

In the control situation, with $\alpha = 0.48$, $\gamma = 0.5$, $M = 8.17 \times 10^{-4}$ ml m⁻¹ s⁻¹, $h = 1.83 \times 10^{-4}$ and $l = 2.4 \times 10^{-2}$ ml m⁻¹ kPa⁻¹ (see Teppema et al. 1995), we calculate a value for $\alpha$ of $1.23 \times 10^{-4}$ ml m⁻¹ s⁻¹ kPa⁻¹.

Since acetazolamide crosses the blood–brain barrier only very slowly we assume that under the present experimental conditions the parameters $\gamma$, $M$, $h$, and $S_i$ remained constant after administration of the drug. Since infusion of 4 mg kg⁻¹ was not followed by a P₀ₕ-ETCO₂ 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 \( Q \) to \( P_{\text{ACO}_2} \) (eqn (A2)). Introducing the subscripts ’d’ for the parameters after drug administration and ’i’ for the control situation, the ratio \( \sigma_d/\sigma_i \) can be written as:

\[
\frac{\sigma_d}{\sigma_i} = \frac{(S_d/S_i)(1 - \alpha_i)}{1 - (S_d/S_i)\alpha_i},
\]

where we have used the relation:

\[
\alpha_i = S_i \frac{\sigma_i}{\bar{S}_i}.
\]

In the carotid body-denervated cats we found for the ratio \( S_d/S_i \) a value of 0.63. Taking a value of \( 1.23 \times 10^{-2} \text{ ml}^{-1} \text{s}^{-1} \text{kPa} \) for \( \sigma_i \) (see above) it can now be calculated that \( \sigma_d = 0.0057 \text{ ml}^{-1} \text{s}^{-1} \text{kPa} \). It also follows from eqn (A8) that if \( \alpha = 0.48 \), the value of \( \sigma_d \) should be 0.30. Using eqns (A5) and (A9), taking a value of 4.45 for \( \beta_n \) (see Teppema et al. 1995) and assuming that \( B \) remained constant, it can be calculated that the observed change in the intercept on the \( P_{\text{ACO}_2} \) 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 \( Q-B_{\text{ACO}_2} \) relation) from 8.6 to 7.5 kPa, together with the change in \( \alpha \) from 0.48 to 0.30. It is thus possible to attribute the effect of 4 mg kg\(^{-1}\) acetazolamide on the \( Q \) 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 Hansson 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 \( \text{CO}_2 \) 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 \( 2.4 \times 10^{-2} \) to \( 6.9 \times 10^{-3} \text{ ml}^{-1} \text{s}^{-1} \text{kPa}^{-1} \). 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 this infusion of that large dose of the drug should result in a 77% decrease in slope of the \( Q \) 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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