

**Reversal of drug-affected breathing** Bijl, J.H.L.

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# **6** Effects of Low-Dose Methazolamide on the Control of Breathing in Cats

Inhibitors of carbonic anhydrase (CA) have complex effects on respiration. Many cells and tissues that are involved in the control of breathing contain various isoforms of CA, e.g., red cells, carotid bodies, lung and brain capillary endothelial cells, muscle and neurons closely associated with central chemoreceptors.<sup>1-9</sup> In human and cats, low intravenous doses of acetazolamide have both stimulatory and inhibitory effects on the control of breathing.<sup>10,11</sup> One of the inhibitory effects applies to the peripheral chemoreceptors because acetazolamide has been shown to reduce the hypoxic response and also the O<sub>2</sub>-CO<sub>2</sub> interaction that is known to reside in the carotid bodies.<sup>10,12,13</sup> The mechanism by which this occurs is unclear: however, due to its physical-chemical properties acetazolamide does not easily cross biological membranes,<sup>1,2</sup> so that at low dose this inhibiting effect is unlikely due to inhibition of an intracellular isoform of CA in the carotid bodies. Methazolamide, another CA inhibitor with an about equal affinity for sulfonamide-sensitive CA isoforms, is much more lipophilic and rapidly permeates into cells.<sup>1,2</sup> Therefore, this agent would be a suitable tool to study the effect of intracellular CA inhibition on carotid body-mediated responses. Another difference between acetazolamide and methazolamide refers to their effects on large-conductance Ca<sup>2+</sup>-dependent potassium (BK) channels: while acetazolamide specifically opens these channels, methazolamide is without any stimulating effect on them.<sup>14</sup> Because BK channels may play a crucial role in the hypoxic response of type I carotid body cells,<sup>5</sup> it is therefore interesting to compare the effects of both agents on the carotid body responses to both hypoxia and hypercapnia.

Dynamic end-tidal  $CO_2$  forcing (DEF) is a suitable means to study the separate effects of pharmacological agents on the  $CO_2$  sensitivity of the peripheral and central chemoreflex loops.<sup>16</sup> In this study we have applied this technique to study the effects of low-dose methazolamide on the control of breathing in the cat.

#### Methods

Experiments were performed in nine female adult cats (weight 2.5–4.1 kg). The Ethical Committee for Animal Experiments of the University of Leiden approved the use of animals. The animals were sedated with 10 mg.kg<sup>-1</sup> ketamine hydrochloride i.m. Anesthesia was induced with 2% sevoflurane in 30% O<sub>2</sub> in N<sub>2</sub>. Both femoral arteries and the right femoral vein were cannulated, 20 mg.kg<sup>-1</sup>  $\alpha$ -chloralose and 100 mg.kg<sup>-1</sup> urethane were slowly

administered intravenously, and the volatile anesthetic was gradually withdrawn. About 1 h later, an infusion of an  $\alpha$ -chloralose-urethane solution was started at a rate of 1.0–1.5 mg.kg<sup>-1</sup>.h<sup>-1</sup>  $\alpha$ -chloralose and 5.0–7.5 mg.kg<sup>-1</sup>.h<sup>-1</sup> urethane.

#### Respiration

The trachea was cannulated at midcervical level and connected to a respiratory circuit. Tidal volume was measured electronically by integrating airway gas flow obtained from a pneumotachograph (number 0 flow transducer, Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (PM 197, Statham, Los Angeles, CA, USA). The respiratory fractions of  $O_2$  and  $CO_2$  were continuously measured with a gas monitor (Multicap, Datex, Helsinki, Finland), which was calibrated with gas mixtures of known composition. The inspiratory gas concentrations were made with computer-steered mass flow controllers (AFC 260, Bronkhorst High-tech BV, Veenendaal, The Netherlands). The end-tidal PCO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) and end-tidal PO<sub>2</sub> (P<sub>ET</sub>O<sub>2</sub>) were controlled independently by a PC by adjusting the inspiratory gas fractions. Arterial blood pressure was measured using a pressure transducer (P23ac, Statham). Arterial blood samples were taken for blood gas analysis (ABL 700, Radiometer Copenhagen, Brønshøj, Denmark).

#### **Experimental Design**

Using the DEF technique, we performed step changes in  $P_{ET}CO_2$  before and after intravenous infusion of 3 mg.kg<sup>-1</sup> methazolamide (Sigma, Zwijndrecht, The Netherlands), dissolved in 0.1 N NaOH and 0.1 N HCl (pH was adjusted to 7.3–7.4).  $P_{ET}O_2$  was kept constant throughout the experiments at a normoxic level of 14 kPa. Both before and after methazolamide administration, 2 to 4 DEF runs were performed and the dynamic ventilatory responses were analyzed (see below). The  $P_{ET}CO_2$  pattern during a DEF run was as follows. After a 10 to 15 min period of steady-state ventilation at constant  $P_{ET}CO_2$  (about 0.5 kPa above the apneic threshold), the  $P_{ET}CO_2$  was increased by 1–1.5 kPa in a step-wise fashion and kept constant for 7 min. Thereafter, the  $P_{ET}CO_2$  was returned to its previous value and maintained for another 7 min.

#### **Dynamic End-Tidal Forcing**

The steady-state relation of inspiratory ventilation (V<sub>i</sub>) to  $P_{ET}CO_2$  at constant  $P_{ET}O_2$  can be described by:

$$\mathbf{V}_{i} = (\mathbf{S}_{p} + \mathbf{S}_{c})(\mathbf{P}_{ET}\mathbf{CO}_{2} - \mathbf{B})$$

where  $S_p$  and  $S_c$  are the carbon dioxide sensitivities of the peripheral and central chemoreflex loops, respectively, and B is the apneic threshold or extrapolated  $P_{ET}CO_2$  at zero  $V_i$ . The sum of  $S_p$  and  $S_c$  is the overall carbon dioxide sensitivity.

For the analysis of the dynamic response of ventilation to a step-wise change in  $P_{ET}CO_2$  we used a two-compartment model:<sup>16</sup>

$$V_p(t) + \tau_p d/dt V_p(t) = S_p (P_{ET}CO_2[t - T_p] - B)$$
$$V_c(t) + \tau_c d/dt V_c(t) = S_c (P_{ET}CO_2[t - T_c] - B)$$

where  $\tau_p$  and  $\tau_c$  are the time constants of the peripheral and central chemoreflex loops, respectively,  $V_p(t)$  and  $V_c(t)$  are the outputs of the peripheral and central chemoreflex loops, respectively,  $P_{ET}CO_2[t - T_p]$  is the stimulus to the peripheral chemoreflex loop delayed by the peripheral transport delay time  $(T_p)$ , and  $P_{ET}CO_2[t - T_c]$  is the stimulus to the central chemoreflex loop delayed by the central transport delay time  $(T_c)$ .

To allow the time constant of the ventilatory on transient to be different from that of the off transient,  $\tau_c$  is written as:

$$\tau_{\rm c} = {\rm x}.\tau_{\rm on} + (1-{\rm x}) \tau_{\rm off}$$

where  $\tau_{on}$  is the time constant of the ventilatory on transient,  $\tau_{off}$  is the time constant of the off transient, and x = 1 when  $P_{ET}CO_2$  is high, while x = 0 when  $P_{ET}CO_2$  is low.

In most experiments a small drift in ventilation was present. We therefore included a drift term (C·t) in our model. The total ventilatory response  $V_i(t)$  is made up of the contributions of the central and peripheral chemoreflex loops and C·t:

$$V_i(t) = V_p(t) + V_c(t) + C \cdot t$$

The parameters of the model were estimated by fitting the model to the breath-by-breath data with a least-squares method. To obtain optimal time delays, a grid search was applied, and all combinations of  $T_p$  and  $T_c$ , with increments of 1 s and with  $T_p$  smaller than or equal to  $T_c$ , were tried until a minimum in the residual sum of squares was obtained. The minimum time delay was chosen, arbitrarily, to be 1 s, 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 obtained after methazolamide infusion, analysis of variance was performed on individual data. The level of significance was set at P = 0.05. Results are given as mean of the mean per cat  $\pm$  SD.

#### Results

The dose of 3 mg.kg<sup>-1</sup> methazolamide did not cause an appreciable arterial-to-end-tidal PCO<sub>2</sub> ( $P_{(a-ET)}CO_2$ ) gradient indicating the absence of effective erythrocytic CA inhibition ( $P_{(a-ET)}CO_2$  differences were -0.14 ± 0.27 kPa in control and 0.18 ± 0.27 kPa after

methazolamide, P = 0.11). Altogether 57 DEF runs (32 before and 25 after methazolamide) were analyzed and the results are summarized in table 1.

	Control	Methazolamide	Р
B (kPa)	$3.60\pm0.72$	$1.77 \pm 1.41$	0.00006
S <sub>c</sub> (l.min.kPa <sup>-1</sup> )	$0.68\pm0.27$	$0.44\pm0.22$	0.013
S <sub>p</sub> (l.min.kPa <sup>-1</sup> )	$0.08\pm0.04$	$0.06\pm0.03$	0.13
Base excess (mM)	$-6.65 \pm 1.75$	$-7.84 \pm 1.90$	0.009

**Table 1.** Effects of methazolamide on respiratory parameters. Values are given as mean of the mean per cat  $\pm$  SD (n = 9).

Methazolamide reduced the apneic threshold and the  $CO_2$  sensitivity of the central chemoreflex loop. The  $CO_2$  sensitivity of the peripheral chemoreflex loop was not significantly reduced. The individual data are shown in figure 1. Time constants, delays and drift were not significantly influenced by methazolamide (data not shown).

### Discussion

Our data show a clear decrease in apneic threshold and central  $CO_2$  sensitivity by low-dose methazolamide. However, we could not demonstrate a significant decrease in peripheral  $CO_2$  sensitivity in our animals. The agent caused a small but significant decrease in base excess.

Previously, we have attributed the decrease in apneic threshold by low-dose acetazolamide to a possible effect on the relationship between cerebral blood flow (CBF) and brain tissue PCO<sub>2</sub> (PtCO<sub>2</sub>).<sup>11</sup> Similar to our previous experiments with low-dose acetazolamide, a significant  $P_{(a-ET)}CO_2$  gradient was also absent after methazolamide, albeit at a somewhat lower dose. Methazolamide, however, is a much more permeable inhibitor with an about equal affinity for carbonic anhydrase II,<sup>1,2</sup> so that compared to acetazolamide (4 mg.kg<sup>-1</sup>) after 3 mg.kg<sup>-1</sup> methazolamide the fractional inhibition of intracellular CA can be expected to be at least equal if not larger. Avoiding complete inhibition is important to prevent large tissue acidosis, which then could have explained the large decrease in apneic threshold. Our data show that the decrease in mean apneic threshold was considerably larger than after low-dose acetazolamide, and this may be caused by additional inhibition of intracellular CA in brain capillary cells.



Figure 1. Scatter diagrams of the respiratory parameters before (control) and after methazolamide.

A change in the CBF–PtCO<sub>2</sub> relationship may also result in a change in CO<sub>2</sub> sensitivity of the central chemoreflex loop. Compared to acetazolamide, we now find a smaller decrease in central CO<sub>2</sub> sensitivity. After low-dose methazolamide, however, inhibition of brain carbonic anhydrase could have altered central chemoreceptors CO<sub>2</sub> sensitivity for at least two reasons. First, carbonic anhydrase has been shown to be present in rostroventrolateral medullary structures associated with central chemoreceptors,<sup>8,9</sup> and second, in a previous study we showed that a high-dose methazolamide caused *an increase* in CO<sub>2</sub> sensitivity independently of extracellular and erythrocytic CA inhibition.<sup>17</sup> Inhibition of brain carbonic anhydrase is followed by decrease in intracellular buffer capacity against CO<sub>2</sub>.<sup>18</sup> Intracellular pH changes play a crucial role in central CO<sub>2</sub> chemoreception (references see 19). In conclusion, the lower decrease in central CO<sub>2</sub> sensitivity of the former on the CBF–PtCO<sub>2</sub> relationship (tending to decrease the CO<sub>2</sub> sensitivity of the central chemoreflex loop) and on the buffer capacity of central chemoreceptors (tending to increase their sensitivity to changes in PCO<sub>2</sub>).

Chapter 6

An interesting finding was that, in contrast to acetazolamide,<sup>11</sup> methazolamide did not significantly reduce the CO<sub>2</sub> sensitivity of the peripheral chemoreflex loop. The carotid bodies contain several CA isoforms.<sup>4-6,20</sup> Because 3 mg.kg<sup>-1</sup> methazolamide will be sufficient for complete inhibition of sulfonamide-sensitive carbonic anhydrases within the carotid bodies (but not in erythrocytes due to their very large CA content),<sup>1,2</sup> its failure to reduce peripheral CO<sub>2</sub> sensitivity may seem surprising. Causing extracellular rather than extracellular and intracellular CA inhibition, low-dose acetazolamide induces a clear reduction in peripheral CO<sub>2</sub> sensitivity, while the steady-state hypoxic response is reduced by 50%.<sup>11,12</sup> Our findings are reminiscent of data obtained from *in vitro* carotid body preparations in which complete CA inhibition appeared to reduce the fast initial rather than the steady-state CO<sub>2</sub> response.<sup>21</sup> One possible explanation of the different effects of methazolamide and acetazolamide on the peripheral chemoreflex loop may be related to a specific effect of acetazolamide on Ca<sup>2+</sup>-dependent large-conductance potassium (BK) channels that is not shared by methazolamide.<sup>14</sup> While acetazolamide has a specific, powerful stimulating effect on these channels (*i.e.*, BK channels from skeletal muscles of  $K^+$ -deficient rat), methazolamide entirely lacks such an opening effect.<sup>14</sup> As recently shown by Williams et al.,<sup>15</sup> BK channels may play a crucial role in the response of type I carotid body cells to hypoxia. Unpublished data from our lab indicate that in contrast to acetazolamide, low-dose methazolamide does not reduce the steady-state hypoxic response in the cat indicating that BK channels may indeed be involved in the inhibiting effect of acetazolamide and that CA inhibition in the carotid bodies not necessarily reduces their steady-state response to changes in PO<sub>2</sub> and PCO<sub>2</sub>.

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