Effect of low-dose acetazolamide on the ventilatory CO\textsubscript{2} response during hypoxia in the anaesthetized cat

M. Wagenaar\*, L. Teppema\*, A. Berkenbosch\*, C. Olievier\*, H. Folgering**


ABSTRACT: Acetazolamide, a carbonic anhydrase inhibitor, is used in patients with chronic obstructive pulmonary disease and central sleep apnoea syndrome in the prevention and treatment of the symptoms of acute mountain sickness. In these patients, the drug increases minute ventilation (V'E), resulting in an improvement in arterial oxygen saturation. However, the mechanism by which it stimulates ventilation is still under debate.

Since hypoxaemia is a frequently observed phenomenon in these patients, the effect of 4 mg·kg\textsuperscript{-1} acetazolamide (i.v.) on the ventilatory response to hypercapnia during hypoxaemia (arterial oxygen tension (P\textsubscript{a,O2})=6.8±0.8 kPa, mean±SD) was investigated in seven anaesthetized cats. The dynamic end-tidal forcing (DEF) technique was used, enabling the relative contributions of the peripheral and central chemoreflex loops to the ventilatory response to a step change in end-tidal carbon dioxide tension, (P\textsubscript{ET,CO2}) to be separated.

Acetazolamide reduced the CO\textsubscript{2} sensitivities of the peripheral (Sp) and central (Sc) chemoreflex loops from 0.22±0.08 to 0.11±0.03 L·min\textsuperscript{-1}·kPa\textsuperscript{-1} (mean±SD) (p<0.01) and from 0.74±0.32 to 0.40±0.10 L·min\textsuperscript{-1}·kPa\textsuperscript{-1} (p<0.01), respectively. The apnoeic threshold B (x-intercept of the ventilatory CO\textsubscript{2} response curve) decreased from 2.88±0.97 to 0.95±0.92 kPa (p<0.01). The net result was a stimulation of ventilation at P\textsubscript{ET,CO2}<5 kPa.

The effect of acetazolamide is possibly due to a direct effect on the peripheral chemoreceptors as well as to an effect on the cerebral blood flow regulation. Possible clinical implications of these results are discussed.


The carbonic anhydrase inhibitor acetazolamide stimulates ventilation, resulting in an improvement in arterial oxygen tension (P\textsubscript{a,O2}) in patients with chronic obstructive pulmonary disease (COPD) or central sleep apnoea syndrome and in those suffering from acute mountain sickness [1–10]. The ventilatory effect with the drug is believed to be mediated by a metabolic acidosis, induced by inhibition of renal carbonic anhydrase [11–14]. However, other local effects of acetazolamide could also contribute to the observed ventilatory effects, since carbonic anhydrase is present in several tissues of the pathways involved in the control of breathing. For example, the enzyme is present in the peripheral and possibly also the central chemoreceptors [15–18], erythrocytes [16] and muscles [19] and in lung as well as brain capillary endothelium [20–22]. Usually, acetazolamide is administered in doses which do not completely inhibit red cell carbonic anhydrase. Complete inhibition of erythrocytic carbonic anhydrase occurs at a fractional inhibition >99.8%, for which doses >10 mg·kg\textsuperscript{-1} acetazolamide are required [16, 23]. In COPD patients, this situation would result in impeded washout of CO\textsubscript{2} from the lungs, leading to CO\textsubscript{2} accumulation in the tissues. Such an undesired complication can be avoided by administering small doses, preventing an increase in the arterial to end-tidal carbon dioxide tension (P\textsubscript{a-ET,CO2}) gradient.

In a previous study in anaesthetized cats, it was found that doses of up to 4 mg·kg\textsuperscript{-1} acetazolamide (i.v.) did not cause a P\textsubscript{a-ET,CO2} gradient [24]. In the same study the effect of 4 mg·kg\textsuperscript{-1} acetazolamide on the ventilatory response to CO\textsubscript{2} during normoxaemia was also investigated: utilizing the technique of dynamic end-tidal forcing (DEF) [25], decreases in the CO\textsubscript{2} sensitivities of the peripheral (Sp) and central (Sc) chemoreflex loops and in the apnoeic threshold (extrapolated carbon dioxide tension (P\textsubscript{CO2}) at zero ventilation) were found. These effects were attributed to a possible direct action of acetazolamide on the peripheral chemoreceptors and to a change in the relation between brain tissue P\textsubscript{CO2} (P\textsubscript{br,CO2}) and arterial P\textsubscript{CO2} (P\textsubscript{a,CO2}), due to a possible effect of the drug on cerebral blood flow regulation.

Since this previous study was performed during normoxia, its results may not be directly relevant to a situation of hypoxaemia, such as frequently occurs in patients with COPD.

During hypoxaemia, both cerebral blood flow and the relative contribution of the peripheral chemoreceptors to total ventilation are different from that in normoxaemia. Therefore, the aim of this study, in anaesthetized cats, was
to investigate the acute ventilatory effects of 4 mg·kg⁻¹ acetzolamide on the peripheral and central chemoreflex loops on a background of moderate hypoxaemia (PaO₂ ~6.8 kPa).

Owing to different pharmacokinetics, the ventilatory effect of oral acetazolamide in patients with CO₂ retention may differ from that after an acute i.v. infusion of a low dose, as performed in the present study. However, both situations are the same to the extent that the effects of the drug will be mediated independently of erythrocytic carbonic anhydrase inhibition, since in both cases the red cell enzyme will not be inhibited effectively. So, despite different pharmacokinetics to those after chronic oral administration, it was decided to study the effect of a low dose of acetazolamide in an acute animal preparation which was made moderately hypoxaemic, a condition which frequently occurs in the clinical situations in which the drug is used.

Materials and methods

Animals, surgery and measurements

Seven adult cats (body weight 4.0–5.6 kg) were premedicated with 15 mg·kg⁻¹ ketamine hydrochloride (i.m.) and atropine sulphate (0.5 mg s.c.). Anaesthesia was induced via inhalation of a gas mixture containing 0.5–1% halothane and 30% O₂ in N₂. After cannulation of the femoral veins and arteries, an initial dose of 20 mg·kg⁻¹ α-chloralose and 100 mg·kg⁻¹ urethane was slowly infused i.v. and the addition of halothane to the inspirate was discontinued. Anaesthesia was maintained with a continuous infusion of 1–1.5 mg·kg⁻¹·h⁻¹ α-chloralose and 5.0–7.5 mg·kg⁻¹·h⁻¹ urethane. This anaesthetic regimen provides a constant level of ventilatory control [26]. Rectal temperature was monitored with a thermistor, kept within 1°C in each cat and ranged from 36.3–38.2°C among the animals. The trachea was cannulated and connected to a respiratory circuit. One femoral artery and vein were connected to an extracorporeal circuit (ECC) (flow 6 mL·min⁻¹) for continuous blood gas measurement. The ventilatory responses to CO₂ were studied before and 1 h after i.v. administration of 4 mg·kg⁻¹ acetazolamide, using the DEF technique (see below). The end tidal PCO₂ (PET,CO₂) was forced stepwise, while the end-tidal oxygen tension (PET,O₂) was kept constant. This was achieved by manipulating the inspired CO₂ and O₂ concentrations by feedback control with a computer. Respiratory airflow was measured with a Fleisch No 0 flow transducer (Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Statham PM197; Statham, Los Angeles, CA, USA) and was electronically integrated to yield tidal volume. The composition of the inspirate was regulated by computer-controlled mass-flow controllers (type AFC 260; Advanced Semiconductor Materials, De Bilt, The Netherlands), using pure O₂, CO₂ and N₂. The CO₂ and O₂ concentrations in the tracheal gas were continuously measured with an infrared analyser (MK2 Capnograph; Gould Godard, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger O₂-test; Jaeger, Würzburg, Germany), respectively.

Arterial pH, Pa,CO₂ and Pa,O₂ in the blood passing through the ECC, were measured continuously with a pH electrode (Radiometer E-5037-0; Radiometer, Copenhagen, Denmark), calibrated with phosphate buffers, a PCO₂ electrode (General Electric A312AB; General Electric, Milwaukee, WI, USA) and a home-made Clark-type oxygen tension (PO₂) electrode. The PCO₂ and PO₂ electrodes were calibrated with water equilibrated with CO₂/O₂/N₂ gas mixtures delivered by a gas-mixing pump (Wösthoff, Bochum, Germany). The PO₂ electrode was recalibrated approximately every 2 h and corrections were made for drift, when necessary. Arterial blood pressure was measured using a pressure transducer (Statham 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 disk. Values for ventilation, tidal volume, respiratory frequency, arterial blood pressure, end-tidal and arterial blood gas tensions (PET,CO₂, PET,O₂, Pa,CO₂ and Pa,O₂) were stored on a breath-by-breath basis.

Experimental protocol and data analysis

Each DEF run was started after a steady-state period of ventilation of about 2 min. Next, the PET,CO₂ was elevated by about 1–1.5 kPa within one or two breaths, maintained constant for a period of 6–7 min and then lowered stepwise to the previous value and kept constant for a further 6–7 min (fig. 1). The Pa,O₂ was kept constant at 6.8±0.8 kPa throughout all runs. In each cat, three to five control DEF runs were performed. After the control runs, all cats received an i.v. injection of 4 mg·kg⁻¹ acetazolamide (Dia- mox; AHP Pharma, Hoofddorp, The Netherlands), dissolved in saline (2 mg·ml⁻¹). PET,CO₂ and PET,O₂ were kept constant during infusion. Sixty minutes after infusion, another three to five DEF runs (acetazolamide runs) were performed.

For the analysis of the breath-to-breath data obtained in the DEF runs, a two-compartment model was used [25]:

\[
\tau_c \frac{d}{dt} V_c(t) + V_c = Sc[PET,CO_2(t-t_c)-B] \tag{1}
\]

\[
\tau_p \frac{d}{dt} V_p(t) + V_p = Sp[PET,CO_2(t-t_p)-B] \tag{2}
\]

\[
\tau = \text{ton} x + (1-x) \text{toff} \tag{3}
\]

\[
V(t) = V_c(t) + V_p(t) + Ct. \tag{4}
\]

Equation 1 [11] describes the ventilatory dynamics of the slow central chemoreflex loop, with the contribution of the central chemoreceptors to the ventilation (V_c). Sc, time constant (\(\tau_c\)) and transport delay time (t_c) of the CO₂ change from lungs to central chemoreceptors, where t represents time; similarly, Equation 2 describes the ventilatory dynamics of the fast peripheral chemoreflex loop, with the contribution to the ventilation (V_p), Sp, time constant (\(\tau_p\)) and delay time (t_p). The offset B (Equations 1 and 2) represents the apnoeic threshold, i.e. the extrapolated ventilatory response to PET,CO₂ at zero ventilation. Equation 3 was used to model the difference in the central time constant of the on-transient \(\text{ton} versus\) the off-transient \(\text{toff}\). When PET,CO₂ is raised (on-transient) we use
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In some experiments a small drift in the ventilation ($V_I$) was present. Therefore, a drift term $Ct$ was included (Equation 4). The parameters of the model were estimated by fitting the model to the data with a least squares method. A grid search was performed to obtain optimal time delays. All combinations of ($t_c, t_p$) (increments of 1 s and $t_c - t_p$) were tried until a minimum in the residual sum of squares was found. The minimal and maximal time delays were, somewhat arbitrarily, chosen to be 1 s and 15 s, respectively, and $\tau_p$ was constrained to be at least 0.3 s.

Statistical analysis

To compare the means of 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 (ANOVA) was performed, using a fixed model. The level of significance was set at $p=0.05$. Results are given as mean of the means±SD.

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

Results

After an initial transient decrease in minute ventilation all cats responded, with a slow increase in ventilation, to an i.v. infusion of 4 mg·kg$^{-1}$ acetazolamide at the prevailing $P_{ET,CO_2}$ level. An example is shown in figure 2.

Thirty-one DEF runs were performed during the control situation and 31 runs after infusion of 4 mg·kg$^{-1}$ acetazolamide. Two examples of DEF runs in the same cat are shown in figure 1, together with the computer analysis: one run before and one after administration of the drug. This figure illustrates that $Sc$ and $Sp$ were decreased after acetazolamide infusion. The effects of acetazolamide on $Sc$ and $Sp$ and on the apnoeic threshold of all individual cats are shown in the scatter diagrams of figure 3. As shown, in all individual cats, each parameter decreased after infusion of 4 mg·kg$^{-1}$ acetazolamide. Table 1 summarizes all of the parameters obtained by the analysis of the DEF responses before and after infusion of 4 mg·kg$^{-1}$ acetazolamide as well as the effect on standard bicarbonate ($P_{CO_2}=5.32$ kPa, pH=7.4) and on the $P_{o_{2}}-ET,CO_2$ difference.

The apnoeic threshold $B$ diminished significantly, by about 2 kPa (from 2.88±0.97 to 0.95±0.92 kPa). $Sp$ and $Se$ decreased significantly to about half their control values (from 0.22±0.08 to 0.11±0.03 L·min$^{-1}$·kPa$^{-1}$ and from 0.74±0.32 to 0.40±0.10 L·min$^{-1}$·kPa$^{-1}$ respectively).

Of all remaining DEF parameters, only $tp$ and $\tau_on$ changed significantly. A small, but significant, increase in the $P_{o_{2}}-ET,CO_2$ gradient and a slight decrease in standard bicarbonate were observed.

Fig. 2. – Acute effect of an i.v. infusion of 4 mg·kg$^{-1}$ acetazolamide in a hypoxaemic cat (arrow). An initial decrease in ventilation ($V_I$), when a constant end-tidal $P_{CO_2}$ was maintained, was followed by a slow secondary increase. $P_{o_{2}}$: arterial oxygen tension; $P_{CO_2}$: carbon dioxide tension in the respiratory air; $P_{o_{2}}$: arterial carbon dioxide tension.
As shown in figure 4, the decrease in total CO₂ sensitivity (Sp+Sc) combined with a diminished apnoeic threshold imply that the ventilatory response curves to CO₂ intersect at a P<sub>ET,CO₂</sub> of about 5 kPa. At a P<sub>ET,CO₂</sub> below this value acetazolamide stimulates ventilation during moderate hypoxaemia in anaesthetized cats.

Discussion

In this study the effects of acetazolamide on the ventilatory CO₂ response curve were investigated in hypoxaeemic cats. A dose of 4 mg·kg<sup>-1</sup> caused an increase in the P<sub>a–ET,CO₂</sub> difference as small as 0.14 kPa, indicating marginal inhibition of erythrocytic carbonic anhydrase [11, 25]. Based on this finding it was concluded that the effects of acetazolamide could be studied without the complication of significant tissue CO₂ retention.

The main results of this study are that, in hypoxaemic cats, 4 mg·kg<sup>-1</sup> acetazolamide causes a decrease in both the Sp and Sc and a decrease in the value of the apnoeic threshold B, resulting in a ventilatory stimulation at P<sub>ET,CO₂</sub> levels below 5 kPa.

The decrease in Sp from 0.22±0.08 to 0.11±0.03 L·min<sup>-1</sup>·kPa<sup>-1</sup> could possibly be explained by a direct effect of the drug on the carotid bodies, since they contain the enzyme carbonic anhydrase [15]. The decrease in Sp found in the present study is in agreement with our previous observation in normoxaemic cats [24]. The absolute values of Sp and Sc in this study are lower than the ones in the normoxaemic study [24]. For unknown reasons, the cats in the present study needed more supplemental anaesthesia than the previous group. This made both Sp and Sc decline by the same percentage. This means that the ratio Sp/Sc, is unaffected by anaesthesia, as shown by VAN DISSEL et al. [27].

After a bolus infusion of 4 mg·kg<sup>-1</sup> acetazolamide a transient initial fall in ventilation was observed (fig. 2). This may indicate that acetazolamide acts directly on the carotid bodies since, during hypoxaemia, the contribution of the peripheral chemoreceptors to the ventilation is larger than in normoxaemia. This is illustrated by the fact that in the present study the ratio Sp/Sc was 0.30, while in the previous study in normoxaemic cats, where the initial fall in ventilation induced by acetazolamide was smaller, this ratio was 0.18 [24]. In hypoxaemic cats, 50 mg·kg<sup>-1</sup> acetazolamide causes a short initial period of apnoea, while the ventilatory response to hypoxia is virtually abolished at this dose [28, 29]. Several other studies have shown that
acetazolamide at doses of 25–100 mg·kg\(^{-1}\) (i.v.) causes a decrease in chemosensitivity of the carotid bodies [30, 31].

The central nervous system, particularly the glial cells and possibly also the central chemoreceptors, contain carbonic anhydrase [16, 17, 21]. Consequently, the decrease in \(S_c\) could be due to a direct effect on the central chemoreceptors, affecting the central chemoreflex loop. This is unlikely for the following reasons: 1) because of its physicochemical properties, acetazolamide passes the blood–brain barrier very slowly [16, 18, 32] and 2) to achieve the full physiological effect, more than 99% inhibition of the carbonic anhydrase is needed [16, 18]. Therefore, 1 h after administration of 4 mg·kg\(^{-1}\) acetazolamide insufficient inhibitor will have reached the central chemoreceptors to cause effective inhibition of local carbonic anhydrase. Inhibition of central nervous system carbonic anhydrase by the more lipophilic drug methazolamide results in an increase in \(S_c\) [33], the opposite effect to that observed in the present study.

It was previously suggested that the effect of acetazolamide on \(S_c\) is probably due to an indirect effect on the central chemoreflex loop, namely by a direct effect on cerebral vessels and consequently on cerebral blood flow regulation. This results in a change in the relationship between \(P_{bt,CO_2}\) (which is considered as the direct stimulus to the central chemoreceptors) and \(P_{a,CO_2}\) [24]. This relationship was previously described by Beekensbosch et al. [34], Read and Leigh [35] and Tepema et al. [33] and is simplified to:

\[
P_{bt,CO_2} = \alpha P_{a,CO_2} + \beta.
\]

Among other factors, the slope \(\alpha\) depends on the cerebral blood flow response to changes in \(P_{a,CO_2}\). The intercept \(\beta\) includes brain metabolism density and the slope of the linearized blood CO\(_2\) dissociation curve, which was assumed to be constant [24].

During hypoxaemia, cerebral blood flow is increased, resulting in a parallel shift in this linear relationship between \(P_{bt,CO_2}\) and \(P_{a,CO_2}\) to lower levels of \(P_{bt,CO_2}\) without a change in slope (i.e. no change in parameter \(\alpha\) in the above formula) [36]. So, the present finding that during hypoxaemia the decrease in \(S_c\) by acetazolamide was about equal to that observed in normoxaemic cats is not unexpected and indicates that the effect of low-dose acetazolamide on the slope of the relation between \(P_{bt,CO_2}\) and \(P_{a,CO_2}\) is not influenced by the level of \(P_{a,CO_2}\) and the concomitant change in cerebral blood flow.

There is no clear explanation for the fact that the decrease in the apnoeic threshold in the hypoxaemic cats of the present study was larger than in normoxaemic animals [2 versus 1 kPa] [24]. The DEF technique is unable to separate the apnoeic threshold B into a peripheral and a central part. As shown previously, the value of B depends on the relation between \(P_{bt,CO_2}\) and \(P_{a,CO_2}\). (i.e. parameter \(\alpha\) in the above formula) [24], but also on \(S_p\) [37].

**Comparison with human studies**

In a previous study in the authors’ laboratory [4] in which hypercapnic and hypoxaemic COPD patients were examined, an increase in the slope of the expiratory minute ventilation (\(\dot{V}E\))–\(P_{et,CO_2}\) response curve was observed after administration of acetazolamide. This was also found in healthy volunteers [13, 38]. These results differ from the present data obtained in anaesthetized animals, since a decrease was found in both slope and apnoeic threshold.

Apart from the use of anaesthesia [39] and from species differences [16] there are several other possible explanations for these differences.

**The use of \(P_{et,CO_2}\) as independent variable.** In most human studies, ventilatory CO\(_2\) response curves are measured using the \(P_{et,CO_2}\) as the independent variable. In COPD patients, however, \(P_{et,CO_2}\) will not be representative of \(P_{a,CO_2}\) because of the presence of a \(P_{a-ET,CO_2},CO_2\) gradient. Furthermore, after acetazolamide infusion the relationship between \(P_{a,CO_2}\) and \(P_{et,CO_2}\) may change, due to both a higher level of ventilation and a change in ventilation/perfusion ratios. So, to find out whether the sensitivities of the chemoreceptors to changes in \(P_{CO_2}\) are altered, \(P_{et,CO_2}\) seems to be an inappropriate independent variable. Illustrative in this context may be the study of Lercie et al. [12] who reported a parallel shift in the CO\(_2\) response curve in healthy subjects. Reanalysing their data by means of linear regression, using \(P_{a,CO_2}\) as the independent variable, reveals that acetazolamide reduced the slope of the \(\dot{V}E–P_{a,CO_2}\) relationship by approximately 30%.

**The use of the rebreathing method for the CO\(_2\) response.** In several human studies the Read rebreathing method was used to estimate ventilatory CO\(_2\) sensitivity [1, 9, 13]. During chronic use of acetazolamide, however, a considerable metabolic acidosis ensues, resulting in a decrease in \(P_{a,CO_2}\) (and \(P_{et,CO_2}\). As argued by Linton et al. [40] and Beekensbosch et al. [34] this may lead to a considerable overestimation of the CO\(_2\) response slope in this situation.

**Fig. 4.** – Effect of 4 mg·kg\(^{-1}\) acetazolamide on the ventilatory CO\(_2\) response curve, calculated from the mean data in eight cats. The continuous line represents the control situation and the dashed line the situation after 4 mg·kg\(^{-1}\) acetazolamide. The dotted line is a hypothetical line representing the CO\(_2\) response curve when an additional metabolic acidosis is allowed to develop as in chronic administration in humans. Note that a metabolic acidosis causes an appreciable left shift of the curve, resulting in a widening of the therapeutic range in which acetazolamide induces an increase in ventilation \((\dot{V}E)\). \(P_{et,CO_2}\): end-tidal carbon dioxide tension.
Acute versus chronic use of acetazolamide. Acetazolamide is used in COPD patients in a chronic setting and administered orally. Swenson and Hughes [38] showed that chronic and acute administration of acetazolamide affected the slope of the $\VE^* - \PCO_2$ response curve differently: chronic use resulted in an increase in slope of the hypoxic $\VE^* - \PCO_2$ response curve, whereas acute administration of the drug had the opposite effect. Therefore acute and chronic administration of acetazolamide may have different effects on the CO$_2$ response slope and this may explain why acute administration in cats, as in humans, results in a decrease on the slope of the hypoxic $\VE^* - \PCO_2$ response curve.

Another difference in the effect of chronic and acute administration of acetazolamide is that in the former case a metabolic acidosis ensues, while this is absent in the latter [38]. This agrees with the present study in which acute administration of acetazolamide only caused a very limited metabolic acidosis.

Clinically, the chronic use of acetazolamide can be advantageous since even in the case of a decrease in slope of the $\VE^* - \PCO_2$ range in which acetazolamide increases the level of ventilation will be extended considerably (see fig. 4). This is caused by a shift in the ventilatory CO$_2$ response curve to lower PCO$_2$ values induced by the ensuing metabolic acidosis.

Further clinical studies are needed to document the effects of chronic use of acetazolamide in patients with chronic obstructive pulmonary disease, using arterial carbon dioxide tension as an independent variable.

References

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