Methazolamide does not impair respiratory work performance in anaesthetized rabbits

Heidrun F. Kiwull-Schöne*, Yi Li*, Peter J. Kiwull *, and Luc J. Teppema**

* Dept. of Physiology, Ruhr-University, Faculty of Medicine, 44780 Bochum, Germany
** Dept. of Anesthesiology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Correspondence:
Dr. Heidrun Kiwull-Schöne M.D.
Dept. of Physiology
Faculty of Medicine
Ruhr-University
44780 Bochum, Germany
tel: +49 234-701285
mail: Heidrun.Kiwull-Schoene@rub.de

Running head: Methazolamide and respiratory muscle function
Abstract

In human medicine, the carbonic anhydrase (CA) inhibitor acetazolamide is used to treat irregular breathing disorders. Previously, we demonstrated in the rabbit that this substance stabilized closed-loop gain properties of the respiratory control system, but concomitantly weakened respiratory muscles. Among others, the highly diffusible CA-inhibitor methazolamide differs from acetazolamide in that it fails to activate Ca\(^{2+}\)-dependent potassium channels in skeletal muscles. Therefore, we aimed to find out, whether or not methazolamide may exert attenuating adverse effects on respiratory muscle performance as acetazolamide. In anaesthetized spontaneously breathing rabbits (N=7), we measured simultaneously the CO\(_2\) responses of tidal phrenic nerve activity, tidal transpulmonary pressure changes and tidal volume before and after intravenous application of methazolamide at two mean (±SEM) cumulative doses of 3.5 ±0.1 and 20.8 ±0.4 mg·kg\(^{-1}\). Similar to acetazolamide, low- and high-dose methazolamide enhanced base-line ventilation by 52 ±10% and 166 ±30%, respectively (P<0.01) and lowered the base excess in a dose-dependent manner by up to 8.3 ±0.9 mmol·l\(^{-1}\) (P<0.001). The transmission of a CO\(_2\)-induced rise in phrenic nerve activity into volume and/or pressure and hence respiratory work performance was 0.27 ±0.05 ml·kg\(^{-1}\)·kPa·unit\(^{-1}\) under control conditions, but remained unchanged upon low- or high-dose methazolamide, at 0.30 ±0.06 and 0.28 ±0.07 ml·kg\(^{-1}\)·kPa·unit\(^{-1}\), respectively. We conclude that methazolamide does not cause respiratory muscle weakening at elevated levels of ventilatory drive. This substance (so far not used for medication of respiratory diseases) may thus exert stabilizing influences on breathing control without adverse effects on respiratory muscle function.

Keywords: methazolamide – control of breathing – metabolic acidosis - respiratory muscle function - rabbits
Introduction

In cardio-respiratory medicine, the carbonic anhydrase (CA) inhibitor acetazolamide (ACTZ) is known for beneficial effects in patients with central sleep apnea (12, 41). Furthermore, it is used to improve blood gases in obstructive lung disease and to restore acid-base values in metabolic alkalosis, reviewed by (30). ACTZ is also widely used against Acute Mountain Sickness (1, 18, 29), most likely because it is able to reduce hypoxic pulmonary vasoconstriction (HPV) and to prevent high altitude pulmonary edema (2, 32). However, there is growing evidence from recent studies in different species that ACTZ may prevent hypoxic pulmonary vasoconstriction entirely independent of carbonic anhydrase inhibition (11) and rather may diminish the hypoxia-induced rise in intracellular Ca$^{2+}$ in pulmonary arterial smooth muscle cells (25).

Among possible side effects of ACTZ, skeletal muscle weakness and fatigue are sometimes reported (9, 28), and repeated oral doses in humans led to impaired exercise endurance or weakened isometric muscle force, mainly under normoxic conditions and more or less also during hypoxia (4, 5, 8, 24).

As far as striated respiratory muscles are concerned, we found that a low (clinical) intravenous dose of ACTZ (~4.5 mg·kg$^{-1}$) reduced the efficiency of respiratory neuro-mechanical transmission by as much as 40% in spontaneously breathing rabbits (14). Surprisingly, even the maximum dose, which completely inhibited both intra- and extracellular CA, did not appreciably add to this effect (15), suggesting that the observed effects may not be due to CA inhibition.

In rat skeletal muscle cells, ACTZ stimulates large-conductance Ca$^{2+}$ dependent potassium (BK) channels (38), a property not shared by methazolamide (MTZ), which is another even more effective sulfonamide CA-inhibitor. Therefore, the question arises, whether respiratory muscle weakening possibly will not occur upon treatment with MTZ. To explore the effects of
MTZ on respiratory muscle function, we used the same protocol as in previous studies on ACTZ (14, 15), whereby we assessed neuromuscular transmission and respiratory work performance from the responses of tidal volume and transpulmonary pressure to CO₂-induced changes of phrenic nerve activity. We have demonstrated for the first time that MTZ in contrast to ACTZ did not impair respiratory muscle function, probably due to properties of both sulfonamides that are unrelated to CA inhibition. Part of this study has been published as an abstract (13).

**Materials and Methods**

The experiments were officially approved according to the “German Law on the Protection of animals”. They were performed in seven male rabbits (mean body weight ±SEM: 3.08 ±0.11 kg), anaesthetized by intravenous sodium pentobarbital (initial dose: 57.7 ±5.5 mg·kg⁻¹, followed by continuous infusion of 5.72 ±0.47 mg·kg⁻¹·h⁻¹) for about 3 hours (192 ± 13 min) until the start of measurements. Materials and methods used in this study are similar to those described previously in detail (14, 15). Briefly, the tracheal cannula was connected to a Fleisch-tube (size 0) and pneumotachograph (SensorMedics, Bilthoven, The Netherlands), transpulmonary pressure was assessed in the oesophagus by manometer/amplifier ( Validyne, Northridge, USA)) and airway CO₂ by infrared-absorption (Binos 1, Leybold-Heraeus, Hanau, Germany). Compound action potentials of the right phrenic nerve were obtained only from one distally cut C3-C5 root to minimize hemi-diaphragmatic paralysis, using a bipolar silver electrode, amplified and integrated with a time constant of 150 ms (DAM 50, WPI, Sarasota, FL; self-constructed leakage integrator). Arterial blood pressure and heart rate were determined by pressure transducer and bridge amplifier (Statham P 23Gb; AWP4 DC, Astro-Med, Warwick, RI). All these variables
were continuously recorded (Chart recorder MT95K2, Astro-Med, Warwick, RI).
Arterial blood gases and pH were measured at (the controlled body temperature of) 38° by conventional equipment (ABL 5, Radiometer, Copenhagen, Denmark)). Standard bicarbonate and base excess (BE) were estimated directly from pH of samples equilibrated with two (4% and 8% CO2 in O2) precise gas mixtures (BMS2 Mk2 blood microsystem, Radiometer, Copenhagen, Denmark; Precision gas-mixing pump, Wösthoff, Bochum, Germany). Concentrations of hemoglobin and lactate were determined photometrically (Spectrophotometer: Hitachi-100-10, Tokyo, Japan, with standard test combinations from Merck, Darmstadt and Labor+Technik Eberhard Lehmann, Berlin, Germany).

Experimental protocol. Throughout the experiment, the animals inhaled oxygen-enriched air (FIO2=0.37). We measured respiratory variables under control conditions at zero inspired CO2 and at four elevated levels of about 3, 4, 6 and 8%, yielding arterial PCO2 values between 5.48 ±0.22 and 7.85 ±0.19 kPa. These CO2 steps were repeated twice, first after intravenous low-dose infusion (0.5 ml/min for 10 min) of methazolamide up to 3.5 ±0.1 mg·kg⁻¹, and second after high-dose infusion (2.5 ml/min for 10 min) up to 20.8 ±0.4 mg·kg⁻¹. The substance was dissolved in saline plus 1N NaOH plus 1N HCl, adjusted to pH 7.35-7.45 (solute concentration: 2.14 ±0.10 mg·ml⁻¹). The total period of measurements took about 5 hours (322 ± 8 min).

Data processing and statistical Analysis. Respiratory rate (fR=60/TI+TE), pulmonary ventilation (V=VT·fR) and tidal respiratory work (VT·ΔPTP) were calculated. The integrated tidal phrenic amplitude (IPNA) was normalized to the maximum arbitrary value achieved in each animal (14, 15). Unless otherwise indicated, data are means ± SEM. Group mean values resulting from application of methazolamide were compared with those under control conditions by paired-samples t-tests. In each animal, linear regression analysis was performed for various relationships between VT and ΔPTP and IPNA during CO2-inhalation. The resulting
individual slopes (and intercepts) likewise underwent paired t-tests to detect significant differences between experimental conditions. Differences were regarded as significant with $P_D \leq 0.05$. Statistical analysis was performed by SPSS 11.0 for Windows (SPSS, Chicago, IL).

**Results**

*General effects of methazolamide on pulmonary ventilation and related variables.* Table 1 summarizes the effects of methazolamide (MTZ) on eucapnic base-line ventilation, gas exchange and acid-base conditions. At low dose, the agent increased ventilation and tidal volume by about 50% and 45%, respectively, with no change in respiratory rate, and lowered the mean end tidal PCO$_2$ by 1.73 kPa. At high dose, the relative rises in ventilation and tidal volume were in the range of 165% and 112 %, respectively, along with about 3.6 kPa reduction in end tidal P$_{CO_2}$. Upon low-dose application, the arterial to end tidal CO$_2$ difference (D(a-et)CO$_2$) rose only transiently and reached the control range again (0.15 ±0.12 kPa) after about one hour. However, with high doses, the increases in D(a-et)CO$_2$ were large (2.64 ±0.14 and 1.64 ±0.15 kPa; Fig. 1) and persisted for the duration of the trial. Whereas the base excess (BE) remained stable for more than two hours during the control phase, MTZ reduced BE values in a dose-dependent manner by up to 8.3 mmol·l$^{-1}$, without significant change in blood lactate concentration. The substance did not exert any effect on mean arterial blood pressure. Likewise, there was no significant effect of MTZ on the dynamic lung compliance within the investigated range of respiratory drive: the mean $V_T$-ΔP$_{TP}$-relationship showed a non-linear characteristic that remained unaffected by MTZ at either dosage (Fig. 2).

Fig. 3 shows original recordings of tidal phrenic amplitude (IPNA), tidal volume ($V_T$) and transpulmonary pressure changes (ΔP$_{TP}$) at zero and elevated (6%) levels of inspired CO$_2$.
before and after application of MTZ. This example demonstrates that the substance does not impair the transmission of a CO\textsubscript{2}-induced phrenic drive into respiratory volume and pressure responses. When related to 10 units of a CO\textsubscript{2}-induced rise in tidal phrenic amplitude, the volume and pressure responses were 12.7 ml and 0.36 kPa under control conditions and 12.2 ml and 0.49 kPa or 11.6 ml and 0.55 kPa upon treatment with low- or high-dose MTZ, respectively.

Effect of low-dose methazolamide on the transmission of tidal phrenic nerve activity into tidal volume, transpulmonary pressure and respiratory work during hypercapnia. Fig. 4 depicts the translation of phrenic neuronal drive into mechanical respiratory responses as group mean values. The upper panel shows that after about 3.5 mg·kg\textsuperscript{-1} cumulative application of MTZ, the conversion of a CO\textsubscript{2}-induced rise of IPNA into V\textsubscript{T} amounted to 0.18 ±0.02 ml·kg\textsuperscript{-1}·unit\textsuperscript{-1}, being statistically not distinguishable from 0.20 ±0.02 ml·kg\textsuperscript{-1}·unit\textsuperscript{-1} under control conditions (P=0.47). Likewise, the middle panel does not show different slopes of the relationship between ΔPTP and IPNA under control conditions (0.014 ±0.002 kPa·unit\textsuperscript{-1}) and after low-dose MTZ (0.015 ±0.003 kPa·unit\textsuperscript{-1}, P=0.43). These data allow us to calculate respiratory work performance (V\textsubscript{T}·ΔPTP) as a function of IPNA (lower panel). Individual regression analysis revealed 0.27 ±0.05·ml·kg\textsuperscript{-1}·kPa·unit\textsuperscript{-1} as the mean slope of this relationship under control conditions, and demonstrates again that low-dose MTZ did not significantly change the mean slope to 0.30 ±0.06 ml·kg\textsuperscript{-1}·kPa·unit\textsuperscript{-1} (P=0.55).

Effect of high-dose methazolamide on the transmission of tidal phrenic nerve activity into respiratory work performance during hypercapnia. Fig. 5 compares the effects of MTZ and ACTZ at high doses on respiratory work performance (V\textsubscript{T}·ΔPTP) as a function of tidal phrenic nerve discharge (IPNA). To ensure complete inhibition of red cell carbonic anhydrase by both substances, high cumulative doses were chosen such that similarly large and persisting P(a-et)CO\textsubscript{2} differences resulted (Fig. 1). In much the same way as described for low-dose MTZ,
there was no attenuating effect of high-dose MTZ on the slope of the $V_T$-$\Delta P_{TP}$ versus IPNA relationship, changing from the control mean (0.27 ±0.05·ml·kg$^{-1}$·kPa·unit$^{-1}$, see above) to a nearly identical value of 0.28 ±0.07 ml·kg$^{-1}$·kPa·unit$^{-1}$ (P=0.83). For comparison, the right panel of Fig. 5 shows that high-dose ACTZ distinctly attenuates the mean respiratory work performance in response to a given neural phrenic drive, namely by more than 50% (15). Since the average control-line in the ACTZ study is steeper than that in the present study on MTZ, it is important to note that the different effects of both substances were independent of individual control slopes.

**Discussion**

The present study explores respiratory effects of methazolamide (MTZ) in spontaneously breathing rabbits, making use of the same protocol as previously (14, 15) to study effects of acetazolamide (ACTZ). Thereby, we assessed respiratory neuro-mechanical coupling by simultaneous recordings of phrenic nerve activity, tidal volume and transpulmonary pressure. The main result of this study was that neither low nor high doses of the more lipophilic CA inhibitor MTZ significantly impaired the transmission of a CO$_2$-induced rise in phrenic nerve activity into adequate changes of volume, pressure or respiratory work. These results suggest that respiratory muscles are able to function normally independent of local CA activity. In retrospect, it follows that the impairment of respiratory neuromuscular coupling observed previously with low and high dose ACTZ likewise may have been largely independent of CA inhibition.

*Effects of MTZ on pulmonary ventilation, gas-exchange and acid-base conditions.* Similar to ACTZ in clinical use, MTZ at a low cumulative dose of 3.5 mg·kg$^{-1}$ gave rise to a small but
transient arterial-to-end tidal PCO₂ gradient that disappeared after 60 min, indicating that red
cell carbonic anhydrase (CA) was not yet completely inhibited. Higher cumulative doses of
MTZ up to ~20 mg·kg⁻¹ caused a large and persistent arterial-to-end-tidal PCO₂ gradient (Fig.
1), which can be ascribed to complete red cell CA inhibition. Although this initially will cause
CO₂/H₂CO₃ disequilibrium in the blood after lung passage, samples obtained from the femor-
al artery had time to achieve high PCO₂ values despite the delayed chemical equilibrium (Ta-
ble 1).

In much the same way as ACTZ, both low and high dose MTZ increased steady state ventila-
tion. However, MTZ elicited more pronounced respiratory responses at much lower plasma
centations than those required for ACTZ. Since MTZ and ACTZ have about equal affini-
ties for CA isoforms II and IV, the higher efficacy of MTZ to drive pulmonary ventilation
may be because this highly lipophilic sulfonamide more easily permeates through cell mem-
branes. Therefore MTZ is distributed rather rapidly and even in many tissues including brain
cells, where it accumulates about 4-fold compared to ACTZ (20). Thus, besides the complete
inhibition of red cell CA, effective blockade of membrane-bound CA at the luminal surface of
brain capillary endothelium will impair removal of CO₂ from brain tissue and thus add to the
chemical drive from central chemoreceptors (29, 33). The latter explanation also applies to
low-dose MTZ as smaller amounts of this highly diffusible inhibitor may effectively block the
easily accessible brain endothelial CA as well and cause some degree of tissue acidosis (29).

Apart from respiratory (brain) tissue acidosis, we cannot exclude additional driving forces on
ventilation by the considerable dose-dependent non-respiratory acid accumulation. In humans,
metabolic acidosis normally leads to an increase in ventilation, for which the peripheral che-
moreceptors may be responsible (26, 27, 30, 36). Already at low-dose, ACTZ largely reduced
the ventilatory response to hypoxia in humans and cats (35, 37), but even a high dose of the
much more effective CA inhibitor MTZ entirely failed on that score at least in cats (34). In the
same species, only ACTZ, but not MTZ, diminished also the CO₂ sensitivity of the peripheral chemoreflex loop (3). In our experiments, we maintained relatively high oxygen tensions to minimize influences from the carotid bodies. However, since MTZ does not seem to inhibit peripheral chemoreceptors, we cannot completely neglect some additional activation by metabolic acidosis.

**Effect of MTZ on blood pressure.** In rabbits, MTZ did not affect the mean blood pressure, whereas ACTZ in high doses led to about 20% reduction (15). For ACTZ, vasodilatation at various locations in the systemic circulation could be demonstrated, in humans already occurring at low doses (31), whereby evidence is strengthened that ACTZ exerts a direct vasodilator effect mediated by vascular K_Ca channel activation (22). On the other hand, MTZ was not able to counteract hypoxic pulmonary vasoconstriction, as did ACTZ by inhibiting the rise of intracellular [Ca^{2+}] in pulmonary artery smooth muscle cells (11, 25). Thus, at least in the pulmonary vascular bed and under hypoxic conditions a vasodilator effect independent of CA inhibition seems to be lacking for MTZ. It needs further investigation to decide, whether this observation also extends to other regions of the circulatory system and thus may explain why MTZ does not induce general systemic hypotension.

**Effect of MTZ on dynamic compliance.** There was no significant effect of low- or high-dose MTZ on the dynamic lung compliance within the investigated range of respiratory drive. This agrees with our earlier finding that also ACTZ at neither dosage did significantly affect the relationship between V_T and ΔP_{TP} (14, 15) and is speaking against any role of carbonic anhydrase for the adjustment of bronchial smooth muscular tone. The fact that MTZ has no adverse effect on lung stiffness is important for assessing the transmission of phrenic nerve activity into respiratory muscle performance from volume and pressure responses (see below).

**No attenuating effect of MTZ on respiratory muscle performance.** Low-dose MTZ did not affect the transmission of phrenic neuronal drive into respiratory volume and pressure res-
responses, thus bearing a striking difference to ACTZ that at a comparable level of CA inhibition, already accomplished respiratory muscle weakening by more than two thirds (14, 15). Furthermore, our finding that even high-dose MTZ, despite complete inhibition of intra- and extracellular CA, did not impair respiratory neuro-mechanical transmission seems rather unexpected in the light of the well-documented role of this enzyme in the regulation of intracellular pH in skeletal muscle (10). However, the efficiency of neuro-mechanical coupling seems to be independent of (CO₂-induced) pH changes already in the control situation, since Vₜ or tidal ΔPₜₚ and IPNA showed rather sound linear correlations with growing hypercapnia, whereby correlation coefficients in the seven animals ranged from 0.89 to 1.00 or from 0.91 to 0.98, respectively. Likewise, in humans, diaphragmatic contractility remained unaffected by hypercapnic acidosis, in contrast to that of non-respiratory skeletal muscle, e.g. adductor pollicis (19).

Although we cannot differentiate between synergistic contributions from the diaphragm and the intercostal muscles (6), we attempted to assess the effect of MTZ on respiratory muscle performance with sufficient accuracy from the relationships between phrenic neural discharge and overall volume/pressure responses to hypercapnia. These relationships showed fair linearity not only under control conditions and upon low-dose MTZ, but also in the majority of animals upon the largest respiratory drive elicited by high-dose MTZ. Thereby, linear correlation coefficients were highest in 6 out of 7 animals (ranging from 0.80 to 0.99), and only in one case the linear compared to the non-linear correlation was smaller (r =0.96 versus 0.98).

Our present data raise the question, by which mechanisms MTZ and the more commonly used CA inhibitor ACTZ may act on respiratory muscles. Previously we demonstrated with the same protocol that a maximum dose of ACTZ, safely assumed to inhibit also intracellular CA completely, did not appreciably add to the effect of a low (clinical) dose (14, 15). This fairly agrees with our present observation that normal respiratory muscle function persisted despite
effective CA inhibition by MTZ. Thus, the present data add to the growing evidence that the sulfonamides ACTZ and MTZ may not only act as CA-inhibitors, but also by other properties may exert different additional effects on the (cardio-) respiratory system.

Possible mechanisms to explain different modes of action of MTZ and ACTZ. In retrospect, it follows that the previously observed respiratory muscle weakening by ACTZ (14, 15) is most probably due to a side effect of this agent. In skeletal muscles of rats, such an effect could be demonstrated for ACTZ but not for MTZ (38), consisting in a strong activation of large conductance calcium-stimulated potassium (BK) channels, which would limit intracellular Ca\(^{2+}\) accumulation upon depolarization and prolong hyperpolarisation. In line with this, ACTZ but not MTZ was able to prevent an insulin-induced hypokalaemic periodic muscle paralysis in rats (40). Similarly independent of CA inhibition, ACTZ improved symptoms of vacuolar myopathy upon experimental K\(^{+}\)-depletion, not only by stimulating BK channels but also by reducing lactate efflux (39). Normally, lactate efflux from the muscle decreases upon inhibition of extracellular CA, as shown in rats (42) and in exercising humans (17, 23), implying the high importance of muscular CA iso-enzymes in lactate (and pH) regulation for work capacity in exercising humans (21). Therefore, it remains unclear, whether adverse effects of ACTZ on skeletal muscles in healthy subjects, such as muscle fatigue and decreased exercise tolerance, are related to CA inhibition and should thus occur on treatment with MTZ as well, or whether they are a matter of additional specific effects of ACTZ on lactate efflux or BK channels.

General aspects for a role of MTZ in respiratory medicine. Unfortunately, data on the respiratory effects of MTZ in humans are scarce. However, MTZ leads to metabolic acidosis like ACTZ and improves the symptoms of Acute Mountain Sickness (7, 43). The different effects of ACTZ and MTZ on respiratory muscle function that we found must also have consequences for the control of breathing, e.g. during sleep or in obstructive lung disease. With
respect to control theory, a higher gain of the closed feedback loop implies a growing risk to develop ventilatory instability. In our animal model, loop gain analysis revealed that both substances contributed to ventilatory stability in different ways, in case of ACTZ via a decreased controller gain, but in case of MTZ by an improved “restoring function”, in the sense of a decreased “plant” gain (16).

_Perspectives and Significance._ The present study demonstrates for the first time that methazolamide (MTZ) unlike acetazolamide (ACTZ) does not impair respiratory muscle performance in rabbits. This finding adds to the growing evidence that different sulfonamide inhibitors of carbonic anhydrase (CA) may exert important pharmacological effects besides CA inhibition. The inhibitory effect of ACTZ on (striated) respiratory muscles may relate to activation of large-conductance Ca^{2+} dependent potassium (BK) channels, a property not shared by MTZ. The presented animal model opens perspectives for future research on the possible significance of respiratory muscle function for the control of breathing. Analysis of the respiratory control system under closed loop conditions would be a useful tool to predict whether MTZ, so far not used for medication of respiratory diseases, may exert stabilizing influences on breathing without adverse respiratory muscle weakening.
Acknowledgment: We would like to thank Professor Dr. Martin Wiemann for his helpful and critical comments on our manuscript. The expert computer-aided data processing by Arne Sandfort is also gratefully acknowledged.
References


[35] Teppema LJ, Bijl H, Romberg RR, Dahan A. Antioxidants reverse


Legends

Figure 1
Dose dependent effect of methazolamide (MTZ) on the PCO$_2$ gradient between blood and the alveolar space.
Values are means ± SEM (N=7) of arterial (closed circles) and end tidal (open circles) PCO$_2$ from repeated measurements under eucapnic conditions. For comparison, the broken lines refer to previous data on acetazolamide (15).
The large PCO$_2$ gradient between blood and alveolar space with the high cumulative dose of MTZ indicates complete inhibition of red cell carbonic anhydrase.

Figure 2
The role of methazolamide (MTZ) in CO$_2$-induced changes of dynamic compliance.
Tidal volume (V$_T$) is depicted as function of tidal transpulmonary pressure change (∆P$_{TP}$).
Values are means ± SEM of N=7 rabbits obtained at different levels of inhaled CO$_2$ under control conditions (closed circles) and after application of MTZ, either at low-dose (open circles) or at high-dose (open triangles).
There is a uniform non-linear relationship between V$_T$ and ∆P$_{TP}$, representing the dynamic compliance at different levels of respiratory drive before and after application of low- or high-dose MTZ.
Figure 3

Recordings of different respiratory variables before and after application of methazolamide.

From top to bottom: Phrenic nerve compound potential (PNA), integrated tidal phrenic nerve activity (IPNA), tidal volume (Vₜ), tidal transpulmonary pressure changes (ΔPₜₚ) and end-tidal PCO₂ (PetCO₂). Variables are recorded in a male rabbit (3.25 kg) inhaling oxygen-enriched air (left panels) and additionally CO₂ (right panels), both under control conditions and after application of low-dose (LD) or high-dose (HD) methazolamide (MTZ). In case of reduced amplification, bars added to the right panels indicate 40 units for IPNA, 50 ml for Vₜ and 1 kPa for ΔPₜₚ.

Methazolamide does not impair the transmission of a hypercapnic neural drive into changes of Vₜ and ΔPₜₚ.

Figure 4

Effect of low-dose methazolamide (MTZ) on respiratory neuro-mechanical transmission.

Upper panel: Tidal volume (Vₜ) as a function of integrated tidal phrenic nerve activity (IPNA). Middle panel: Tidal transpulmonary pressure change (ΔPₜₚ) as a function of IPNA. Lower panel: Respiratory work performance (Vₜ·ΔPₜₚ) as a function of IPNA. Values are means ± SEM (N=7) obtained at different levels of inhaled CO₂ under control conditions (closed circles) and after application of low-dose (LD) MTZ (open circles).

Note that low-dose MTZ does not impair neuro-mechanical transmission, neither in terms of the tidal volume response nor with respect to that of transpulmonary pressure or respiratory work performance.
Figure 5

Effects of high-dose methazolamide (MTZ) and acetazolamide (ACTZ) on respiratory work performance.

Neuro-mechanical transmission is expressed as tidal respiratory work ($V_T \cdot \Delta P_{TP}$) related to the integrated tidal phrenic nerve activity (IPNA). Data are means ± SEM (N=7) obtained at different levels of inhaled CO$_2$ before and after treatment with high-doses (HD) of either MTZ (this study) or ACTZ from a previous study (15).

At doses sufficient for complete CA inhibition, MTZ does not attenuate neuro-mechanical transmission, whereas ACTZ reduces it by about 50%.
Table 1. Effects of methazolamide on eucapnic base-line values before hypercapnia.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>METHAZOLAMIDE Low-dose</th>
<th>METHAZOLAMIDE High-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>V [ml·kg⁻¹·min⁻¹]</td>
<td>186.3 ± 16.7</td>
<td>279.5 ± 24.5**</td>
<td>493.3 ± 71.0**</td>
</tr>
<tr>
<td>V_T [ml·kg⁻¹]</td>
<td>5.6 ± 0.3</td>
<td>8.0 ± 0.6***</td>
<td>11.8 ± 0.7***</td>
</tr>
<tr>
<td>f_R [min⁻¹]</td>
<td>34.2 ± 4.1</td>
<td>36.3 ± 4.7</td>
<td>42.3 ± 5.8</td>
</tr>
<tr>
<td>ΔP_TP [kPa]</td>
<td>0.43 ± 0.05</td>
<td>0.48 ± 0.06*</td>
<td>0.76 ± 0.09**</td>
</tr>
<tr>
<td>IPNA [units]</td>
<td>27.1 ± 5.0</td>
<td>38.4 ± 4.3*</td>
<td>63.5 ± 2.8**</td>
</tr>
<tr>
<td>PaCO₂ [kPa]</td>
<td>5.55 ± 0.24</td>
<td>4.75 ± 0.19**</td>
<td>4.48 ± 0.15***</td>
</tr>
<tr>
<td>PetCO₂ [kPa]</td>
<td>5.35 ± 0.23</td>
<td>3.62 ± 0.17***</td>
<td>1.76 ± 0.14***</td>
</tr>
<tr>
<td>pHa</td>
<td>7.425 ± 0.007</td>
<td>7.419 ± 0.019</td>
<td>7.356 ± 0.014**</td>
</tr>
<tr>
<td>BE [mmol·l⁻¹]</td>
<td>2.6 ± 1.1</td>
<td>-0.7 ± 1.3*</td>
<td>-5.7 ± 1.0***</td>
</tr>
<tr>
<td>Lac [mmol·l⁻¹]</td>
<td>2.8 ± 0.6</td>
<td>2.7 ± 0.4</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>PaO₂ [kPa]</td>
<td>24.5 ± 0.9</td>
<td>27.1 ± 0.7**</td>
<td>29.5 ± 0.7***</td>
</tr>
<tr>
<td>MAP [kPa]</td>
<td>15.0 ± 0.5</td>
<td>15.0 ± 0.9</td>
<td>14.7 ± 0.6</td>
</tr>
</tbody>
</table>

Means ±SEM (N=7) of minute ventilation (V), tidal volume (V_T), respiratory rate (f_R), trans-pulmonary pressure changes (ΔP_TP), integrated phrenic nerve activity (IPNA), end-tidal and arterial PCO₂ (PetCO₂, PaCO₂), arterial pH (pHa), base excess (BE), lactate concentration (Lac), arterial PO₂ (PaO₂) and mean arterial blood pressure (MAP) under control conditions and measured up to 20 min after low-dose (3.5 ±0.1 mg·kg⁻¹) and high-dose (20.8 ±0.4 mg·kg⁻¹) application of methazolamide. Significant difference of means by paired samples t-test: *P ≤0.05, ** P ≤0.01 and *** P ≤0.001.
Cumulative dose of methazolamide [mg·kg⁻¹] vs. PCO₂ [kPa]

- Endtidal
- Arterial

Ctl

Cumulative dose of methazolamide [mg·kg⁻¹]
Eucapnia

Hypercapnia

- **PNA [µV]**
  - Control
  - LD MTZ
  - HD MTZ

- **IPNA [units]**
  - Control
  - LD MTZ
  - HD MTZ

- **Vₜ [ml]**
  - Control
  - LD MTZ
  - HD MTZ

- **ΔPₜₚ [kPa]**
  - Control
  - LD MTZ
  - HD MTZ

- **PetCO₂ [kPa]**
  - Control
  - LD MTZ
  - HD MTZ

Control              LD MTZ            HD MTZ                  Control              LD MTZ            HD MTZ