Arterial [H\(^+\)] and the ventilatory response to hypoxia in humans: influence of acetazolamide-induced metabolic acidosis

Luc J. Teppema, Eveline L. A. van Dorp, and Albert Dahan

Department of Anesthesiology, Leiden University Medical Centre, Leiden, The Netherlands

Submitted 3 August 2009; accepted in final form 27 October 2009

Teppema LJ, van Dorp EL, Dahan A. Arterial [H\(^+\)] and the ventilatory response to hypoxia in humans: influence of acetazolamide-induced metabolic acidosis. Am J Physiol Lung Cell Mol Physiol 298: L89–L95, 2010. First published October 30, 2009; doi:10.1152/ajplung.00255.2009.—In this study, we investigated possible separate effects of H\(^+\) ions and CO\(_2\) on hypoxic sensitivity in humans. We also examined whether hypoxic sensitivity, conventionally defined as the ratio of (hypoxic – normoxic) ventilation over (hypoxic – normoxic) Hb oxygen saturation can also be estimated by taking the ratio (hypoxic – normoxic) ventilation over (logPa\(_{O_2}\) hypoxia – logPa\(_{O_2}\) normoxia), enabling one to measure the hypoxic response independently from potential confounding influences of changes in position of the Hb oxygen saturation curve. We used acetazolamide to induce a metabolic acidosis. To determine the acute hypoxic response (AHR), we performed step decreases in end-tidal PO\(_2\) to ~50 Torr lasting 5 min each at three different constant end-tidal PCO\(_2\) levels. Nine subjects ingested 250 mg of acetazolamide or placebo every 8 h for 3 days in a randomized double-blind crossover design. The metabolic acidosis was accompanied by a rise in ventilation, a substantial fall in PA\(_{CO_2}\), and a parallel leftward shift in the ventilatory CO\(_2\) response curve. In placebo, PCO\(_2\) induced equal relative increases in hypoxic sensitivity (O\(_2\)-CO\(_2\) interaction) regardless of the way it was defined. Acetazolamide shifted the response line representing the relationship between hypoxic sensitivity and arterial [H\(^+\)] (H\(^+\)) to higher values of [H\(^+\)] without altering its slope, indicating that it did not affect the O\(_2\)-CO\(_2\) interaction. So, in contrast to an earlier belief, CO\(_2\) and H\(^+\) have separate effects on hypoxic sensitivity. This was also supported by the finding that infusion of bicarbonate caused a leftward shift of the hypoxic sensitivity-[H\(^+\)] relationship in placebo and acetazolamide. A specific inhibitory effect of acetazolamide on hypoxic sensitivity was not demonstrated.

The hypoxic ventilatory response in humans is biphasic and consists of an initial rise in ventilation initiated by the carotid bodies followed by a secondary roll-off, usually designated as hypoxic ventilatory decline (HVD) (8, 13, 16, 25). The magnitude of this decline is proportionally related to the initial rise in ventilation in response to acute hypoxia and thus also to the intensity of the hypoxic stimulus (8, 18, 25). Measurement of the ventilatory response to hypoxia without the “confounding” influence of HVD is possible by exposing subjects to brief step-wise changes in end-tidal PO\(_2\) lasting 3–5 min, a period too short for HVD to develop. Consequently, the ventilatory response to such an acute hypoxic challenge (the acute hypoxic response, AHR) is attributed to the peripheral chemoreceptors in the carotid bodies. The magnitude of the AHR can be influenced by many factors such as the background arterial PCO\(_2\). A rise in PA\(_{CO_2}\) augments the AHR, and this is known as multiplicative O\(_2\)-CO\(_2\) interaction (6, 7, 10). This phenomenon is thought to reside in the carotid bodies (15, 22, 26), but its mechanism at the molecular level in oxygen-sensitive type I cells remains to be elucidated (29). A rise in PA\(_{CO_2}\) not only leads to extracellular acidosis (acidemia) but also to increases in PCO\(_2\) and [H\(^+\)] in carotid body cells. The relative contribution of these three changes during hypercapnia to the increase in carotid body activity remains a controversial issue, some studies suggesting a specific effect of the extracellular (i.e., arterial) pH supporting virtually no effect of molecular CO\(_2\) (12, 19, 23, 44), whereas others showed carotid body stimulation by CO\(_2\) at constant extracellular pH (2, 14) or type I cell activation by both isocapnic changes in external pH and CO\(_2\) (4).

In humans, the interaction between hypoxia and metabolic acidosis and/or hypercapnia has been ascribed to a unique effect of arterial [H\(^+\)] on hypoxic sensitivity (17, 24). However, metabolic acidosis does not always lead to an augmented hypoxic response. For example, the carbonic anhydrase inhibitors acetazolamide and benzolamide produce a metabolic acidosis without increasing the isocapnic AHR (20, 37, 38, 43). Apart from a substantial acidemia, acetazolamide also causes a substantial drop in arterial PCO\(_2\) induced by a carotid body-mediated rise in ventilation (35, 38, 41). When the PA\(_{CO_2}\) is kept at the much higher pre-drug resting value, the AHR after acetazolamide becomes higher than control (43). These findings suggest that unless these carbonic anhydrase inhibitors have specific effects on the carotid bodies, arterial H\(^+\) and CO\(_2\) may have independent effects on the AHR. The aim of this study was to reinvestigate this issue. To quantify the O\(_2\)-CO\(_2\) interaction on ventilation, we measured the AHR at three levels of constant arterial PCO\(_2\) in healthy volunteers after placebo and after induction of a metabolic acidosis with acetazolamide. In both placebo and metabolic acidosis, we determined the relationship between hypoxic sensitivity and the measured arterial [H\(^+\)] at these three CO\(_2\) levels.

Due to the linear relationship between ventilation and arterial hemoglobin oxygen saturation, hypoxic sensitivity is usually expressed as the ratio delta ventilation over delta saturation (31, 32). A potential problem, however, with metabolic acidosis is that the Hb saturation curve may shift to the right (Bohr effect) so that the ratio \(\Delta\) ventilation/\(\Delta\) Sa\(_{O_2}\) may underestimate “true” hypoxic sensitivity, due to the fact that, at the level of arterial blood, the Pa\(_{O_2}\), rather than Sa\(_{O_2}\), represents the stimulus to the carotid bodies (21, 30). Therefore, in this regard, it would be preferable to express hypoxic sensitivity with the aid of a parameter directly derived from the measured arterial PO\(_2\). In the cat, it has been demonstrated that in the hypoxic range, ventilation is a linear function of log Pa\(_{O_2}\) (Ref. 33; also Teppema, unpublished observations). In this study, we examined whether it would be justified to use the ratio delta ventilation over delta log Pa\(_{O_2}\) (in the range below 100 Torr) to determine...
relative increases in hypoxic sensitivity induced by alterations in arterial PCO2. To this aim, both in the placebo condition and following acetazolamide, hypoxic sensitivity was either defined in the conventional way, namely as the ratio Δventilation/ΔSaO2, or as the ratio Δventilation/Δlog PaO2, and CO2-induced relative increases in sensitivities were compared. In the placebo condition, both scenarios yielded almost identical outcomes, suggesting that a realistic estimation of changes in hypoxic sensitivity can be made without possible confounding effects of changes in saturation.

METHODS

Subjects and apparatus. Nine healthy volunteers (ages 20–23 yr; 4 men; 5 women using contraceptives) participated in this study after approval from the Leiden University Medical Centre human ethics committee. Written informed consent was obtained from all subjects, and the protocol was performed conforming to standards in the latest version of the Declaration of Helsinki. Throughout the testing schedule, subjects were instructed to abstain from caffeine and alcohol. On test days, a catheter was inserted into the right antecubital vein for infusion of a bicarbonate solution and into the left radial artery for arterial blood gas analysis and continuous blood pressure measurement.

Details of measuring ventilation, respiratory gases, and oxygen saturation and the technique of dynamic end-tidal forcing are provided elsewhere (40, 41).

Study design. Following a randomized crossover double-blind design, subjects ingested either acetazolamide (250 mg/dose) or placebo every 8 h for 3 days ending the morning of outcome measurements. Each treatment period was separated by a 14-day washout period to overcome potential crossover effects of acetazolamide. On test days, 4 h after the last dose was taken, resting ventilation, blood pressure, end-tidal PO2 and PCO2 (PET O2 and PET CO2), and arterial acid base and electrolyte status were measured. Subsequently, the following protocols were performed.

Experiments in hyperoxia, normoxia, and hypoxia. At three different levels of constant PET CO2, we first followed a protocol aimed to determine steady-state ventilation in normoxia and mild hypoxia after the subjects were exposed to a 5-min bout of hypoxia. First, subjects breathed quietly in normoxia (PET O2 100–110 Torr) at a PET CO2 2–3 Torr above resting (level 1). After ~6 min, PET O2 was increased to ~150 Torr for 5–8 min and then lowered stepwise to 49–50 Torr for 5 min to enable the AHR to develop. The hypoxic exposures were limited to 5 min to prevent HVD from developing (e.g., see Ref. 8). Arterial samples were collected at the end of the normoxic and hypoxic periods. Hypoxia was terminated by returning to mild hyperoxia (PET O2 ~ 150 Torr) for 5 min, at which time the subjects breathed room air for 15 min. This procedure was repeated at constant PET CO2 values that were higher than level 1 by 2–3 Torr (level 2) and 4–6 Torr (level 3), respectively. This whole sequence was repeated after infusion of bicarbonate.

Bicarbonate infusion. The subjects were allowed to breathe quietly at a PET CO2 ~3 Torr above resting level in normoxia (PET O2 100–110 Torr). Then, at this constant PET CO2, sodium bicarbonate (8.4%) was infused in a quantity anticipated to change base excess by ~5–6 meq/l using the formula dose = 0.3 × body weight × 6. This is based on the assumption that the extracellular fluid is ~33% of total body weight (e.g., see Ref. 45). In most subjects, the infusion lasted 30–45 min. Between subjects, infused volumes varied between 120 and 180 ml.

Data analysis. At all PET CO2 levels, breath-to-breath inspired ventilation of the last minute of normoxia and hyperoxia and of the fourth minute of hypoxia were averaged. By plotting ventilation against PET CO2, we determined CO2 response curves in terms of slope and x-intercept, using the formula V i = S(PCO2 – B), where S = total ventilatory CO2 sensitivity (unit l·min−1·Torr−1) and B = the extrapolated PaCO2 at zero ventilation (apnoeic threshold; unit Torr).

As outlined above, hypoxic steps were performed starting from a mild hyperoxic level. It is our experience that mild hyperoxia can cause a small rise in ventilation, possibly due to a diminished Haldane effect and/or a small decrease in cerebral blood flow (e.g., Ref. 7). Consequently, the difference between hyperoxic and hypoxic ventilation can be smaller than that between normoxic and hypoxic ventilation. Therefore, to limit underestimation of the contribution of the carotid bodies to the initial increase in ventilation during the first 3 min of hypoxia, we considered the difference in ventilation between normoxic and hypoxic ventilation as the response mediated by the carotid bodies. Normoxic levels of ventilation were those obtained over the last minute of normoxia immediately preceding the mild hyperoxic step (Fig. 1, C, F, and I). In placebo, hypoxic sensitivity was first defined as [Vi (hypoxia) – Vi (normoxia)]/(SaO2 (hypoxia) – SaO2 (normoxia)]. Then, hypoxic sensitivity was plotted as a function of arterial [H+] measured at the three isocapnic PaCO2 levels. Subsequently, the ratio [Vi (hypoxia) – Vi (normoxia)]/[logPaO2 (hypoxia) – logPaO2 (normoxia)] was taken as a measure of hypoxic sensitivity; also, this ratio was plotted as a function of arterial [H+] at the three isocapnic PaCO2 levels (note that the normoxic PaCO2 was always lower than 100 Torr). The relative CO2-induced changes in hypoxic sensitivities were determined in both cases and appeared to be almost identical (see RESULTS). Therefore, in comparing placebo and metabolic acidosis, hypoxic sensitivities were presented using the ratio ΔV/ΔlogPaO2, avoiding its possibly erroneous estimation in a condition of metabolic acidosis.

To detect significant effects of acetazolamide on resting parameters and slopes and intercepts of the ventilatory CO2 response curves, a paired t-test was performed. An analysis of variance was performed on hypoxic sensitivities at different CO2 values. Differences between CO2 levels were tested with the Student-Newman-Keuls test. P values < 0.05 were considered significant. Paired comparisons were made (t-tests) in placebo and acetazolamide between relative changes in hypoxic sensitivities, either defined as ΔV/ΔSaO2 or ΔV/ΔlogPaO2. Sometimes hypoxic sensitivities were not normally distributed and were indicated. A nonparametric test (Wilcoxon signed-rank test) was used. Unless otherwise indicated, values are means ± SD.

RESULTS

Resting conditions. In accordance with previous studies (38, 41), acetazolamide induced a metabolic acidosis and a significant rise in mean resting ventilation coupled to a rise in end-tidal and arterial PO2 and a fall in arterial and end-tidal PCO2 (Table 1).

CO2 responses in normoxia, hyperoxia, and acute hypoxia. Table 2 summarizes the effect of acetazolamide on the steady-state ventilatory CO2 response curves during normoxia and mild hyperoxia. In normoxia, acetazolamide shifted the curve to lower PET CO2 values without changing the slope, an effect expected to result from a metabolic acidosis. In mild hyperoxia, ventilatory CO2 sensitivity showed a small but significant increase following acetazolamide, whereas the apnoeic threshold (x-intercept extrapolated down to zero ventilation) showed a small significant fall.

Hypoxic sensitivity as ΔV/ΔlogPaO2 or ΔV/ΔSaO2 in placebo and following acetazolamide. Table 3 summarizes the percentages by which hypoxic sensitivity, either defined as ΔV/Δlog PaO2 or ΔV/ΔSaO2, augmented with a rise in PaCO2. Because following acetazolamide, one subject did not respond to hypoxia at the lowest isocapnic PCO2 level, we calculated individual hypoxic sensitivities in eight subjects enabling us to perform paired comparisons with t-tests. In placebo, no signif-
ACETAZOLAMIDE-INDUCED METABOLIC ACIDOSIS AND HYPOXIC RESPONSE

**Table 1. Effect of acetazolamide on resting parameters**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Acetazolamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PetO₂, Torr</td>
<td>108.9±4.9</td>
<td>114.0±3.6</td>
<td>0.028</td>
</tr>
<tr>
<td>PaO₂, Torr</td>
<td>91.8±6.4</td>
<td>95.8±4.1</td>
<td>0.042</td>
</tr>
<tr>
<td>PetCO₂, Torr</td>
<td>36.5±3.5</td>
<td>31.1±4.2</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>PaCO₂, Torr</td>
<td>39.0±3.5</td>
<td>32.4±3.3</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Vₑ, l·min⁻¹</td>
<td>8.8±1.3</td>
<td>10.2±1.4</td>
<td>0.011</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>96.8±0.6</td>
<td>97.0±0.0</td>
<td>ns</td>
</tr>
<tr>
<td>SₐO₂ (%)</td>
<td>96.7±0.6</td>
<td>96.9±3.8</td>
<td>ns</td>
</tr>
<tr>
<td>pH</td>
<td>7.392±0.02</td>
<td>7.305±0.02</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>[HCO₃⁻], mM</td>
<td>24.0±2.6</td>
<td>16.1±1.2</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Base excess, mM</td>
<td>−1.0±2.5</td>
<td>−10.0±1.1</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>[Na⁺], mM</td>
<td>138.1±1.7</td>
<td>139.0±1.9</td>
<td>ns</td>
</tr>
<tr>
<td>[K⁺], mM</td>
<td>3.8±0.3</td>
<td>3.5±0.1</td>
<td>0.018</td>
</tr>
<tr>
<td>[glucose], mg/dl</td>
<td>101.3±9.2</td>
<td>101.1±7.1</td>
<td>ns</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>36.6±3.8</td>
<td>38.1±3.1</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Effect of acetazolamide on acid base status and respiratory parameters in 9 subjects. Mean values ± SD are shown; ns, not significant.

**Table 2. Effect of acetazolamide on CO₂ responses under normoxia and mild hyperoxia**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Acetazolamide</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxic S (l·min⁻¹·Torr⁻¹)</td>
<td>1.88±0.89</td>
<td>2.68±1.56</td>
<td>0.040</td>
</tr>
<tr>
<td>Hyperoxic B, Torr</td>
<td>30.8±3.5</td>
<td>26.4±4.0</td>
<td>0.006</td>
</tr>
<tr>
<td>Normoxic S (l·min⁻¹·Torr⁻¹)</td>
<td>1.75±0.78</td>
<td>2.06±1.03</td>
<td>0.207</td>
</tr>
<tr>
<td>Normoxic B, Torr</td>
<td>30.6±3.0</td>
<td>24.6±4.0</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Effect of acetazolamide on ventilatory CO₂ response curves. S, ventilatory CO₂ sensitivity; B, apnoeic threshold, i.e., the extrapolated end-tidal PCO₂ at zero ventilation. Data are means ± SD from 9 subjects.

Fig. 1. Acute hypoxic responses at 3 end-tidal PCO₂ levels. A–J show the experimental protocol (steady-state normoxia, steady-state mild hyperoxia, and acute hypoxia) at 3 levels of end-tidal PCO₂ in 1 representative subject after placebo. A–C refer to the lowest PCO₂ level (level 1, ~2 Torr above resting), D–F to a level ~7 Torr above resting. The acute hypoxic response increases with rising PCO₂ indicating positive O₂–CO₂ interaction. N, last minute in normoxia over which normoxic ventilation was averaged to calculate the ventilatory response from hypoxia.

significant differences were found, so with normal acid base status relative increases in hypoxic sensitivities are the same regardless of how the sensitivity is defined. Following acetazolamide, going from the lowest to the intermediate PCO₂ level, the relative increase in sensitivity was significantly smaller when defined as the ratio $\frac{\Delta V}{\Delta S_{A,o}}$ (Table 3), an effect that could be anticipated if the HbO₂ saturation curve would undergo a rightward shift due to the acidosis, thus resulting in an underestimation of hypoxic sensitivity (we were not able to measure $P_{50}$ values). The overall (step 1→3) relative sensitivity was also smaller when defined as $\frac{\Delta V}{\Delta S_{A,o}}$. So, an absence of a rightward shift of the HbO₂ saturation curve cannot be excluded.

Effect of acetazolamide on the AHR. Because the placebo data showed that hypoxic sensitivity can also be estimated by using $\frac{\Delta V}{\Delta \log P_{a,o}}$, we used this ratio in our further data analysis. Figure 1 shows hypoxic experiments in a representative subject at three levels of $P_{\text{ET,CO₂}}$. The influence of acetazolamide on hypoxic sensitivity is shown in Fig. 2. Data points in this figure represent mean values for hypoxic sensitivity defined as $\Delta V/\Delta \log P_{a,o}$ at three different average arterial H⁺ concentrations obtained by changing $P_{a,CO₂}$ in eight
Table 3. Relative increases in hypoxic sensitivities defined as $\Delta V/\Delta S_{O_2}$ and $\Delta V/\Delta \log Pa_{O_2}$, respectively, in eight subjects

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Increase in $\Delta V/\Delta S_{O_2}$</th>
<th>% Increase in $\Delta V/\Delta \log Pa_{O_2}$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Pco2 level 1 $\rightarrow$ 2</td>
<td>102±26</td>
<td>101±20</td>
</tr>
<tr>
<td></td>
<td>Pco2 level 2 $\rightarrow$ 3</td>
<td>32±6</td>
<td>38±11</td>
</tr>
<tr>
<td></td>
<td>Pco2 level 1 $\rightarrow$ 3</td>
<td>159±24</td>
<td>172±30</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>Pco2 level 1 $\rightarrow$ 2</td>
<td>75±14</td>
<td>90±14</td>
</tr>
<tr>
<td></td>
<td>Pco2 level 2 $\rightarrow$ 3</td>
<td>96±45</td>
<td>80±41</td>
</tr>
<tr>
<td></td>
<td>Pco2 level 1 $\rightarrow$ 3</td>
<td>231±69</td>
<td>219±45</td>
</tr>
</tbody>
</table>

Percentage increase in hypoxic sensitivities with rising Pco2. Data are means ± SE from 8 subjects. *Lack of normal distribution so that a nonparametric test was used.

Subjects. Similar to the cat, hypoxic sensitivity is a linear function of arterial [H+] (33). Obviously, the lines representing hypoxic sensitivity as a function of arterial [H+] were shifted to higher values of [H+]a (or to lower Pco2 values) following acetazolamide without changing the slope (slope values were 8.70 l min$^{-1}$ mmol$^{-1}$ in placebo and 9.05 l min$^{-1}$ mmol$^{-1}$ after acetazolamide, respectively). This clearly suggests that at least when changes in [H+]a are induced by alterations in Pco2, the O2-H+ interaction (or the effect of CO2 to increase the response to acute hypoxia) is not reduced by acetazolamide. Thus, consistent with earlier observations (43), the magnitude of the AHR depends on the Pco2 level at which it is measured; at a fixed Pco2, it will be greater after acetazolamide. To investigate if the rightward shift of the hypoxic sensitivity-[H+]a line could, at least partly, be caused by an inhibiting effect of acetazolamide per se rather than by the substantially lower Pco2 that accompanies the metabolic acidosis, we also measured AHR after infusion of bicarbonate. This resulted in temporary increases in base excess of 6.1 ± 2.0 and 5.9 ± 1.3 meq l$^{-1}$ in placebo and acetazolamide, respectively. During the measurement protocols, slow infusion rates were sometimes maintained to prevent base excess from too quickly restoring to preinfusion level. Figure 3 shows that after bicarbonate infusion, the response lines shifted to lower levels of [H+]a and that this occurred in an approximately parallel way in placebo but with a decrease in slope after acetazolamide.

DISCUSSION

The main results of this study can be summarized as follows. 1) Acetazolamide caused a partly compensated, metabolic acidosis; 2) in steady state normoxia, the ventilatory CO2 response curve showed a parallel shift to the left following acetazolamide, consistent with the picture of a metabolic acidosis; in mild hypoxia, ventilatory CO2 sensitivity was slightly increased; 3) acetazolamide displaced the line relating hypoxic sensitivity to arterial [H+] to higher values of [H+]a indicating that CO2 and H+ may have separate effects on hypoxic sensitivity; and 4) in placebo and acetazolamide, isocapnic bicarbonate infusion shifted these response lines leftward, also suggesting separate effects of H+ and CO2 and not directly supporting an inhibitory effect of acetazolamide on hypoxic sensitivity.

Possible limitations of the study and methodological considerations. The metabolic acidosis induced by acetazolamide can be considered “classic” in the sense that the normoxic ventilatory CO2 response curve displayed a parallel shift to the right.

Fig. 2. Hypoxic sensitivity as a function of arterial [H+] and Pco2. A: acetazolamide produces arterial acidosis and induces a parallel shift of the line relating hypoxic sensitivity (HS) to arterial [H+] to the right. Linear equations were as follows: in placebo, HS = 8.73 × [H+] − 304; following acetazolamide, HS = 9.05 × [H+] − 432. B: HS-Pco2 relationship before and after acetazolamide. Absolute values of hypoxic sensitivity, defined as $\Delta$ventilation/$\Delta$log PaO2, are shown. Data are means ± SE from 8 subjects.

Fig. 3. Influence of bicarbonate infusion on the hypoxic sensitivity-[H+] relationship. After both placebo and acetazolamide, an infusion of bicarbonate reduces arterial [H+] and shifts the hypoxic sensitivity-[H+] relationship to the left. Linear equations were as follows: for placebo, hypoxic sensitivity = 8.73 × [H+] − 304 and 9.06 × [H+] − 316 before and after bicarbonate, respectively. For acetazolamide, hypoxic sensitivity = 9.05 × [H+] − 432 and 6.07 × [H+] − 235 before and after bicarbonate, respectively. Numbers added to data points are mean arterial Pco2 values in Torr. Absolute values of hypoxic sensitivity are given. Data are means ± SE from 8 subjects.
and the subjects had a $\Delta$PCO$_2$/\Delta[HCO$_3^-$] of 0.90 ± 0.2 Torr·mmol·L$^{-1}$, which perfectly agrees with a ratio of 0.9 during a partly compensated metabolic acidosis in healthy subjects (10, 24). However, apart from inducing a metabolic acidosis, acetazolamide may have rather complex effects on both the control of breathing and the volume status of the body, and the present data cannot be discussed without taking these into consideration.

First, acetazolamide is a mild diuretic and will therefore cause extracellular fluid (ECF) volume depletion (3). Both changes in ECF volume and osmolality may have an effect on respiratory control (1, 34). The effects on volume status appear complex: decrements in total body, and extracellular, water, but an increase in intracellular water without significant changes in plasma osmolality (3) (osmolality in our subjects, calculated as 2 [Na$^+$] + [glucose]1/18 did not change significantly). Assuming no effect of the dose regimen on erythropoietin production and an initial plasma volume of 51, we estimate an $\sim$4% volume depletion in our subjects (see Table 1). The effects on electrolyte status are also complex: a kaliuretic effect along with an intra-to-extracellular shift of potassium ions and opposite shifts of Na$^+$ and H$^+$ ions (3). We did not monitor and correct volume status and hence we cannot exclude that changes in water balance may have influenced our results. On the other hand, however, we remark that we were able to reverse the effects of acetazolamide with relatively low volumes of a bicarbonate solution (120–180 ml), too low to cause appreciable changes in plasma volume. This suggests to us that the parallel shift of the hypoxic sensitivity-[H$^+$]$_a$ line following acetazolamide is due to a pH effect, rather than to changes in volume status of the body.

Second, acetazolamide is a carbonic anhydrase inhibitor that may cause chemical disequilibrium in blood and tissues provided the inhibition of erythrocytic carbonic anhydrase is $>99.95%$ (28). However, the (clinical) dose that we administered is too low to achieve a fractional inhibition $>0.995$, and hence blood gases measured in vitro may be considered to represent in vivo values (28, 36).

Third, acetazolamide may have inhibitory effects on the hypoxic ventilatory response, but as explained below, we have reasons to believe that these are limited to cases in which it is administered intravenously.

Shortly after bicarbonate infusions, bicarbonate ions will be distributed over the extracellular space tending to restore arterial pH to its preinfusion value. To prevent this from occurring too quickly, infusion rates were sometimes maintained at low rates. This means, steady state in the subjects may not have been reached and the question arises as to how this may have influenced our results. Intra-to-extracellular H$^+$ gradients may have been unstable, altering the stimulus level in carotid body cells. However, at least in the placebo condition, CO$_2$-induced changes in hypoxic sensitivity were not altered by bicarbonate infusions given the finding that the slopes of the hypoxic sensitivity-[H$^+$]$_a$ lines did not obviously change (see Fig. 3). Following acetazolamide, the line became less steep after bicarbonate infusion, and it is possible that this is due to less stable H$^+$ gradients at the background of a metabolic acidosis. However, an alternative explanation is also possible (see below).

Expressing hypoxic sensitivity as $\Delta V$/\Delta Sa$_{O_2}$ vs. $\Delta V$/\Delta logPa$_{O_2}$:

The difference in ventilation during acute hypoxia and steady-state normoxia was considered to be due to activation of the carotid bodies. The acute hypoxic ventilatory response was defined as the ratio of this difference over $\Delta$logPa$_{O_2}$ (both during normoxia and hypoxia blood samples were taken). This does not follow from the conventional exponential relationship between ventilation and Pa$_{O_2}$ or from the linear ventilation-Sa$_{O_2}$ relationship, but was done for practical reasons and based on empirical data in cats (33). At least in the placebo condition with normal acid-base status, CO$_2$-induced relative changes in hypoxic sensitivities were equal regardless of its definition ($\Delta V$/\Delta Sa$_{O_2}$ vs. $\Delta V$/\Delta logPa$_{O_2}$), and for this reason we presented hypoxic sensitivity as $\Delta V$/\Delta logPa$_{O_2}$. When using $\Delta V$/\Delta Sa$_{O_2}$ in a state of acidosis or alkalosis, possible changes in the position of the Hb saturation curve should always taken into consideration in estimating true hypoxic sensitivity. The smaller relative increase in hypoxic sensitivity estimated from $\Delta V$/\Delta Sa$_{O_2}$, from the lower to the intermediate CO$_2$ level in acidosis (Table 3) might be an illustration of this.

Hypoxic sensitivity is not a unique function of arterial [H$^+$].

In all subjects, acetazolamide shifted the hypoxic sensitivity $- [H^+]$ response lines to higher values of arterial H$^+$ without changing the slope indicating that after chronic use, acetazolamide did not affect the O$_2$-CO$_2$ interaction (the means of individual slopes in placebo and acetazolamide, respectively, were 9.80 ± 1.96 and 8.34 ± 1.67 l·min$^{-1}$·mmol$^{-1}$; means ± SE, $P = 0.052$). It is also clear that although the relationship between hypoxic sensitivity and CO$_2$-induced alterations in [H$^+$]$_a$ is linear (confirming data obtained in the cat, see Ref. 33), it is not a unique function of [H$^+$]$_a$ at the level of the arterial blood. Figures 2 and 3 show that a given level of arterial [H$^+$] can be associated with different magnitudes of hypoxic sensitivities, and the same applies to a given level of arterial Pco$_2$. Figure 2 shows that at a given Pa$_{CO_2}$, the AHR will be greater after acetazolamide; if following acetazolamide administration the hypoxic response is measured at about resting Pa$_{CO_2}$, it will be about equal or somewhat lower than at resting Pa$_{CO_2}$ in placebo, despite the much higher resting [H$^+$]$_a$, (see also Ref. 43). Thus according to our data, the notion that generally a rise in arterial [H$^+$] will act to increase the hypoxic response (17, 24) is not tenable. In retrospect, from Fig. 2 we anticipate that this would be unlikely since it would imply an infinitely high AHR in a metabolic acidosis with a pH of $\sim$7.30.

Our data do not allow us to draw conclusions as to the mechanism of the O$_2$-CO$_2$ interaction, but they provide some ground for speculation. For example, considering points d and d’ and e and f in Fig. 3, hypoxic sensitivity appears to be considerably less at somewhat lower H$^+$, but equal Pa$_{CO_2}$ levels. Because isocapnic changes in arterial pH penetrate slowly and only partly into the brainstem extracellular space (39), we assume the central chemoreceptor extracellular pH to be about equal in d and d’ and e and f’, respectively. Points d’ and f are isohydric points, and the lower hypoxic sensitivity in f could be due to the lower carotid body Pco$_2$ (peripheral O$_2$-CO$_2$ interaction) combined with a diminished efficacy of afferent carotid body output to increase ventilation due to the also lower central Pco$_2$ (i.e., a negative peripheral-central interaction for which there is no experimental evidence in...
In slope: the mean of the individual slope decreased from before and after bicarbonate, respectively. Following acetazolamide administration in both animals and humans when both in normoxia and hypoxia acetazolamide increases ventilation. Inhibitory effect, however, is not supported by the finding that effect of chronic acetazolamide on the carotid bodies. An so that our measurements would not reflect real steady-state conditions, the difference between the prevailing PCO2 and however, from the perspective of a unique relationship between the AHR and [H+]a (17, 24), the horizontal shift of the response lines in Fig. 2 after acetazolamide could be due to an inhibitory effect on the carotid bodies without affecting the O2-CO2 interaction. In placebo, bicarbonate shifted the hypoxic sensitivity-[H+]a response line leftward in an approximately parallel way: the means of the individual slopes were 9.98 ± 1.96 and 9.08 ± 2.30 l·min−1·mmHg−1 (P = 0.446) before and after bicarbonate, respectively. Following acetazolamide, the line also shifted leftward, but not with a decrease in slope: the mean of the individual slope decreased from 8.34 ± 1.67 to 5.17 ± 1.09 l·min−1·mmHg−1 (P = 0.014).

The lower slope after bicarbonate could be related to less stable H+ gradients at the background of a metabolic acidosis, so that our measurements would not reflect real steady-state situations. But we also cannot entirely exclude an inhibitory effect of chronic acetazolamide on the carotid bodies. An inhibitory effect, however, is not supported by the finding that both in normoxia and hypoxia acetazolamide increases ventilation (36, 41). This is clearly different from the effect of intravenous administration in both animals and humans when acetazolamide, in a dose too low to have direct effects on the central nervous system, has inhibitory effects on both the hypoxic response and the O2-CO2 interaction (36, 40, 42). Why in humans an inhibitory effect seems solely present after acute administration aimed at reaching equal plasma levels of the agent (36) remains unclear. One difference between acute and chronic application is the induction of a metabolic acidosis with the latter. Perhaps for some reason the metabolic acidosis prevents or neutralizes inhibitory effects of acetazolamide on the carotid bodies, and the finding that bicarbonate infusion decreased the slope of the hypoxic sensitivity-[H+]a line could be a manifestation of this. Future studies are warranted to investigate this further. Also, more insight is needed into the mechanism of CO2-O2 interaction in oxygen-sensing (type I) cells in the carotid body and the way in which hypoxia and hypercapnia have more-than-additive effects on Ca2+ influx in type I cells (9, 29).

Significance for the use of acetazolamide at high altitude. The low PaCO2 after chronic acetazolamide will have a stabilizing effect on breathing for at least three reasons that may also play a role in its known beneficial effects on sleep at high altitude. First, if the ventilatory CO2 response curve shows a parallel leftward shift, the CO2 reserve, i.e., the difference between the prevailing PaCO2 and the PaCO2 where apnea ensues (11), will increase. Note that in our study in resting and awake conditions, the difference between the prevailing PCO2 and apneic threshold did not increase, but this was probably due to a small (insignificant) increase in the slope of the ventilatory CO2 response curve (see the Tables 1 and 2). More relevant, however, is what happens during sleep when ventilatory CO2 sensitivity is reduced resulting in an increase in the CO2 reserve (11). Second, a lower PaCO2 implies a location on a steeper part of the metabolic hyperbola for CO2, so that for a given decrease in PaCO2, to occur, a larger increase in ventilation is required. And third, the lower PaCO2 prevents an enormous increase in hypoxic sensitivity that would occur if H+ and CO2 had no separate effects on the carotid bodies and if hypoxic sensitivity were a unique function of arterial [H+]. Benzolamide and spiranolactone, two diuretic agents that also induce a metabolic acidosis, appeared to reduce periodic breathing during sleep at high altitude and prevented the symptoms of acute mountain sickness, respectively (27, 37). Thus, acetazolamide’s effects at high altitude may at least partly be due to the metabolic acidosis. Further studies are warranted to see if additional effects of acetazolamide, for example on pulmonary vessels (38), may also contribute to its beneficial effects.

In summary, we showed separate effects of H+ and CO2 on hypoxic sensitivity in humans. Expressing hypoxic sensitivity as ∆ventilation/∆logPaO2 avoids possible confounding influences of changes in position of the HbO2 saturation curve. With a clinically relevant dosing, acetazolamide has no inhibitory effects on the carotid bodies.

DISCLOSURES
No conflicts of interest are declared by the author(s).

REFERENCES


