Acetazolamide suppresses the prevalence of augmented breaths during exposure to hypoxia

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Bell HJ, Haouzi P. Acetazolamide suppresses the prevalence of augmented breaths during exposure to hypoxia. Am J Physiol Regul Integr Comp Physiol 297: R370–R381, 2009. First published June 3, 2009; doi:10.1152/ajpregu.00126.2009.—Augmented breaths, or “sighs,” commonly destabilize respiratory rhythm, precipitating apneas and variability in the depth and rate of breathing, which may then exacerbate sleep-disordered breathing in vulnerable individuals. We previously demonstrated that hypocapnia is a unique condition associated with a high prevalence of augmented breaths during exposure to hypoxia; the prevalence of augmented breaths during hypoxia can be returned to normal simply by the addition of CO₂ to the inspired air. We hypothesized that counteracting the effect of respiratory alkalosis during hypocapnic hypoxia by blocking carbonic anhydrase would yield a similar effect. We, therefore, investigated the effect of acetazolamide on the prevalence of augmented breaths in the resting breathing cycle in five awake, adult male rats. We found a 475% increase in the prevalence of augmented breaths in animals exposed to hypocapnic hypoxia compared with room air. Acetazolamide treatment (100 mg/kg ip bid) for 3 days resulted in a rapid and potent suppression of the generation of augmented breaths during hypoxia. Within 90 min of the first dose of acetazolamide, the prevalence of augmented breaths in hypoxia fell to levels that were no greater than those observed in room air. On cessation of treatment, exposure to hypocapnic hypoxia once again caused a large increase in the prevalence of augmented breaths. These results reveal a novel means by which acetazolamide acts to stabilize breathing and may help explain the beneficial effects of the drug on breathing stability at altitude and in patients with central forms of sleep-disordered breathing.

sighs; respiratory instability; carbon dioxide; acidosis; alkalosis; Diacox

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OCCASIONAL RESPIRATORY DISTURBANCES, in the form of augmented breaths, are a typical feature of the resting breathing rhythm of all mammalian species studied, including humans (4, 34, 35). Augmented breaths, also referred to as “sighs,” are distinct from normal background eupneic breaths for a number of reasons. The most obvious quantitative difference is the volume of air mobilized during the augmented breath, which is far greater than that during the prevailing background eupneic breathing cycles. Indeed, the tidal volume corresponding to an augmented breath is commonly more than three times as large as that of the background breathing cycles. Augmented breaths also differ from normal eupneic breaths because of their two-phase inspiratory flow profile. The beginning of the flow profile of an augmented breath typically appears to be much the same as that of a normal eupneic breath, but at a point close to what would normally be the peak flow of the breath, a second phase is “superimposed,” and flow rapidly increases.

In the integrated system, sensory feedback from the carotid body chemoreceptors and vagal lung afferents appears to provide regulatory influence over the prevalence of augmented breaths (1, 15, 33). However, augmented breaths can also be recorded in vitro preparations, in which these peripheral sources of sensory feedback are no longer intact (28). Isolated medullary brain stem slice preparations containing the pre-Bötzinger complex within the ventral respiratory group are known to generate fictive eupnea and also augmented breaths. A further reduced isolated “ventral respiratory group island preparation” will also exhibit these same respiratory neuronal discharge patterns (56). Therefore, although peripheral inputs appear to be important in regulating the central generation of augmented breaths, they may not be essential.

In terms of physiological function, it is believed that augmented breaths primarily provide a means to maintain healthy lung function, because they recruit hypoventilated regions of the alveolar space. In this way, augmented breaths maintain or improve lung compliance, improve gas exchange, and stimulate the secretion of lung surfactant (3, 36, 41, 54). Such periodic large lung inflations are especially important during early development, when the mechanical properties of the lung tissues and thorax leave the pulmonary system vulnerable to progressive atelectasis.

On the other hand, augmented breaths represent a respiratory disturbance in the eupneic breathing cycle and are often followed by a period of apnea (14, 17, 42, 60) or, at least, a change in tidal volume and/or frequency of breathing that may last for many subsequent breath cycles (4, 9, 13, 17, 18, 42, 60). Whether this perturbation in breathing rhythm is due to an acute alteration in arterial blood gas composition or a central respiratory phenomenon related to the generation of eupneic breathing rhythm remains unclear. Nevertheless, augmented breaths have been implicated in exacerbating sleep-disordered breathing in populations ranging from infants (7, 13, 21), to heart failure patients (40), to healthy adults at simulated altitude (6).

Since augmented breaths are important in maintaining healthy lung function, yet they predispose the respiratory control system to instability, it is essential to develop a better understanding of the mechanisms that regulate their frequency of occurrence in the breathing cycle.

In our recent studies of breathing in awake, freely behaving rats, we found that the prevalence of augmented breaths is dramatically increased in response to hypoxia, but only when hypocapnia is allowed to develop (2). In other words, when hypocapnia is prevented during hypoxia by supplementation of 5% CO₂ into the inspired air, we found that the prevalence of
augmented breaths is no greater than that observed during normal room air exposures.

This observation led us to propose that a hypoxic O2 status does not lead to the dramatic increase in the rate of occurrence of augmented breaths, unless hypocapnia occurs. We then reasoned that preventing or counteracting the development of respiratory alkalosis during hypoxia by interventions other than CO2 supplementation should also suppress the increased generation of augmented breaths that would otherwise occur.

We therefore examined the effect of acetazolamide (Diamox) on the generation of augmented breaths during exposure to hypoxia. Acetazolamide, a potent sulfonamide carbonic anhydrase inhibitor, is the treatment of choice for symptoms of acute mountain sickness (AMS) (31, 59). AMS, a condition often observed in healthy adults on arrival at altitude (>2,500 m), is related to the physiological effects of lower barometric pressure and PO2 in the atmosphere. Symptoms will typically include headache, malaise, anorexia, nausea and vomiting, and difficulty sleeping (44). Periodic breathing, or Cheyne-Stokes respiration, is also a common feature of AMS and is believed to exacerbate the other symptoms of this condition (58). Acetazolamide has a complex mechanism of action that leads to its efficacy in treatment of the symptoms of AMS (27, 32): the drug causes metabolic acidosis via the inhibition of renal HCO3_3 reabsorption (57) and increased H+ retention (46). The resulting metabolic acidosis and tissue retention of CO2 can thereby offset the respiratory alkalosis that normally occurs during exposure to hypoxia.

We therefore hypothesized that acetazolamide would decrease the number of augmented breaths that are generated during exposure to hypoxia. In this study, we examined the effect of 3 days of acetazolamide treatment on the respiratory response to hypoxia in five awake, adult male rats. We specifically focused on the effect of acetazolamide on the prevalence of augmented breaths during exposure to 10% hypoxia.

**METHODS**

**Animals and Procedures**

Experiments were performed using five adult male Sprague-Dawley rats weighing 591, 683, 822, 668, and 708 g (Charles River, Wilmington, MA). Fully mature adult rats were preferentially used in this study to avoid any influence of hypoxia-induced metabolic depression (39) and related complications in the interpretation of respiratory measurements performed in this study. All procedures received prior approval from the Institutional Animal Care and Use Committee of the Penn State College of Medicine (protocol no. 2008-096). Respiratory variables were obtained from tests performed on five different occasions, over 4 separate days.

On day 1, animals were studied to establish a baseline response during exposure to room air and hypoxia. This testing session took place between ~1000 (10 AM) and 1200 (noon). Animals were monitored in the chamber in which they were exposed to a 2.5 l/min bias flow of room air for ~0.5 h before data collection began. Generally, grooming and sniffing behaviors were minimal after this time had passed. Respiratory data were recorded for ~20–25 min in room air before the fresh gas supply provided to the animal was replaced with a hypoxic (10% O2-balance N2) mixture. The animals were monitored in hypoxia for 20–25 min and then returned to a background of room air for 20–25 min. The fresh gas supply was then switched to provide a background of hypoxia with supplemental CO2 (10% O2-5% CO2-balance N2), so that we could document the effect of supplemental CO2 on the generation of augmented breaths in hypoxia. This condition primarily served to provide information regarding the consistency of response with respect to our previous findings (2). For this reason, the order of exposure to hypoxia and hypoxia + CO2 was not randomized; rather, the hypoxia + CO2 exposure was always performed later in the testing session. After the hypoxia + CO2 exposure, animals were returned to a background of room air and monitored for 20–25 min before they were returned to their cages.

Later on day 1, at ~1430, the animals received their first injection of acetazolamide (Ben Venue Laboratories, Bedford, OH; 100 mg/kg ip). The drug was obtained in powder form and was reconstituted for injection in isotonic saline solution (0.9% NaCl; Baxter Healthcare, Deerfield, IL). At ~30 min after this first injection, the animals were placed in the monitoring chamber for a second testing session, which was identical to the first session (see above).

On days 2 and 3, the animals were injected with acetazolamide (100 mg/kg ip) at ~0830 and again at 1430. At ~30 min after the second injection, the respiratory responses to the different background conditions were monitored.

On day 4, the animals were injected with an equivalent volume of isotonic saline that contained no acetazolamide at 0830 and 1430 (the times at which acetazolamide was injected on days 2 and 3). Respiratory responses were monitored ~30 min after the second saline injection.

These testing sessions are designated baseline, D1, D2, D3, and recovery. D1, D2, and D3 coincided with acetazolamide treatment. The time line of the protocol is shown in Fig. 1.

Animals were weighed (700/800 series, Ohaus, Pine Brook, NJ), and their core (rectal) temperature was assessed (model 524928, Becton Dickinson, Franklin Lakes, NJ) immediately before each testing session began.

**Equipment**

The methodology used to monitor respiratory variables of interest was identical to that described previously in detail (2). During the testing sessions, each animal was individually monitored using a custom-designed animal chamber and air control circuit that allowed unrestrained whole body open-flow plethysmography. This technique, which is used to noninvasively monitor respiratory activity, along with
with its experimental benefits and limitations, has been discussed at length (29, 38, 43, 50).

Our animal chamber consisted of a sealed, leak-proof acrylic cylinder (1.9 liter internal volume, 120 mm diameter), with 5-mm air inlet and outlet ports on opposite ends. The chamber was of sufficient diameter that air could pass freely around all sides of the animal without interference. Gas mixtures were supplied from dry, premixed tanks of known composition. Fresh inlet gas entered the animal chamber through an internal diffuser that distributed the gas evenly over the cross-sectional area. Mixed respired gases were exhausted from the chamber through the outlet port, which passed gas into a section of low-resistance, noncompliant chlorinated polyvinylchloride tubing (11.5 mm ID) and through a downstream pneumotachograph (model 000, Fleish) that vented into room air.

CO₂ and O₂ levels in air leaving the chamber were continuously measured (models 17630 infrared and 17620 fuel cell analyzers, respectively, Vacumed, Ventura, CA). The flow of air through the animal chamber that contained the respiratory signal was continuously monitored via the Fleish pneumotachograph, which was interfaced to a pressure transducer (Sensym, DCLX O1DN, Honeywell, Morris- town, NJ) housed in a custom-designed electronic demodulator. Temperature in the animal chamber was continuously monitored via a fast-responding thermocouple (Thermalert TH5; Physiotemp, Clifton, NJ).

Data Analysis

Analog signals representing flow through the chamber, percent CO₂ and percent O₂ at the inlet and outlet ports of the box, and box temperature were fed into a 14-bit analog-to-digital converter (model USB6009, National Instruments, Austin, TX) that was interfaced with an Intel/Windows Vista-based computer system (Compaq 8510w; Hewlett Packard, Palo Alto, CA) running custom-written data acquisition software (LabView, National Instruments; source code available on request). Analog signals were sampled at 200 Hz and displayed in raw form while being stored for subsequent analysis. ASCII data files were imported for visualization and analysis using Chart software (version 5.5.4; ADInstruments, Colorado Springs, CO).

For extraction of respiratory variables from the raw flow trace, the signal was treated using a high-pass (>0.2 Hz) filter to determine and subtract the direct-current component. The respiratory signal was used for calculations of breathing frequency and integrated over each breath cycle to obtain the changes in volume associated with each breath. These volume changes were then temperature corrected to obtain an index of tidal volume (estVT) and the product of tidal volume and breathing frequency, i.e., minute ventilation (estV˙E). The temperature correction took into account the difference between ambient temperature in the chamber (Tc, K), as determined immediately before and then after the experiment. This temperature correction was based on the following equation estVT = [ΔV*(Tc/Tb)](1 – (Tc/Tb)) where Tb is body core temperature (K), Tc is chamber temperature (K), and ΔV is result of integrated plethysmographic flow signal for each breath cycle.

Because of the complexity of factors involved in the quantitative interpretation of an open-flow plethysmographic signal (12, 29, 38, 43, 50), this determination of estVT and estV˙E provided a semiquan-

tative index that is represented in the units that are appropriate for direct measurements of tidal volume and minute ventilation.

Each testing session provided data from animals monitored in three separate background conditions (room air, hypoxia, and hypoxia + 5% CO₂). In each condition, the animals were monitored for 20–25 min. For the purpose of analysis, a 5-min window was used to determine estV˙E, estVT, breathing frequency, CO₂ production (V˙CO₂), O₂ consumption (V˙O₂), Tc, the O₂ and CO₂ composition of air leaving the chamber, and the number of augmented breaths. The specific 5-min window was chosen as a quiet period of breathing during which unwanted sniffing activity or movement was minimal. The default interval used for analysis in a given condition was 10–15 min during each exposure. Only if there was obvious disruption in the respiratory trace that totaled >15–20 s (<10% of the observation window), another 5-min window was chosen. In no case did the interval used for analysis start any earlier than 5 min or later than 20 min after the beginning of any given exposure. The great majority of intervals used for analysis, across all animals and conditions for all days, was the default 10- to 15-min interval [51 of 60 (85%) of cases]. Moreover, there was no systematic tendency for any one condition, animal, or day to require the use of an analysis interval outside the default window.

Because of their unique characteristics, augmented breaths were easily identified in the respiratory flow trace (Fig. 2). In our previous study (2), we defined an identified augmented breath as a spontaneous large breath with an amplitude >300% of the background eupneic breathing rhythm. The same criterion was applied without issue throughout the great majority of our data analysis. However, in one animal, augmented breaths often occurred during a large tidal volume, which was just slightly smaller than three times the average background tidal volume (commonly 2.6–2.7 times). This was apparently not due to any pulmonary limitation, since this animal occasionally demonstrated augmented breaths much more than 300% of the volume of background rhythmic breaths. In every other regard, however, the morphology of the augmented breaths was stereotypical: a spontaneous large breath with a large biphasic inspirational flow profile. For this reason, we modified our objective criterion for determining augmented breaths as follows: any spontaneous large breath with an amplitude ≥2.5 times that of the background rhythmic breathing pattern. This simple criterion easily discriminated augmented breaths from other large eupneic breaths, even when ventilation was stimulated.

The concentration of O₂ and CO₂ in the air entering the chamber was also determined before and after exposure to each condition. While the animals were in the chamber, production of carbon dioxide (V˙CO₂) and oxygen consumption (V˙O₂) were calculated in STPD conditions, as previously described (2, 20): V˙O₂ = V˙inlet FIO₂ – V˙outlet FEO₂ and V˙CO₂ = V˙outlet FCO₂ – V˙inlet FICO₂. Since only V˙outlet was directly measured, V˙inlet was calculated as follows. V˙inlet = V˙outlet [(1 – Fio₂ + Fco₂)/(1 – Fio₂ – Fco₂)]. The difference between V˙inlet and V˙outlet results from the gas exchange ratio (V˙CO₂/V˙O₂) and was therefore compensated for in these conditions.

The first room air exposure was used for data analysis, as were the exposures to hypoxia (10% O₂–balance N₂) and hypoxia + CO₂ (10% O₂–5% CO₂–balance N₂). The composition of fresh gas supplied to the animal chamber, as well as the composition of inspired gas within the animal chamber, remained consistent across the five testing sessions within each condition. Specific values are summarized in Table 1.

Statistical Analysis

Changes in all variables were assessed using two-factor repeated-measures ANOVA (SigmaStat version 3.5, SPSS, Chicago, IL). Specifically, the two factors included in analysis were background condition (room air, hypoxia, and hypoxia + CO₂) and testing session (baseline, D1, D2, D3, and recovery). The α priori value for acceptability of a type I (α) error in any statistical comparison was set to 0.05. Post hoc testing was performed where indicated using the Holm-Sidak method for multiple comparisons. This analysis provided
RESULTS

Augmented Breaths

The effect of background conditions and acetazolamide (i.e., testing session) on the prevalence of augmented breaths in all five animals tested is summarized in Fig. 3. Two-way repeated-measures ANOVA revealed a significant interaction effect between background condition and testing session on the number of augmented breaths ($P < 0.001$).

Baseline measurements. Our finding that exposure to hypocapnic hypoxia caused an increase in the prevalence of augmented breaths (ABs) from $2.6 \pm 0.5$ ABs/5 min in room air to $15.0 \pm 4.9$ ABs/5 min during poikilocapnic hypoxia, a change of $12.4 \pm 4.8$ ABs/5 min, or 475%, confirms our previous findings. In addition, exposure to hypoxia + 5% CO$_2$ suppressed the increase in the prevalence of augmented breaths triggered by hypocapnic hypoxia, such that it remained virtually unchanged from room air conditions ($3.0 \pm 0.0$ ABs/5 min).

Effect of acetazolamide. Figure 2 shows a plethysmographic flow recording from one animal monitored in hypocapnic hypoxia during each of the five separate testing sessions across the 4 days of the protocol. The effect of acetazolamide on the prevalence of augmented breaths was dramatic. Clearly, acetazolamide eliminated the increased prevalence of augmented breaths that normally occurred during hypocapnic hypoxia. This effect was also rapid. During the D1 testing session, i.e., exposure to hypoxia ~1.5 h after the initial acetazolamide injection, the prevalence of augmented breaths in hypocapnic hypoxia was dramatically reduced compared with the baseline exposure to hypoxia (from $15.0 \pm 4.9$ to $3.4 \pm 1.7$ ABs/5 min, $P < 0.001$). This trend continued
throughout the D2 and D3 testing sessions, where the prevalence of augmented breaths during exposure to hypoxia was powerfully suppressed. Consequently, the prevalence of augmented breaths was similar in room air and hypoxia (both tests, $P < 0.001$). Figure 3 shows the effect of acetazolamide on the prevalence of augmented breaths in each individual rat, across the three background conditions, during each of the 5 testing sessions.

During the recovery testing session, ~24 h after the last acetazolamide injection, the prevalence of augmented breaths during hypoxia ($9.6 \pm 3.2 \text{ ABS/5 min}$) returned toward baseline levels and was significantly more frequent than during the D1, D2, or D3 testing session ($P < 0.001$ for all comparisons).

**Ventilation**

The effect of background conditions and acetazolamide treatment on est$\dot{V}E$ in all five animals is summarized in Fig. 4. Two-way repeated-measures ANOVA revealed a significant interaction effect between background condition and testing session on the level of est$\dot{V}E$ ($P < 0.001$).

**Baseline measurements.** In room air, est$\dot{V}E$ was $58 \pm 10 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1}$. On exposure to hypoxia (10% O$_2$-balance N$_2$), est$\dot{V}E$ increased to $98 \pm 19 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1}$ ($P < 0.001$). During exposure to hypoxia + 5% CO$_2$ (10% O$_2$-5% CO$_2$-balance N$_2$), est$\dot{V}E$ increased to $129 \pm 15 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1}$ ($P < 0.001$), a level that was also significantly greater than during hypoxia alone ($P < 0.001$).

**Effect of acetazolamide.** Acetazolamide provided a source of respiratory drive that increased est$\dot{V}E$. This stimulation was observed as an increase in est$\dot{V}E$ in room air during the D1 testing session ($78 \pm 6$ vs. $58 \pm 10 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1}$, $P = 0.008$), where data for this condition were collected ~90 min after the first acetazolamide injection. Ventilation in room air conditions remained elevated above baseline for the duration of acetazolamide treatment (i.e., baseline vs. D2 and D3, $P < 0.001$). The level of ventilation in hypoxia was not different during the D1, D2, or D3 testing session compared with baseline. As a result, the increase in ventilation in hypoxia compared with room air during D1, D2, and D3 testing sessions was dramatically reduced compared with baseline.

**Relationship between the number of augmented breaths and ventilation.** The relationship between minute ventilation and the prevalence of augmented breaths per unit of ventilation is shown in Fig. 5 during baseline and D3 testing sessions. Clearly, there is a complete dissociation between the number of augmented breaths and the level of ventilation. For instance, although similar levels of ventilation were reached during baseline hypoxia compared with room air and hypoxia during the D3 testing session, the number of augmented breaths per unit of ventilation was almost five to six times lower during acetazolamide treatment.

**Breathing Frequency**

Breathing frequency in the three background conditions and the effect of acetazolamide treatment are summarized in Fig. 6. Two-way repeated-measures ANOVA revealed a significant
interaction effect between background condition and testing session on the level of breathing frequency ($P < 0.001$).

**Baseline measurements.** Breathing frequency in room air was $82 \pm 13$ breaths/min. Breathing frequency was increased during exposure to hypoxia ($146 \pm 22$ breaths/min) and during exposure to hypoxia + 5% CO$_2$ ($140 \pm 12$ breaths/min, both $P < 0.001$). There was no apparent difference in breathing frequency at baseline between hypoxia and hypoxia + 5% CO$_2$.

**Effect of acetazolamide.** Acetazolamide changed the breathing frequency response in animals during hypoxia. Although breathing frequency continued to increase significantly during exposure to hypoxia compared with room air during D1, D2, and D3 testing sessions, as for ventilation, this increase became smaller than during the baseline testing session, so that the response of breathing frequency to hypoxia appeared to be reduced after acetazolamide injection. This effect was likely due to the fact that acetazolamide decreased breathing frequency during hypoxia compared with baseline and recovery (all $P < 0.001$). Despite the smaller increase in breathing frequency in hypoxia during D1, D2, and D3 testing sessions, these changes remained significant ($P \leq 0.01$ for all tests).

**Relationship between the number of augmented breaths and breathing frequency.** The relationship between the number of augmented breaths for every 100 breath cycles and breathing frequency is shown in Fig. 7. Acetazolamide dramatically altered the relationship between breathing frequency and the normalized prevalence of augmented breaths, regardless of the level of breathing frequency.

**Tidal Volume**

The effect of background conditions and acetazolamide on estVr in all five animals is summarized in Fig. 8. Two-way repeated-measures ANOVA revealed a significant interaction
Effect between background condition and testing session on the level of estVT ($P = 0.004$).

**Baseline measurements.** In room air, animals demonstrated an estVT of $5.1 \pm 1.1$ ml. On exposure to hypoxia (10% $O_2$-balance $N_2$), estVT did not change significantly, averaging $4.7 \pm 0.6$ ml ($P = 0.244$). During hypoxia + 5% $CO_2$ (10% $O_2$-5% $CO_2$-balance $N_2$), estVT increased to $6.4 \pm 0.9$ ml ($P < 0.001$), a level that was also significantly greater than during hypoxia alone ($P < 0.001$).

**Effect of acetazolamide.** Acetazolamide provided a source of respiratory drive, which increased estVT. In room air, estVT showed a trend toward an increase above the baseline testing session during the D1 testing session ($5.9 \pm 1.1$ ml, not significant), and this increase became significant during D2 and D3 testing sessions ($6.4 \pm 1.0$ ml ($P < 0.001$) and $6.9 \pm 0.9$ ml ($P < 0.001$, respectively). Acetazolamide also caused an increase in estVT during hypoxia (10% $O_2$-balance $N_2$) and hypoxia + $CO_2$ (10% $O_2$-5% $CO_2$-balance $N_2$). This was observed as an increase in estVT above the baseline testing session during D1, D2, and D3 testing sessions ($P < 0.002$ for all sessions; Fig. 8).

**$V_O2$.** Two-way repeated-measures ANOVA showed no significant interaction effect between condition and testing session on the level of $V_O2$ ($P < 0.001$). However, a significant difference in the mean values among the different background conditions was observed after we allowed for the effects of differences in testing session ($P = 0.008$). No independent effect of testing session could be resolved in $V_O2$ data ($P = 0.091$). Across all room air tests, $V_O2$ averaged $11.0 \pm 2.6$ ml·kg$^{-1}$·min$^{-1}$. During hypoxia, $V_O2$ increased slightly to $12.0 \pm 2.5$ ml·kg$^{-1}$·min$^{-1}$ ($P = 0.004$). During hypoxia + 5% $CO_2$, $V_O2$ increased slightly above that observed in room air conditions, to $11.9 \pm 2.3$ ml·kg$^{-1}$·min$^{-1}$ ($P = 0.007$).

**$V_{CO2}$.** Two-way repeated-measures ANOVA showed no significant interaction effect between condition and testing session on the level of $V_{CO2}$ ($P = 0.199$). However, significant independent effects were present for condition ($P < 0.001$) and testing session ($P = 0.008$). When averaged across all days, $V_{CO2}$ increased by $\sim 10\%$ from $9.1 \pm 1.1$ ml·kg$^{-1}$·min$^{-1}$ in room air to $9.9 \pm 1.4$ ml·kg$^{-1}$·min$^{-1}$ during hypoxia. During hypoxia + 5% $CO_2$, however, $V_{CO2}$ decreased slightly, by $\sim 5\%$, to $8.5 \pm 1.1$ ml·kg$^{-1}$·min$^{-1}$ compared with room air. Comparisons also showed that $V_{CO2}$ decreased slightly as a result of acetazolamide treatment. When averaged across all conditions for a given day, $V_{CO2}$ was significantly lower than baseline ($10.2 \pm 1.6$ ml·kg$^{-1}$·min$^{-1}$) during D1 ($9.0 \pm 1.2$ ml·kg$^{-1}$·min$^{-1}$), D2 ($8.9 \pm 1.3$ ml·kg$^{-1}$·min$^{-1}$), and D3 ($8.6 \pm 0.8$ ml·kg$^{-1}$·min$^{-1}$) testing sessions ($P \leq 0.006$ for all comparisons).

**DISCUSSION**

**Hypocapnic Hypoxia and Generation of Augmented Breaths**

Breathing hypoxic air results in a potent stimulation for the generation of augmented breaths. We recently reported that this increased prevalence of augmented breaths only occurs when hypocapnia is allowed to develop (2). In other words,
5% CO₂ to the inspired air. The mechanism through which this effect of hypoxia was eliminated by the simple addition of 475%. Consistent with our previous results, we found that (per 100 breath cycles) decreased on exposure to hypoxia (10% O₂-balance testing revealed that, if anything, the relative number of augmented breaths decreased during hypoxia remained unclear. We previously speculated that ventilation is primarily stimulated by the action of CO₂ supplementation (5%) into the background inspired air source, the stimulation of augmented breaths during hypoxia is effectively eliminated. This observation led us to propose that hypoxia requires hypocapnia to stimulate the production of augmented breaths.

In the present study, we have confirmed our previous findings; in baseline tests, hypoxia caused breathing to increase by ~70% compared with room air conditions. By comparison, V₀₂ increased by only ~6%; therefore, hypoxia caused a significant hyperventilation, resulting in hypocapnic hypoxia. Hypocapnic hypoxia caused a potent stimulation of augmented breaths: from ~3 every 5 min to ~15 every 5 min, an increase of 475%. Consistent with our previous results, we found that this effect of hypoxia was eliminated by the simple addition of 5% CO₂ to the inspired air. The mechanism through which hypocapnia increases the prevalence of augmented breaths during hypoxia remains unclear. We previously speculated that a number of mechanisms, including a differential signal between the peripheral and central chemoreceptors, decreased brain blood flow, and increased neuronal excitability due to respiratory alkalosis, or, less likely, stimulation of lung afferents, may be involved. Our present observations confirm that hypocapnia, or respiratory alkalosis, is essential to the mechanism by which hypoxia causes a potent stimulation of augmented breaths.

Acetazolamide Prevents Increased Prevalence of Augmented Breaths

The main goal of the present study was to test the effectiveness of acetazolamide as a means of “preventing” the normal increase in the prevalence of augmented breaths during exposure to hypocapnic hypoxia. We hypothesized that acetazolamide would offset the effects of hyperventilation-induced respiratory alkalosis during exposure to hypoxia. Our results clearly demonstrate that acetazolamide does lead to a profound reduction in the prevalence of augmented breaths, which occurs in the resting breathing cycle during exposure to hypocapnic hypoxia. Just 90 min after the first injection of acetazolamide, the normal increase in the prevalence of augmented breaths during hypocapnic hypoxia was virtually eliminated. This potent effect of acetazolamide persisted for the duration of treatment. On cessation of acetazolamide treatment, the prevalence of augmented breaths during exposure to hypocapnic hypoxia returned toward pretreatment levels. Since augmented breaths are transient respiratory disturbances that often lead to respiratory instability and apneas (4, 13, 17, 18, 42, 60), this effect of acetazolamide may reveal a novel means by which acetazolamide exerts a stabilizing effect on breathing rhythm.

Mechanism of Action

The known effects of acetazolamide treatment as they pertain to respiratory control have been extensively reviewed (27, 48). Briefly, acetazolamide is a potent carbonic anhydrase inhibitor, and it also exerts renal effects, including a decrease in H⁺ excretion and an increase in HCO₃⁻ excretion (46, 57). As such, acetazolamide causes a combined metabolic acidosis and retention of CO₂ in the tissues. These two effects are believed to contribute to the increased respiratory drive and minute ventilation that occurs during acetazolamide treatment (27). In the animals we studied, these effects were clearly apparent. Acetazolamide caused minute ventilation in room air conditions to increase throughout the course of treatment; the D1 tests revealed a 35% increase in estV˙E compared with baseline tests, and this difference increased to 60% during the D2 testing session and to 67% during the D3 testing session. The actions of acetazolamide are complex; however, it is believed that ventilation is primarily stimulated by the action of H⁺ and CO₂ at the level of the central respiratory chemoreceptors.

Our results regarding the effect of acetazolamide on the prevalence of augmented breaths support our hypothesis that counteracting respiratory alkalosis is an effective treatment to “prevent” the increased prevalence of augmented breaths during hypocapnic hypoxia. In our previous study, we proposed that augmented breaths were uniquely stimulated during hypocapnic hypoxia as a result of one of several possible mechanisms (see Ref. 2 for detailed discussion). For example, during hypoxia, the peripheral chemoreceptors are stimulated and exhibit increased afferent discharge, which stimulates breathing. However, the respiratory alkalosis that develops in the absence of CO₂ supplementation means that the central chemoreceptors may not be stimulated to the same extent as in hypoxia. In our current study, we hypothesized that this differential signal may precipitate an increase in the rate of occurrence of augmented breaths. If this is the case, then any intervention that counteracts the development of central hypocapnia and alkalosis should prevent the increased prevalence of augmented breaths during hypoxia. Acetazolamide treatment represents one such approach and effectively does this by producing a state of metabolic acidosis and CO₂ retention in the tissues. We therefore speculate that acetazolamide suppresses the generation of augmented breaths.
tion of augmented breaths during hypoxia by attenuating the disparity between the peripheral and central chemoreflex signals. Given the established importance of interactions between the central and peripheral arms of the respiratory chemoreflexes (5, 10, 45), this possibility warrants further attention.

An alternative explanation of our results also warrants discussion. Acetazolamide is known to depress the responsiveness of the carotid body chemoreceptors. Acetazolamide stimulates breathing; however, a paradoxical inhibition of carotid bodies has been described in response to physiological stimuli in the form of hypoxia and CO2 (51–53). Although the inhibition of carbonic anhydrase in peripheral chemosensory cells is likely involved in the reduced CO2 sensitivity (26, 55), the mechanism that leads to a decreased hypoxic sensitivity is not known. Nevertheless, since afferent traffic from the carotid bodies is believed to be important in mediating the generation of augmented breaths (1, 9, 15, 33), any intervention that decreases traffic from these sensory organs might also decrease their prevalence in the breathing cycle. As such, acetazolamide may decrease the prevalence of augmented breaths during hypoxia by decreasing overall peripheral chemoreceptor responsiveness.

In the present study, we cannot exclude the possibility that acetazolamide is suppressing the prevalence of augmented breaths during hypoxia, at least in part by depressing peripheral chemoreceptor responsiveness. However, it should be remembered that supplemental CO2 provided over the background of hypoxia also suppressed the generation of augmented breaths during hypoxia, in baseline tests in this study and in our previous report (2). On the basis of decades of research reports, one would expect the peripheral chemoreceptors to exhibit greater activity when CO2 is maintained at higher levels during hypoxia (24). Yet, supplemental CO2 decreases the prevalence of augmented breaths during hypoxia, such that augmented breaths are no more prevalent than in normal room air conditions. For this reason, we propose that the primary effect of acetazolamide in suppressing the prevalence of augmented breaths during hypoxia is not related to alteration of carotid chemoreceptor function. Rather, we propose that acetazolamide counteracts the respiratory alkalosis that normally develops during hypoxia by inducing a metabolic acidosis. This study therefore provides corroborative support to our previous report wherein we proposed that hypocapnia is the essential condition precipitating the increased prevalence of augmented breaths during hypoxia.

**Augmented Breaths and Breathing Instability**

In our recordings, augmented breaths are often followed by a period of apnea, which is consistent with earlier observations of many other researchers (14, 17, 42, 60). In the rats we studied, the duration of this apnea can vary considerably: from 1–2 breath cycles to 10 breath cycles. Even when the period of apnea is very short, there are noticeable changes in the depth and rate of the following breath cycles. Although the purpose of this study was not to provide a detailed analysis of breathing variability resulting from augmented breaths, we can provide some interesting generalizations regarding the effect of aug-

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**Fig. 8. Effect of acetazolamide on tidal volume (estVt).** Three line plots show the effect of acetazolamide on tidal volume across all 5 testing sessions in room air, hypoxia (10% O2-balance N2), and hypoxia + CO2 (10% O2-5% CO2-balance N2). Each individual animal is represented by a different thin line (solid, long-dashed, short dashed, and dashed-dotted); average response across all animals is represented by a solid thick line. Bar chart shows differences between tidal volume in room air and hypoxia (10% O2-balance N2) across the 5 consecutive testing sessions. Acetazolamide caused an increase in tidal volume in room air that was significant by the D2 and D3 testing sessions vs. the baseline testing session. Exposure to hypoxia (10% O2-balance N2) did not result in consistent changes in tidal volume compared with room air. Decrease in tidal volume in hypoxia vs. room air did reach significance during the D3 testing session. *P = 0.03.
mented breaths in different background conditions. Generally, there appears to be less tendency for augmented breaths to cause prolonged apneas in hypocapnic hypoxia. Instead, there appears to be a greater trend for destabilization of breathing pattern with respect to variability in the depth and rate of breathing after an augmented breath in this same condition. We should note that the rats we studied were monitored in a state of quiet resting wakefulness; therefore, the effects of augmented breaths on breathing stability may be quite different during sleep. Given the influence of sleep on the control of breathing, it would be interesting to determine whether augmented breaths have a different propensity to destabilize breathing in different stages of sleep vs. wakefulness.

**Study Limitations**

**Breathing pattern and the “prevalence” of augmented breaths.** We have expressed the prevalence of augmented breaths as the number of events during a 5-min observation window. It may be argued that an increase in breathing frequency should increase the number of augmented breaths in 5 min, by virtue of the fact that more breath cycles will occur in any given time period. To exclude this possibility, we examined the prevalence of augmented breaths in room air and hypoxia, at baseline and during the D3 testing session, expressed as the number of augmented breaths for every 100 breath cycles (Fig. 7). Our results and interpretation remain unaffected. Acetazolamide dramatically alters the relationship between breathing frequency and the normalized prevalence of augmented breaths. A similar examination of minute ventilation data reveals the same effect (Fig. 5). In other words, neither the effects of hypocapnic hypoxia nor the effect of acetazolamide can be explained by changes in breathing pattern or the absolute level of ventilation.

**Gas exchange and effects of hypoxia on small animals.** Metabolic rate is an important issue in the study of respiratory responses in small animals, because small animals are able to depress their metabolic rate in response to a decrease in ambient O2 availability (37, 39). However, metabolic depression was not mechanistically involved in the increased prevalence of augmented breaths that we observed during hypocapnic hypoxia. Although rats have the ability to decrease their metabolic rate in response to hypoxia, this response is limited to rats of smaller body size (39). Whether the rat’s ability to depress metabolism in response to hypoxia is related to its body size per se or to developmental changes remains unclear. Nevertheless, we did not expect any significant metabolic depression in the animals we studied, because we purposely used larger (>500 g body wt) adult (~5 mo old) male animals that had achieved full maturity.

Interestingly, our animals demonstrated a small increase in VO2 during exposure to hypoxia. In a previous study (2), we also observed this same slight increase in VO2 in response to hypoxia; therefore, the observations in the present study were not unexpected. Rather, they confirmed our earlier observations. We speculate that this slight (<10%) increase in VO2 during exposure to hypoxia likely results simply from the hyperpnea and related metabolic cost of breathing in this condition.

Consistent with the small increase in VO2 during hypoxia, we found an ~10% increase in VCO2 during hypoxia compared with room air. In animals exposed to hypoxia + 5% CO2, however, VCO2 decreased very slightly (~5%). This slight decrease in VCO2 was likely a simple result of the decreased CO2 gradient in the inspired air. VCO2 also decreased slightly as a result of acetazolamide treatment. This observation agrees well with a reported decrease in VCO2 during acute acetazolamide infusion in anesthetized dogs (8). Using the same dose of acetazolamide used in the present study, Cardenas et al. (8) found a 26% acute decrease in VCO2 at the lung that had still not been compensated for 1 h after injection. Furthermore, they found that an increase in the blood-alveolar CO2 gradient eventually primarily compensates for the decrease in VCO2 following acetazolamide treatment. Our measurements in hypoxia + 5% CO2 were made ~2.5 h after injection of acetazolamide and show a residual decrease in VCO2 of ~5–6%.

**Respiratory status.** In this study, we did not perform blood gas analysis to confirm the presence of a metabolic acidosis due to acetazolamide treatment. However, our observation that ventilation in room air conditions was stimulated to 70% above baseline conditions, combined with the characterization of the effects of acetazolamide in literature, allows us to say with certainty that our intervention had typical effects. Although the precise contribution of a decrease in peripheral chemoreceptor responsiveness could not be determined from the results of our study, the significance and potential implications of our findings remain the same.

**Effects of alterations in behavioral state.** Although we did not monitor indexes of sleep-wake status, we are confident that our results were not specifically related to changes in the state of arousal or state transitions. In our recordings, animals were generally in a state of quiet resting wakefulness. Animals will sleep in the chamber only if left undisturbed for extended periods of time: they assume a rather typical position, with closed eyes, and exhibit a lack of overt motor activity, aside from breathing movements. However, in these experiments, animals were not left undisturbed, and they remained alert, because any movement, noise, or changes in chamber air composition caused the animals to become curious and increase their activity before they resumed their state of quiet resting wakefulness. The duration of our recordings in any given condition was limited to 25 min, and the CO2 and O2 composition of the inlet gas was checked at the beginning and toward the end of the monitoring period in each condition.

Moreover, the generation of augmented breaths does not depend on any specific condition of wakefulness. Augmented breaths are present and observed in all behavioral states, including resting wakefulness, during grooming behavior and movement, and during “obvious” sleep, in the animals we study. In anesthetized animals, augmented breaths remain a typical feature of the breathing rhythm and still become far more prevalent in hypoxia (1, 9, 15, 33). Finally, during periods of observation that clearly cannot include any sleep-wake transitions, the prevalence of augmented breaths remains consistent with quiet periods. Since it is easy to identify periods of activity by changes in VO2, tidal volume, and breathing frequency, we can say that the prevalence of augmented breaths remains consistent with quiet periods.

**Potential strain specificity of responses.** We are aware of only one study that examined strain differences in rats and reported augmented breaths (16). The study of Golder et al.
disturbances due to the severe irregularity in breath amplitude. Other symptoms of AMS, at least in part, may be caused by sleep disturbances: during hypoxia, wherein CO$_2$ was maintained to within 2 Torr of resting levels, augmented breaths were observed in the phrenic nerve recordings of Fischer rats. Augmented breaths were not observed in these same Fischer rats during normoxia or during hypercapnia alone. Moreover, augmented breaths were not observed in any other strain of rat in any condition.

An extremely important methodological consideration in interpreting the results of Golder et al. (16) is that the animals were ventilated, and bilateral vagotomy was performed to prevent the entrainment of respiratory nerve activity with the ventilator activity. On the basis of other studies, one would certainly expect that vagotomy would have caused augmented breaths to largely “disappear” from the respiratory/phrenic traces (1, 15, 33). We have found it especially interesting that, in Fischer rats specifically, augmented breaths actually begin to “appear” in the phrenic recordings during exposure to hypoxia, despite the bilateral vagotomy. Moreover, compared with our results, the prevalence of augmented breaths was relatively high for isocapnic hypoxia (9 ABs/5 min in the trace they provide). Further studies may reveal important strain-related differences in the afferent mechanisms regulating augmented breath production in the integrated system.

Time-based effects of repeated exposures to hypoxia. Separate time-control experiments were not performed in this study. Instead, we used posttreatment “recovery” tests to control for any possible effect of repeated testing and exposures to hypoxia on the measured variables. Acetazolamide treatment has a “reversible” effect, in that the drug is rapidly cleared from the body, and therapeutic effects are no longer present. As such, recovery tests performed in the same animals provided a strong experimental design to control for any time-based factors that may have independently affected outcome measurements. If the effects of the drug were not reversible in this sense or caused permanent or long-lasting effects, then separate time-control studies in separate animals would have been required. The salient finding in this study was that the prevalence of augmented breaths during exposure to hypoxia is dramatically suppressed by Diamox. A comparison of baseline and recovery responses in hypoxia reveals that minute ventilation, breathing frequency, tidal volume, and gas exchange were nearly identical, despite the interposed Diamox treatment. During recovery tests, hypoxia once again caused a large increase in the prevalence of augmented breaths. The recovery tests therefore show that our observations are not related to time-dependent changes resulting from the repeated hypoxic exposures.

**Perspectives and Significance**

Acetazolamide is a drug indicated for use in the treatment of a wide range of conditions, including edema, some forms of epilepsy, and glaucoma. However, it is perhaps best known in pulmonary medicine for the treatment and prevention of AMS during ascent to altitude (31, 59). Periodic breathing, or Cheyne-Stokes respiration, is typically observed in most healthy adults on arrival at altitude (>2,500 m) and exacerbates other symptoms of AMS, at least in part by causing sleep disturbances due to the severe irregularity in breath amplitude and periodic breathing. During hypoxia, wherein CO$_2$ was maintained to within 2 Torr of resting levels, augmented breaths were observed in the phrenic nerve recordings of Fischer rats. Augmented breaths were not observed in these same Fischer rats during normoxia or during hypercapnia alone. Moreover, augmented breaths were not observed in any other strain of rat in any condition.

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One of the most dramatic effects of acetazolamide at altitude is the potent stabilizing effect on breathing rhythm (47). Our results suggest a novel mechanism through which this may occur. Hypoxia-induced hyperventilation at altitude results in hypocapnic hypoxia and respiratory alkalosis. We have shown that this unique condition dramatically increases the prevalence of augmented breaths. Moreover, augmented breaths are known to precipitate periods of breathing instability in hypobaric hypoxia (6, 25). Our present finding that acetazolamide decreases the prevalence of augmented breaths during exposures to hypoxia may partially explain the stabilizing effects of the drug, independent of other putative explanations. Although the mechanism by which acetazolamide is able to stabilize breathing at altitude remains unclear, it is most commonly believed to result from a reduction in the gain of the peripheral chemoreflex and/or an increase in the CO$_2$ reserve (27, 48, 49). A low gain in the peripheral chemoreflex feedback loop is believed to protect high-altitude natives from respiratory instability at altitude (25), and an increased CO$_2$ reserve would mean that larger decreases in arterial PCO$_2$ are required before there is a risk of reaching a putative “threshold” for apnea (11). Although these may be important factors, our finding that acetazolamide decreases the prevalence of transient respiratory disturbances in the form of augmented breaths represents a novel alternative mechanism through which the drug may stabilize breathing.

The problem of Cheyne-Stokes respiration and central sleep apnea is certainly not restricted to those who choose to ascend to altitude; it is a wide-spread problem affecting a large number of people in their everyday lives. For example, an estimated 40% of patients with heart failure suffer from sleep-disordered breathing in the form of Cheyne-Stokes respiration or central sleep apnea (23). Interestingly, there is evidence to suggest that a single dose of acetazolamide is effective in stabilizing breathing during sleep in these patients (22). Since heart failure patients are typically hypocapnic (19, 30, 40), it is possible that they exhibit an increased propensity to generate augmented breaths. We therefore speculate that the beneficial effects of acetazolamide treatment for central sleep apnea may be due in part to a reduction in the prevalence of respiratory disturbances in the form of augmented breaths.

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**REFERENCES**

ACETAZOLAMIDE SUPPRESSES AUGMENTED BREATHS


