Reversal of drug-affected breathing

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7 The Carbonic Anhydrase Inhibitors Methazolamide and Acetazolamide Have Different Effects on the Hypoxic Ventilatory Response in the Anesthetized Cat

Carbonic anhydrase (CA) catalyzes the interconversion of CO_2 and bicarbonate and plays a crucial role in respiration. To date, fourteen iso-enzymes have been characterized in higher vertebrates including humans.[1] A specific role of CA in respiration is indicated by the presence of various isoforms in tissues and cells that are directly or indirectly involved in ventilatory control such as lung and brain capillary endothelium, kidneys, muscles, carotid bodies and central chemosensitive areas in the rostroventrolateral medulla oblongata.[1,2]

Inhibitors of CA have a profound influence on the control of respiration. Due to different physical-chemical properties, various sulfonamide CA inhibitors have distinct dose-dependent effects. Acetazolamide (AZ), a moderately permeable sulfonamide, is the most frequently used inhibitor to study the role of CA in the hypercapnic response of the carotid body and has been shown to diminish its steady-state CO_2 sensitivity,[3,4] although other studies reported only a reduced response speed and/or elimination of over- and undershoots in carotid sinus nerve activity upon (removal of) sudden hypercapnic stimuli.[5,6] AZ reduces the CO_2-induced initial fast depolarization and nerve activity in type I carotid body cells and co-cultured petrosal ganglion neurons, respectively, without changing steady-state responses.[7] This is consistent with a role of CA in regulating the speed and magnitude of the initial CO_2-induced fall in intracellular pH in type I cells.[8] In a superfused carotid body preparation from cat, AZ reduces both the release of dopamine and the increase in carotid sinus nerve discharge upon acidic stimuli.[9] In the cat, AZ exerts inhibitory effects on carotid body-mediated reflexes. For example, low intravenous doses reduce both steady-state hypoxic sensitivity and the O_2–CO_2 interaction.[10,11] In a dose that completely inhibits erythrocytic CA, it causes a much greater rise in ventilation in carotid body denervated than in intact cats,[12] while in the latter the hypoxic ventilatory response is virtually abolished, an observation that also has been reported in man after an intravenous infusion of 500 mg.[12-14] To our knowledge, effects of AZ on the hypoxic response of the carotid sinus nerve or type I cells have not been reported, but the above findings in intact organisms clearly suggest that it may have inhibitory effects.

Methazolamide (MTZ) is a more lipophilic sulfonamide with an about equal K_I as AZ for CA II and IV.[15] MTZ does not seem to affect the magnitudes of the cat carotid sinus nerve responses to hypercapnia and hypoxia.[5,16,17] Whether MTZ affects the hypoxic response in intact organisms is unknown.

It would be interesting to compare the effects of AZ and MTZ on the hypoxic ventilatory response for two reasons. First, both agents may have different pharmacological effects. Recently, Tricarico *et al.* showed a direct, stimulating, action of AZ on voltage-sensitive large-conductance Ca^{2+}-dependent potassium (BK) channels in muscle cells from K^+ depleted rats,[18] an effect that is not shared by MTZ. At least in the rat, BK channels play a role in oxygen sensing by the carotid bodies,[19-21] but this could not be confirmed in type I cells from adult cats.[22] Despite the paucity of data on oxygen sensitive potassium channels in type I cells from cat, it would be interesting to compare the effects of MTZ and AZ in this species, given the different pharmacological properties of these agents. Second, if the elimination of the hypoxic response by high-dose AZ is due to inhibition of one or more CA isoenzymes, a high dose of the more permeable CA inhibitor MTZ should also abolish it. The purpose of the present study in anesthetized cats was to examine if inhibition of carbonic anhydrase in all body tissues by an agent other than AZ would reduce or abolish the hypoxic response and, second, if AZ could reduce it by a mechanism other than by inhibition of CA isoforms. To study the effect of CA inhibition on the hypoxic response, we administered MTZ in a dose (33 $mg.kg^{-1}$) that would inhibit CA in all body tissues. To compare the effects of low doses of both sulfonamides, we infused low doses (3 $mg.kg^{-1}$) of MTZ and AZ (in this order) in a separate group of animals.

Methods

Experiments were performed in fifteen adult cats of either sex (mean body weight 3.55 ± 0.98 kg) after approval by the Ethical Committee for Animal Experiments of the University of Leiden. The animals were sedated with 10 $mg.kg^{-1}$ ketamine hydrochloride i.m. Anesthesia was induced with 2% sevoflurane in 30% O_2 in N_2. Both femoral arteries and the right femoral vein were cannulated, 20 $mg.kg^{-1}$ α-chloralose and 100 $mg.kg^{-1}$ urethane were slowly administered intravenously, and the volatile anesthetic was gradually withdrawn. Then an infusion of a α-chloralose-urethane solution was started at a rate of 1.0–1.5 $mg.kg^{-1}.h^{-1}$ α-chloralose and 5.0–7.5 $mg.kg^{-1}.h^{-1}$ urethane to obtain a stable but light anesthesia.[23]

Respiration

The trachea was cannulated at midcervical level and connected to a respiratory circuit. Tidal volume was measured electronically by integrating airway 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, Veenendaal, The Netherlands). The end-tidal PCO_2 ($P_{ET}CO_2$) and end-tidal PO_2 ($P_{ET}O_2$) were controlled independently by a PC by adjusting the inspiratory gas fractions. Arterial blood samples were taken from the right femoral artery for

blood gas analysis (ABL 700, Radiometer Copenhagen, Brønshøj, Denmark). Arterial blood pressure was measured using a pressure transducer (P23ac, Statham). Rectal temperature was maintained within 1 ºC. All signals were converted to digital values (sample frequency 100 Hz), processed by a PC and stored breath-by-breath.

Study Design

In a first group of ten animals (group I), we measured the steady-state ventilatory O_2 response at constant $P_{ET}CO_2$ in the control situation. Then, 3 $mg.kg^{-1}$ methazolamide (Sigma, Zwijndrecht, The Netherlands; dissolved in NaOH, adjusting the pH to about 7.4 with HCl) was infused in a volume of about 5 ml (~1 $ml.min^{-1}$). About 60 min thereafter, a second isocapnic steady-state hypoxic response curve was determined. After finishing these measurements, 3 $mg.kg^{-1}$ acetazolamide (Diamox, AHP Pharma, Hoofddorp, The Netherlands; 2 $mg.ml^{-1}$ in saline) was infused and after another 60 min a third steady-state isocapnic hypoxic response was measured.

After control measurements in a second group of five animals (group II), an isocapnic hypoxic response curve was determined after 3 $mg.kg^{-1}$ MTZ as described above. Then, to obtain complete inhibition of CA in all tissues, these animals were given an additional dose of 30 $mg.kg^{-1}$ MTZ, whereafter another isocapnic hypoxic reponse curve was determined.

Isocapnic Hypoxic Response and Data Analysis

Near step-wise changes in $P_{ET}O_2$ were achieved by adjusting the O_2 fraction of the inspired air; the $P_{ET}CO_2$ was kept constant by adjusting the inspired CO_2 concentration.[24] In this way, a new steady-state level of ventilation has established after 5–6 min.[24] The last 20 breaths of this period were averaged to yield steady-state ventilation at a given $P_{ET}O_2$. Blood samples were taken at the end of the steady-state periods to analyze blood gases. Using a least square method, inspiratory ventilation (V_i) was fitted to arterial PO_2 (PaO_2) according to the exponential function:[24,25]

$$V_i = G \cdot \exp(-D \cdot PO_2) + A$$

in which G is the overall hypoxic sensitivity ($l.min^{-1}$), D is a shape parameter (kPa^{-1}) and A is the ventilation during hyperoxia ($l.min^{-1}$). None of these three parameters was fixed but all were estimated with the aid of an iteration method.

The statistical analysis was performed using SPSS v11.0 for windows (SPSS Inc., Chicago, IL, USA). To detect significant differences between the three treatments (control, MTZ and AZ), we performed a one-way repeated measures analysis of variance with *post-hoc* Bonferroni correction. *P*-values < 0.05 were considered significant. Unless otherwise indicated, data are presented as mean ± SD.

Results

In the animals of group I (n = 10), the hypoxic responses were measured by forcing the $P_{ET}O_2$ level from hyperoxia to hypoxia resulting in PaO_2 levels that covered the range from 60.5 ± 4.1 to 5.6 ± 1.4 kPa in control, 57.1 ± 5.7 to 5.7 ± 1.0 kPa after MTZ and 57.6 ± 7.4 to 5.9 ± 1.2 kPa after AZ. The dose of 3 $mg.kg^{-1}$ MTZ did not cause a rise in the mean arterial-to-end-tidal PCO_2 ($P_{(a–ET)}CO_2$) gradient indicating the absence of effective erythrocytic carbonic anhydrase inhibition (see table 1). The additional dose of 3 $mg.kg^{-1}$ AZ caused a significant increase in the gradient by about 0.3 kPa, probably too small to cause appreciable tissue acidosis and a further rise in hyperoxic ventilation (see table 1). Table 1 also shows that MTZ caused a mild acidosis that was more pronounced after the subsequent infusion of AZ.

Table 1. Effects of methazolamide (MTZ) and acetazolamide (AZ) on the steady-state hypoxic response in ten cats.

	Control	**MTZ**	**AZ**
G ($l.min^{-1}$)	1.93 ± 1.32	1.89 ± 0.90	1.09 ± 0.92 *
D (kPa^{-1})	0.20 ± 0.07	0.22 ± 0.06	0.14 ± 0.06
A ($l.min^{-1}$)	0.86 ± 0.33	1.30 ± 0.40 †	1.32 ± 0.43 ‡
$PaCO_2$ (kPa)	4.63 ± 0.75	4.55 ± 0.78	4.78 ± 0.81
$P_{(a–ET)}CO_2$ (kPa)	-0.01 ± 0.30	-0.08 ± 0.38	0.29 ± 0.61 §
Arterial pH	7.338 ± 0.04	7.307 ± 0.05 §	7.253 ± 0.05 a
Base excess (mM)	-6.75 ± 1.26	-8.56 ± 1.83 b	-10.53 ± 1.97 a

* P = 0.003 *versus* control and P = 0.01 *versus* MTZ; † P = 0.003 *versus* control; ‡ P = 0.002 *versus* control; § P = 0.007 *versus* control; a P = 0.000 *versus* control; b P = 0.006 *versus* control

An example of the effect of both agents on the hypoxic response in one animal is shown in figure 1. The overall results, summarized in table 1, show that the mean control hypoxic sensitivity in the ten animals studied was not different from that after MTZ (P = 0.88 *versus* control) while AZ reduced it by 44%. Compared to the control situation, all animals except one had a lower hypoxic sensitivity after AZ administration. The shape parameter D was unaffected by MTZ, while AZ tended to reduce it (P = 0.023 *versus* control). The hyperoxic ventilation A increased after MTZ but did not rise further after AZ.

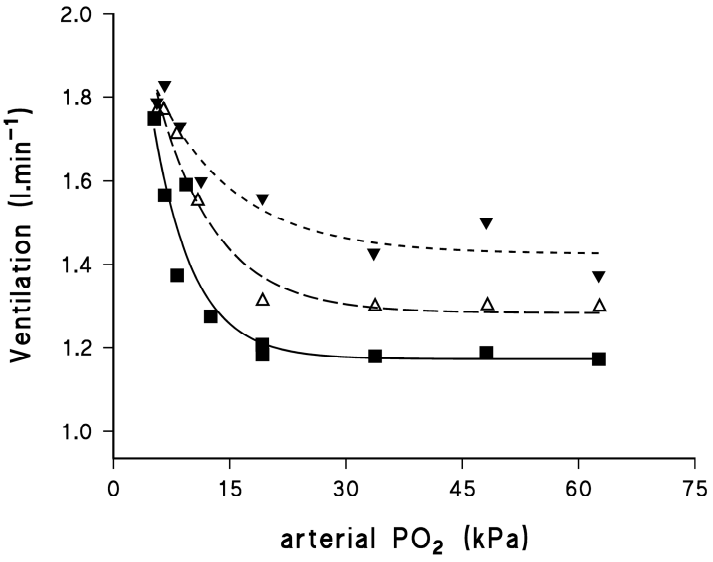

Figure 1. Steady-state hypoxic response curves in one animal before (squares) and after infusions of, respectively, 3 mg.kg^{-1} MTZ (open triangles) and 3 mg.kg^{-1} AZ (closed triangles). Curves are optimal fits (least square method) to a monoexponential equation with residual. Parameter values for G (l.min^{-1}), D (kPa^{-1}) and A (l.min^{-1}) were 1.52, 0.19 and 1.17 in control, 1.1, 0.13 and 1.28 after MTZ and 0.68, 0.10 and 1.43 after AZ, respectively.

The animals of group II (n = 5) were given 3 mg.kg^{-1} MTZ, followed by 30 mg.kg^{-1}. Hypoxic responses in these animals were measured by forcing the $P_{ET}O_2$ level from hyperoxia to hypoxia resulting in PaO_2 levels that covered the range from 58.1 ± 1.1 to 4.9 ± 0.8 kPa in control, 54.4 ± 4.3 to 4.9 ± 0.9 kPa after 3 mg.kg^{-1} MTZ and 56.6 ± 1.5 to 5.0 ± 0.9 kPa after 30 mg.kg^{-1} of the agent. In table 2, note the appearance of a large $P_{(a-ET)}CO_2$ gradient after 33 mg.kg^{-1}, indicating effective inhibition of erythrocytic carbonic anhydrase. An example of the effect of low- and high-dose MTZ in one animal is shown in figure 2. The scatter diagram of figure 3 shows the individual effect of low-dose MTZ in all fifteen animals (group I and II) studied. Note the absence of a systematic increase or decrease of parameter G by MTZ. All five animals of group II showed a substantial response to hypoxia after 33 mg.kg^{-1} MTZ (in three animals parameter G was even larger than in control), which is in sharp contrast after high-dose AZ, when the hypoxic response is abolished.[12,13] Overall, high-dose MTZ did not induce significant changes in hypoxic sensitivity, shape parameter and hyperoxic ventilation (table 2).

Table 2. Effects of methazolamide (MTZ) (3 and 33 mg.kg^{-1}, respectively) on the steady-state hypoxic response in five cats.

	Control	**MTZ 3 mg.kg^{-1}**	**MTZ 33 mg.kg^{-1}**
G (l.min^{-1})	3.26 ± 1.44	3.23 ± 1.16	3.00 ± 1.23
D (kPa^{-1})	0.27 ± 0.07	0.29 ± 0.10	0.27 ± 0.09
A (l.min^{-1})	0.79 ± 0.16	0.59 ± 0.21	0.65 ± 0.21
$PaCO_2$ (kPa)	5.75 ± 0.39	5.76 ± 0.31	6.14 ± 0.21
$P_{(a-ET)}CO_2$ (kPa)	0.40 ± 0.45	0.66 ± 0.72	2.40 ± 0.54 †
Arterial pH	7.262 ± 0.03	7.239 ± 0.04	7.217 ± 0.02 *
Base excess (mM)	-7.03 ± 0.88	-8.23 ± 1.27	-8.38 ± 1.09 *

Note that after 33 mg.kg^{-1} MTZ, the $PaCO_2$, pH and base excess represent equilibrium values *in vitro*; *in vivo* $PaCO_2$ in the blood perfusing the carotid bodies must have been considerably lower and pH considerably higher than the *in vitro* values shown here. * $P = 0.012$ *versus* control; † $P = 0.000$ *versus* control.

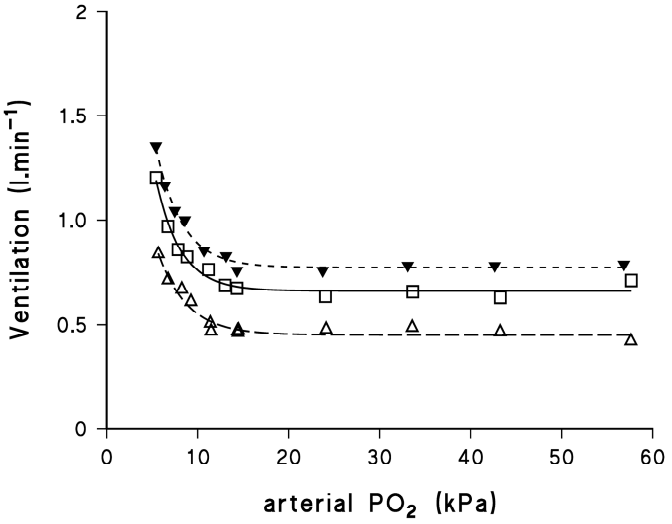

Figure 2. Example of steady-state response curves in one animal in control (squares), 3 mg.kg^{-1} MTZ (open triangles) and 33 mg.kg^{-1} MTZ (closed triangles), respectively. Curves are optimal fits (least square method) to a monoexponential equation with residual. Parameter values for G (l.min^{-1}), D (kPa^{-1}) and A (l.min^{-1}) were 3.99, 0.37 and 0.66 in control, 2.31, 0.31 and 0.45 after low-dose MTZ and 3.70, 0.34 and 0.77 after high-dose MTZ, respectively.

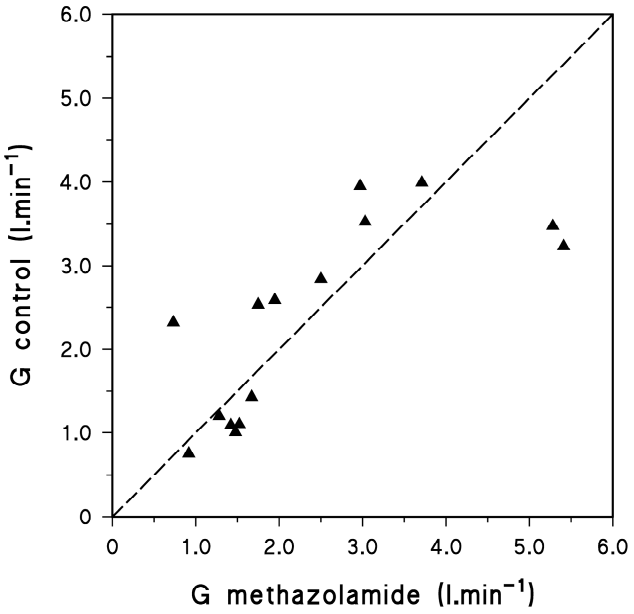

Figure 3. Scatter diagram of the effect of 3 mg.kg^{-1} MTZ on hypoxic sensitivity G in fifteen animals. Low-dose MTZ did not cause a systematic change in G.

Discussion

The main findings of this sudy can be summarized as follows. First, in a dose (33 mg.kg^{-1}) that completely inhibits carbonic anhydrase in all body tissues, methazolamide did not alter the steady-state hypoxic ventilatory response in the cat. Second, in a dose of 3 mg.kg^{-1}, the less lipophilic sulfonamide acetazolamide reduced hypoxic sensitivity by 44%, while an equal low dose of MTZ lacked this effect. These results indicate that full inhibition of CA does not reduce the steady-state hypoxic response in the cat and that the depressing effect of AZ may be caused by a pharmacological action other than on CA.

Study Design

Full physiological inhibition of CA is reached when 99.99% of the enzyme is inhibited.[26] The concentration of CA II in cat erythrocytes is very high providing a possible explanation why 3 mg.kg^{-1} MTZ did not widen the $P_{(a-ET)}CO_2$ gradient.[27] Because red cells contain much more CA than carotid bodies,[2] the fractional inhibition of the enzyme after 3 mg.kg^{-1} MTZ (which is evenly distributed) will be larger in the latter. In cat choroid plexus, also an organ with a relatively high CA concentration, this dose has shown to result in 99.58% inhibition.[28] We thus speculate that in the carotid bodies the inhibition will then be close to 99.99% if not more, so that a subsequent AZ dose would not exert a physiological effect *via* inhibition of local CA. Although in the absence of concrete data we can not exclude that the additional AZ

dose (group I) could have made the difference between incomplete and full carotid body CA inhibition, our observation of an entirely intact hypoxic response after a MTZ dose that completely inhibits CA in all tissues,[2,28] lends support to the view that the inhibiting effect of AZ in the animals of group I must be due to an effect unrelated to CA inhibition. Note that the AZ dose of 3 $mg.kg^{-1}$ was somewhat smaller than that was previously shown to reduce the hypoxic response.[11]

Tables 1 and 2 show that 3 $mg.kg^{-1}$ MTZ caused a decrease in mean pH of 0.031 in group I and 0.023 in group II. This mild acidosis could have counteracted an inhibitory effect of the agent on the hypoxic response. Low-dose AZ, however, causing a similar degree of mild acidosis, clearly reduces hypoxic sensitivity,[11,29] indicating that MTZ and AZ have different effects on the hypoxic response indeed. In the present study, AZ enlarged the MTZ-induced acidosis, but this did not prevent a clear reduction in hypoxic sensitivity. Consequently, AZ must have a potent inhibitory effect on the hypoxic response that is not shared by MTZ.

Effects of MTZ and AZ on the Hypoxic Ventilatory Response: Parameters A, D and G

In both men and animals, the hypoxic response is biphasic, starting with an initial rise in ventilation mediated by the carotid bodies, followed by a secondary decrease called hypoxic ventilatory depression (HVD). HVD is a poorly understood phenomenon: it is related to the magnitude of the initial carotid body stimulation, but possibly also to an increased washout of CO_2 from brain due to a rise in brain blood flow, and/or a release of inhibitory neurotransmitters.[30-32]

We examined the effects of MTZ and AZ on the steady-state hypoxic response curve, described by the exponential function $V_i = G \cdot \exp(-D \cdot PO_2) + A$, with hypoxic sensitivity G (comprising both the stimulation by the carotid bodies and HVD), shape factor D and hyperoxic ventilation A. Neither low- nor high-dose MTZ changed mean hypoxic sensitivity, while AZ, given after an equal initial MTZ dose (3 $mg.kg^{-1}$), reduced it by 44%. The hyperoxic ventilation A increased after low- but not high-dose MTZ. AZ tended to reduce parameter D.

Parameter A, the ventilation during hyperoxia is a complex variable and is influenced by the CO_2 sensitivity of the central chemoreflex loop, the X-intercept of the CO_2 response curve (apneic threshold), the prevailing (arterial and brain stem tissue) PCO_2/pH and a small, PCO_2-dependent, contribution of the carotid bodies to total ventilation. Similar to AZ,[29] low-dose MTZ causes a decrease in sensitivity of the central chemoreflex loop resulting in a less steep CO_2 response curve (unpublished observations in nine cats) and this would tend to reduce the value of parameter A. At the same time, however, also similar to AZ,[29] it causes a large decrease in the apneic threshold (so CO_2 response curves before and after MTZ intersect). The influence of the latter effect on parameter A will thus depend on the prevailing PCO_2: the lower the PCO_2 the higher the tendency for low-dose MTZ to increase parameter A.

Therefore, because the experiments in the animals of group II were performed at a background PCO_2 considerably higher than in group I, our finding of an unchanged value of A in this group by low-dose MTZ is not necessarily conflicting with the increase in group I.

The physiological significance of the shape parameter D remains unknown and we cannot explain the tendency for AZ to decrease it. In our previous study, we did not find an influence of AZ on D.[11]

The most important and surprising findings of this study are that neither low- nor high-dose MTZ changed the hypoxic sensitivity G. Previously, we ascribed the elimination of the hypoxic response by high-dose AZ solely to a total inhibition of CA isoenzymes in the carotid bodies.[12,13] Now, however, we may have to reconsider this view because the MTZ dose administered in group II (33 $mg.kg^{-1}$) will inhibit all extracellular membrane-bound CA IV as well as intracellular CA I and II.[2,26,28] An action of MTZ other than inhibition of CA is not known to us, so we can not speculate about a scenario in which complete carotid body CA inhibition by MTZ would abolish the O_2 response while at the same time an additional pharmacological action, not shared by AZ, would reverse this. The alternative, however, an action of AZ other than CA inhibition alone that is not shared by MTZ, may open a way to discuss our results against a background of recent studies showing unexpected actions of this agent.

Although there is evidence indicating that AZ has inhibiting effects on the carotid bodies (see introduction), we will discuss possible different effects of MTZ and AZ on *both* components of the hypoxic response namely the initial carotid body-mediated increase in ventilation and the secondary decrease (HVD).

Different effects of MTZ and AZ on HVD?

A hypoxia-induced release of inhibitory neurotransmitters may be one of the mechanisms that contributes to HVD.[30,32] AZ is known to reduce the excitability of neurons that are involved in seizures and as such it is used as an anticonvulsant.[2,33] Changes in extra- and intracellular pH of neurons will influence their excitability, and it is possible that compared to AZ, MTZ, by its larger permeability, may have different effects particularly on intracellular pH (pH_i) of carbonic anhydrase-containing neurons. In hippocampal CA3 neurons, CA inhibitors cause intracellular acidosis, and this effect, which is at least partly responsible for their anticonvulsant action, is larger with membrane permeant inhibitors.[34] Compared to AZ, MTZ has a superior inhibiting effect on seizures,[2,35] and if this would reflect a general decrease in activity in CA-containing neurons, the agent might be expected to promote rather than reduce HVD (compared to AZ), assumed that these neurons (or some of them) play a role in it.

Possible different effects of MTZ and AZ on the hypoxia-induced increase in cerebral blood flow (CBF) may also result in distinct effects on HVD. AZ has a reputation as a dilator of

cerebral vessels, but this effect is clearly dose-dependent and does not seem to be operative with intravenous doses lower than 5 $mg.kg^{-1}$.[36] Also, it remains to been seen whether low-dose AZ would alter the hypoxia-induced rise in CBF. In humans, after a usual oral clinical dose this does not seem to be the case.[37] We are not aware of studies showing a dilating effect of low-dose MTZ on cerebral vessels. At high dose, CBF will rise by the increase in tissue PCO_2 due to inhibition of erythrocytic CA.

Different effects of MTZ and AZ on the Carotid Bodies?

The most likely explanation for our results is a different effect of MTZ and AZ on the carotid bodies. One possibility is that AZ, with its vasodilatory reputation, increases carotid body blood flow, while MTZ lacks this effect. Recently, Pickkers *et al.* ascribed the vasodilatory effects of AZ on the peripheral circulation (forearm) in humans to a stimulating effect on large-conductance calcium-dependent potassium (BK) channels,[38] which they thought might be mediated by intracellular alkalosis that they had previously demonstrated in pig mesenteric arterial smooth muscle cells.[39] Because sulfonamides lacking CA inhibitory effects had a much smaller vasorelaxant effect, the authors argued that the inhibition of a CA isoenzyme results in intracellular alkalosis, followed by opening of BK channels.[38] In an *in vitro* carotid body preparation from cat, the specific BK channel inhibitor charybdotoxin has been reported to induce vasodilation indicating that this channel type may be involved in the regulation of carotid blood flow in this species.[40] For reasons explained above, we believe that if AZ would have increased carotid blood flow in our animals, this would most likely be due to an effect unrelated to CA inhibition (direct opening of BK channels? –see also below). Unfortunately, data on possible effects of MTZ on the peripheral circulation are lacking. From the available data in the literature we would certainly expect high-dose AZ to cause a large increase in carotid body blood flow. Whether this could explain the elimination of the hypoxic response that we showed previously remains to be seen.[12,13] Would high-dose MTZ fail to induce changes in carotid body blood flow? Studies examining the effects of low- and high-dose MTZ on the peripheral circulation and/or carotid body blood flow are warranted.

Finally, we discuss possible different effects of MTZ and AZ on type I carotid body cells. Type I cells contain carbonic anhydrase isoenzymes of which the precise subcellular locations remain to be elucidated except for the cytosolic isoforms CA II and III (possibly a membrane-bound isoform is also involved, see references 41–44). There is ample data to indicate that in the carotid bodies CA regulates the speed and magnitude of changes in pH_i of type I cells upon (removal of) sudden hypercapnic stimuli (reference see above). Hypoxia, however, does not cause a fall in pH_i,[45] and in this respect the absence of an effect of MTZ on the hypoxic response may not be as surprising as it may seem (also, see references 16 and 17). In this context then, we could ascribe the inhibiting effect of AZ to an action that is not related to inhibition of CA. The next issue is whether such an effect of AZ, unrelated to CA inhibition, could be mediated *via* BK channels. While there is ample data on the existence of specific oxygen-sensitive potassium channels in the carotid bodies of other species, *e.g.*, K_v channels

in rabbit and mouse, and TASK-1 and BK channels in rat, the information from cat is scarce.[20,21,46-48] In one report, it was found that a voltage-sensitive potassium current (inhibited by hypoxia) recorded in type I cells from adult cats was insensitive to charybdotoxin.[22] Another study in an *in vitro* perfused carotid body reported a decrease in carotid sinus nerve activity by this agent during hypoxia.[40] Clearly more studies are needed, particularly in type I cells from neonatal cat, to identify the oxygen-sensitive potassium channels in this species. It would also be interesting to compare the effects of MTZ and AZ in rat, because it is now well established that in this species BK channels play a role in oxygen sensing by the carotid bodies.[21] Finally, because AZ also appears to inhibit (directly or indirectly) various types of voltage-sensitive Ca^{2+} channels,[49] we can not exclude that this may also have contributed to the difference in effect of MTZ and AZ on the hypoxic response.

In conclusion, we have shown different effects of MTZ and AZ on the steady-state hypoxic response in the cat that in our opinion are best explained by an action of AZ on carotid body blood flow or type I cells that is not related to inhibition of carbonic anhydrase. Our data indicate that normal CA activity in the carotid bodies is not a prerequisite for a normal steady-state hypoxic response to occur. The abolishment of the hypoxic response by high-dose AZ that we previously reported is probably mediated by an action other than inhibition of carbonic anhydrase.

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