Hypocapnia increases the prevalence of hypoxia-induced augmented breaths

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Hypocapnia increases the prevalence of hypoxia-induced augmented breaths. Am J Physiol Regul Integr Comp Physiol 296: R334–R344, 2009. First published December 17, 2008; doi:10.1152/ajpregu.90680.2008.—Augmented breaths promote respiratory instability and have been implicated in triggering periods of sleep-disordered breathing. Since respiratory instability is well known to be exacerbated by hypocapnia, we asked whether one of the destabilizing effects of hypocapnia might be related to an increased prevalence of augmented breaths. With this question in mind, we first sought to determine whether hypoxia-induced augmented breaths are more prevalent when hypocapnia is also present. To do this, we studied the breath-by-breath ventilatory responses of a group of freely behaving adult rats in a variety of different respiratory background conditions. We found that the prevalence of augmented breaths was dramatically increased during hypocapnic-hypoxia compared with room air conditions. When hypocapnia was prevented during exposure to hypoxia by adding 5% CO2 to the inspired air, the rate of occurrence of augmented breaths was no greater than that observed in room air. The addition of CO2 alone to room air had no effect on the prevalence of augmented breaths. We conclude that in spontaneously breathing rats, hypoxia promotes the generation of augmented breaths, but only in poikilopacalic conditions, where hypocapnia develops. Our results, therefore, reveal a means by which CO2 exerts a stabilizing influence on breathing, which may be of particular relevance during sleep in conditions commonly associated with respiratory instability.

sighs; carbon dioxide; hypocapnia; respiratory instability

The quiet, eupnoic breathing rhythm of mammals is periodically interrupted by spontaneous large breaths that have been called “sighs”, or “augmented breaths” (2, 4, 10). Augmented breaths have been documented and studied across a diverse spectrum of species (46, 47), and their ubiquitous presence alludes to the physiological importance of their function in the animal. Augmented breaths function to prevent atelectasis, which otherwise results in collapsed and hypoventilated regions of the lung (58). In the absence of occasional augmented breaths, lung compliance gradually decreases and efficacy of gas exchange worsens (3, 48, 58, 71). Therefore, an occasional augmented breath provides benefit by helping to maintain effective lung function.

A less favorable attribute of augmented breaths is that they promote instability in subsequent breathing cycles. Augmented breaths are often followed by a period of apnoea (25, 28, 60, 81). Even when apnoea does not result, these large breaths will alter the depth and rate of breathing for several subsequent respiratory cycles (4, 10, 23, 28, 29, 60, 81). Normally, the respiratory system is stable enough to resume a normal rhythmic pattern of breathing shortly after an augmented breath. However, in certain conditions, wherein the respiratory system is vulnerable to instability, an augmented breath can seriously disrupt normal breathing.

The respiratory control system is especially vulnerable to instability during sleep, where an augmented breath can precipitate central apnoeas and periodic breathing. Not surprisingly, the destabilizing consequences of augmented breaths have been directly implicated in contributing to sleep-disordered breathing in many people, including term and preterm infants (8, 23, 35), heart failure patients (57), idiopathic central sleep apnoea patients (79, 80), and in healthy adults at simulated altitude (6). Computational models also show that augmented breaths are transient respiratory disturbances capable of causing periodic breathing in vulnerable systems (11, 22, 38, 72). Despite the known destabilizing influence of augmented breaths, their prevalence in conditions associated with central forms of sleep-disordered breathing remains unclear.

What is clear from the existing body of research, involving central forms of sleep-disordered breathing is that they are commonly associated with abnormally low levels of CO2 in the arterial blood, otherwise referred to as hypocapnia. Indeed, eliminating hypocapnia through the addition of CO2 to the inspired air has been shown to effectively stabilize breathing (6, 9, 44, 69, 78). Presently, the most widely accepted interpretation of this stabilizing effect of CO2 on breathing during sleep involves the existence of an “apnoeic threshold” (18, 19, 82). Briefly, this concept proposes that when PCO2 falls below a critical threshold value, that chemical drive to breathe related to CO2 is no longer sufficient to sustain breathing activity during sleep. While the association between hypocapnia and central sleep disorders may well rely on an apnoeic threshold for CO2, other factors are thought to be involved, such as changes in the gain of feed-forward and feed-back components of the respiratory chemoreflexs, altered hemodynamics, and other as of yet undetermined mechanisms (19, 63, 67, 72).

We asked whether hypocapnia might also have an additional destabilizing effect by increasing the prevalence of transient respiratory disturbances in the form of augmented breaths. Hypoxia is well known to increase the prevalence of augmented breaths. However, while the general effect of CO2 in regulating the prevalence of augmented breaths has received some limited attention in prior studies, the results have been inconsistent and difficult to interpret (2, 10, 62, 65). We, therefore, performed experiments in freely behaving adult rats to study the prevalence of augmented breaths across a broad range of respiratory backgrounds. We provide evidence that the respiratory status of hypocapnic hypoxia, rather than hy-
Continuously monitored via a fast responding thermocouple (Therma-
<10°C; ADInstruments, Colorado Springs, CO). 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 calculations.

Experimental protocol. Rats were placed in the animal chamber and
exposed to a room air composition of fresh gas supplied to the chamber at a flow rate of \( \sim 2.5 \text{ l/min} \). This flow was selected to maintain the fraction of CO\(_2\) in the chamber below \(-0.4\%\) during the poikilocapnic exposures wherein CO\(_2\) was not added to the fresh gas entering the chamber. This ensured that animals would become hypocapnic during the phase of hypoxia-induced hyperventilation. Animals were allowed to acclimatize to the chamber environment for approximately 1 h before the experiments and recording of data began. The chamber was well-tolerated by the experimental animals, and at no time did they display signs of anxiety or fear. Typically, animals demonstrated grooming and sniffing behavior upon initial enclosure within the chamber, but these behaviors were minimal after the acclimatization period had passed.

To examine the influence of background O\(_2\) and CO\(_2\) levels upon the spontaneous generation of augmented breaths, six different fresh gas compositions were delivered to the animals from premixed compressed gas cylinders. These conditions included room air (~21% O\(_2\), hypoxia (~10% O\(_2\)), and hyperoxia (~95% O\(_2\)), as well as these same three conditions with 5% CO\(_2\) added. The backgrounds described above were applied in random order, each at the same mass flow rate as in the room air acclimatization period (~2.5 l/min). In each case, a room air exposure was interpolated between subsequent conditions. Animals were exposed to each condition and each interpolated room air exposure for a period of 10 min. O\(_2\) and CO\(_2\) concentrations in the fresh air being supplied to the chamber were checked before and after exposure to each condition for calculation of gas exchange, as described in the previous section. This protocol

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resulted in rats being exposed to 11 intervals of alternating background conditions.

Data analysis. estVE, f, VCO2, VO2, Tc, the O2 and CO2 composition of air leaving the chamber, and the number of augmented breaths were determined from data collected during the last 5 min of each condition. The concentration of O2 and CO2 in the air entering the chamber was also determined both before and after each condition. Because the protocol involved a total of 6 room air exposures, the third exposure was systematically selected for comparison to other conditions, regardless of the random sequence of conditions that came before.

All aforementioned variables were compared using two-factor repeated-measures analysis of variance procedures (SigmaStat V2.03, SPSS, Chicago, IL). Specifically, the two factors included in the analysis were O2 status and CO2 status. Within O2 status, the conditions of normoxia, hypoxia, and hyperoxia were considered. CO2 status included the conditions of poikilocapnia and hypercapnia. The a 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 paired t-testing procedures with Bonferroni’s correction applied. This analysis provided a conservative approach that determined the independent effects of O2 and CO2 condition on respiratory variables, but also allowed us to determine whether there was evidence for an interaction effect between the factors of O2 and CO2 condition. Averaged data are reported as means ± SE, unless otherwise indicated.

RESULTS

Each of the 5 rats were studied successfully in background conditions, including room air (~21% O2), hypoxia (~10% O2), and hyperoxia (>95% O2), as well as these same three conditions with 5% CO2 added. The chamber gas compositions, recorded and averaged for all animals for the six background conditions, are displayed in Fig. 1. In the hyperoxic condition, the FIO2S were slightly different across CO2 status (98.4 ± 0.4, vs. 93.8 ± 0.8%), by virtue of the fact that premixed tanks composed of 100% O2 and 95% O2 + 5% CO2 were used to provide the hyperoxic backgrounds. At nearly 100% Fio2, a difference of less than 5% is physiologically negligible. There were no observed differences in FICO2 across O2 status within the factor of CO2 status.

Raw flow traces obtained during the study of the 0.464-kg rat are shown in compressed time scale in Fig. 2. These traces correspond to the 5-min intervals, across which respiratory variables were calculated in each condition for this animal. An example of a spontaneous augmented breath with an expanded time scale is also shown, and the characteristics of the augmented breath can clearly be contrasted to those of the breaths proceeding and following the respiratory event. Because of the comparatively large inspiratory and expiratory flows occurring during the augmented breath cycle, these breaths were easily discerned from the background of normal eupnoeic breathing, even in the compressed time scale traces where the augmented breaths appear as episodic spikes in the trace. Obvious differences were also readily apparent with respect to the frequency of occurrence of augmented breaths in the trace obtained during the exposure to hypoxia alone.

Augmented breaths. The number of augmented breaths recorded in all background conditions are displayed in Fig. 3A. Both O2 status and CO2 status had significant independent effects on the number of augmented breaths generated during the 5-min recording intervals, and there was a significant interaction effect observed between these factors (P < 0.001). This interaction effect warranted further comparisons between specific background conditions. As discussed in the following paragraphs (Fig. 3B), conditions of poikilocapnic hypoxia was always associated with an increase in minute ventilation by at least 2 times. This condition will thus be referred to as hypocapnic hypoxia in the rest of the article.

Augmented breaths were far more prevalent in the condition of hypoxic hypoxia (13.0 ± 1.5 5 min−1) compared with either room air (1.0 ± 0.3 5 min−1, P < 0.001) or hyperoxic conditions (1.4 ± 0.5 5 min−1, P < 0.001). However, adding 5% CO2 during exposure to hypoxia to the inspired air had the dramatic effect of restoring the number of augmented breaths back to a level comparable with room air and hyperoxic conditions (2.4 ± 0.6 5 min−1, P < 0.001).

Because each animal was monitored during six separate exposures to room air conditions, we were able to determine the repeatability of their responses, specifically with respect to the rate of occurrence of ABs within an individual condition. All data used for this analysis is shown in Fig. 4. A one-way ANOVA did not reveal evidence of any differences across the six separate exposures to normal room air conditions in terms of the number of augmented breaths that occurred in the 5-min observation window (P = 0.768). The average “within-animal” and “within-trial” variability (i.e., the variability across room air exposures for a given animal and across animals within a given room air exposure) was small and nearly identical. Both within-animal and within-trial variability were ± 0.7 ABs·5 min−1, when expressed as the means of the collective standard deviations.

The relationship between O2 status, CO2 status, and the number of augmented breaths is represented graphically in Fig. 5. Clearly, the only condition wherein there was a distinctive increase in the prevalence of augmented breaths was hypcap-
nic-hypoxia, (again, hypoxia without supplemental CO₂). Since neither fB, estVT, nor estVE were similarly unique in this condition (see Fig. 6), the prevalence of augmented breaths could not have been directly determined by these variables. When the number of augmented breaths is plotted against ventilation, tidal volume, or breathing frequency (Fig. 6, A–C), the distinctiveness of the condition of hypoxia is emphasized. Data points representing the condition of hypoxia with no CO₂ added, appear as “outliers” on the rather horizontal relationship, which would have otherwise described these plots.

These results demonstrate a powerful stimulation of augmented breaths in hypocapnic hypoxia that is effectively reversed by the supplementation of 5% CO₂ in the inspired air. When all room air exposures across all animals were grouped to determine the average rate of occurrence augmented breaths in room air conditions, this average was 1.1 ± 0.1 5 min⁻¹. Thus, our arbitrary choice to use the 3rd room air exposure in each animal for comparison to all other background conditions did not influence our analysis of rate of occurrence of augmented breaths.

Minute ventilation. The levels of minute ventilation achieved during exposure in each of the background conditions for each animal are shown in Fig. 3B. The factor of O₂ status had a significant independent effect on the level of ventilation that was achieved across all background conditions (P < 0.001), as did the factor of CO₂ status (P < 0.003). However, there was no significant interaction effect observed between the factors of O₂ status and CO₂ status (P = 0.159), and so further comparisons between specific background conditions were not justified. Nevertheless, ventilation was greater in the condition of hypoxia (590 ± 40 ml·min⁻¹·100 g⁻¹) than it was in either room air (311 ± 27 ml·min⁻¹·100 g⁻¹; P < 0.001) or hyperoxia (319 ± 51 ml·min⁻¹·100 g⁻¹; P < 0.001), regardless of CO₂ level. Ventilation was not different between room air and hyperoxic conditions. When CO₂ was maintained below ~5%, ventilation was less compared with conditions where CO₂ was maintained at ~5%, regardless of background O₂ condition. These results verify that both hypoxia and hypercapnia provided significant drives to breathe in the animals we studied.

Breathing frequency. The effects of the background inspired gas conditions on breathing frequency are shown in Fig. 3C. Both O₂ condition and CO₂ condition had significant independent effects upon breathing frequency (P < 0.001 and P < 0.016, respectively). There was a significant interaction effect observed between the factors of O₂ and CO₂ status (P =
0.001), which warranted further comparisons between specific background conditions. The addition of 5% CO₂ to the inspired air caused breathing frequency to increase in both room air (from 71 ± 3 min⁻¹ to 119 ± 9 min⁻¹; P < 0.001) and hyperoxia (from 76 ± 3 min⁻¹ to 121 ± 5 min⁻¹; P = 0.001); however, breathing frequency was not significantly affected by the addition of 5% CO₂ in the condition of hypoxia, where breathing frequency was already elevated (137 ± 11 min⁻¹ vs. 131 ± 5 min⁻¹; P = 0.551). Across conditions wherein CO₂ was maintained below 0.5%, hypoxia caused a higher breathing frequency compared with either room air (P < 0.001) or hyperoxia (P < 0.001); however, room air and hyperoxia were no different in this regard (P = 1.000). Across conditions wherein CO₂ was maintained at ~5%, there were no observed differences in breathing frequency during the exposures to room air, hypoxia, or hyperoxia (p, range 0.428 to 1.000). These results show that in hypoxia, the prevention of hypocapnia through the addition of CO₂ to the inspired air had no significant effect on breathing frequency.

**Metabolic rate and chamber temperature.** Metabolic rate of the animals in the different background conditions was estimated by measuring their rates of CO₂ elimination. There was a significant effect of O₂ status on the rate of CO₂ elimination (P = 0.001), and a significant interaction effect between the factors of O₂ status and CO₂ status was observed (P = 0.005). Further comparisons between specific background conditions revealed that in the O₂ status condition of hypoxia, the addition of ~5% CO₂ to the inspired air resulted in a lower rate of CO₂ elimination.

**Fig. 3.** The effect of the six different background conditions on ventilation (VE), breathing frequency (fB), the number of augmented breaths (#AB) occurring, and metabolic rate as determined via measurement of CO₂ production (V̇CO₂). All animals are individually represented by a unique symbol that remains consistent in each condition and across all three panels. The mean values within each condition are also indicated (-x-) with error bars representing the SE. A: number of augmented breaths was dramatically increased by exposure to hypocapnic hypoxia, but this effect was suppressed when CO₂ was added to the inspired air. B: both 10% hypoxia and 5% CO₂ provided potent drives to breathe, but no interaction effects were observed. C: both hypoxia and 5% CO₂ caused breathing frequency to increase. Specifically, the addition of 5% CO₂ to the inspired air caused breathing frequency to increase in both room air and hyperoxic conditions; however, breathing frequency was not significantly affected by the addition of 5% CO₂ in the condition of hypoxia, in which breathing frequency was already elevated.

**Fig. 4.** The rate of occurrence of augmented breaths was the same across six separate room air exposures. In this figure, all five animals are represented by a symbol, which is unique to that animal across all six exposures. There was no difference between any of the individual room air exposure trials with respect to the rate of occurrence of augmented breaths (P = 0.768). The maximum number of augmented breaths observed in the 5-min observation window was 3, and the minimum was none. By far, the most common scenario observed was the occurrence of one augmented breath, which represented 59% of the data points. The distribution frequency of the data points is given to the right of the chart, and the horizontal dashed line indicates the average number of augmented breaths observed, as averaged across all animals and room air exposures.

![Image](image-url)
production compared with hypoxia with no CO2 added (14.6 ± 0.7 ml·kg⁻¹·min⁻¹ vs. 17.9 ± 0.6 ml·kg⁻¹·min⁻¹; P < 0.011). The addition of 5% CO2 to room air or hyperoxic air had no such effect. While similar trends were observed in oxygen consumption data, no interaction effect between O2 status and CO2 status was resolved.

No differences in Tc were observed across any of the conditions. In other words, neither the factor of O2 status nor CO2 status influenced the Tc to any significant extent. Average temperature in the animal chamber during the experiments was 26.8 ± 0.2°C. These results show that CO2 elimination was elevated in animals while exposed to hypoxia without added CO2, compared with other conditions. This increased CO2 output was not related to any observed difference in ambient temperature of the animal chamber.

DISCUSSION

In this study, we found that hypocapnic hypoxia dramatically increased the prevalence of augmented breaths. In normal room air conditions (~20% O2, <0.5% CO2), animals demonstrated an average of 1 augmented breath in a 5-min observation window. When animals were exposed to hypoxia (~10% O2) and allowed to become hypocapnic, the rate of occurrence of augmented breaths increased dramatically to 13 in the 5-min observation window. Most remarkably, when hypocapnia was prevented during exposure to hypoxia by adding 5% CO2 to the inspired air, a powerful reduction in the generation of augmented breaths was observed. When animals were exposed to the same level of hypoxia but with ~5% CO2 added, the number of augmented breaths fell from 13 to an average of 2.4 in the 5-min observation window, representing a suppression of more than 80%. Therefore, our results demonstrate that transient respiratory disturbances in the form of augmented breaths become most prevalent when the respiratory status is hypocapnic and hypoxic. This particular effect of hypocapnia on the generation of transient respiratory disturbances in the form of augmented breaths has not previously been reported.

Comparison with previous studies. Four previous studies have attempted to investigate the effect of background O2 and CO2 levels upon the prevalence of augmented breaths (2, 10, 62, 65). All of these have shown that hypoxia increases the prevalence of augmented breaths; however, the effect of hyper- and/or hypocapnia have been less consistently reported. Renninger and Segall (62) found that the addition of 3% CO2 to the inspired air resulted in a doubling in the rate of occurrence of augmented breaths, regardless of whether oxygen background was hypoxic or normoxic. However, this study, performed in dogs, is difficult to interpret since it was published only in abstract form, and the details regarding methodology and results are unavailable.
Cherniack et al. (10) found that in hypoxia, augmented breaths occurred more often when CO₂ levels were increased from the hypocapnic range (~28 Torr) toward normocapnia (~38 Torr). Increasing arterial CO₂ beyond this point into the hypercapnic range (~60 Torr) had no effect on the rate of occurrence of augmented breaths. Unfortunately, the rate of occurrence of augmented breaths in normal room air conditions is not reported for further comparison. Significant methodological differences in their study include the use of pentobarbital anesthesia and gallamine paralysis with phrenic-driven “servo-respirator” in experiments. The authors mention that, “In most experiments the cats were paralyzed with gallamine either from the beginning or only at a later stage of the experiment”, but it is not clearly stated how many cats were paralyzed and ventilated, and when during the experiments this was done. The use of pentobarbital anesthesia may have affected the mechanisms leading to the generation of augmented breaths, as has been demonstrated in other animals (65). Moreover, because of the essential role of vagal feedback in the generation of augmented breaths (2, 26, 45), their use of the servo-respirator further complicates interpretation of data.

Schwenke and Cragg (65) found that 8% CO₂ in the inspired air resulted in a more than 3-fold increase in the rate of occurrence of augmented breaths, compared with room air conditions. However, the authors concede the fact that guinea-pigs have an atypical chemoreflex feedback system compared with other mammals. This includes a low sensitivity to hypoxia, and moreover, carotid body denervation does not affect the respiratory response to hypoxia. The sensitivity to CO₂ is also relatively modest in these animals (65). It is, therefore, likely that the respiratory control system of guinea-pigs has also developed atypical strategies for regulating the rate of occurrence of augmented breaths.

In one last study, Bartlett (2) found that “... the inspired CO₂ concentration had no influence on the frequency of sighs at any inspired O₂ level.” As was the case in our experiments, Bartlett studied rats while awake. However, there were important methodological differences between our studies in the way the responses to hypoxia and CO₂ were assessed. Bartlett used a closed-system plethysmograph in which the chamber was sealed off during the 2–4 min over which time the augmented breaths were counted. As such, the rat in the chamber became progressively hypoxic and hypercapnic, even when the chamber was initially close to atmospheric conditions. In other words, the background of respiratory gas composition was not presented to the respiratory control system in a steady state, but rather was in a state of dynamic alteration throughout the recording interval. Whether or not the mechanism(s) that control the rate of occurrence of augmented breaths are sensitive to the rate component of changes in background F<sub>CO₂</sub> remains an outstanding question.

Hypocapnic hypoxia and the generation of augmented breaths—mechanism of action. Hypoxia leads to a stimulation of breathing via the peripheral chemoreceptors, which results in a hyperventilation with respect to CO₂ production in the tissues. In our experiments, the condition of hypoxia alone caused animals to nearly double their level of ventilation compared with room air conditions (see Fig. 3B), leading to a condition of hypocapnia. With 5% CO₂ added to the hypoxic background, hypocapnia would have been prevented. This difference in CO₂ status during hypoxia dramatically altered the occurrence of augmented breaths in the two conditions. Several possible mechanisms related to the systemic action of hypocapnia may be implicated in our findings.

Vagal feedback. Vagal feedback is essential to the generation of augmented breaths (2, 26, 45). It could, therefore, be that hypocapnia results in an increase in afferent discharge from relevant receptor fibers. Several different types of afferent receptors in the lungs have been studied with respect to their effect on the generation of augmented breaths, including rapidly adapting receptors (RARs), and slowly adapting receptors (SARs). RARs have been strongly implicated in the mechanisms generating augmented breaths (15, 26, 39, 45, 74). However, what limited data we have available suggest that RAR afferents do not increase their rates of discharge in response to hypocapnia (61). On the other hand, SARs are known to respond to hypocapnia by increasing their rate of discharge (12, 30, 41, 50). However, SARs are not strongly implicated in the generation of augmented breaths, and only limited data suggest that they play any role in determining their rate of occurrence (45). As such, despite the importance of vagal feedback in the generation of augmented breaths, it remains unlikely that hypocapnia facilitates them via the stimulation of either RARs or SARs.

Central-peripheral chemoreceptor interaction. During hypoxia-induced hypocapnia, the peripheral chemoreceptors are stimulated by hypoxia, while the central CO₂-sensitive chemoreceptors experience a reduction in their level of activation. It is an interesting possibility that such a disparity in drive from the peripheral and central respiratory chemoreflexes may precipitate an increase in the rate of occurrence of augmented breaths. In this scenario, the addition of 5% CO₂ during hypoxia would remove the disparity in drive from the peripheral and central respiratory chemoreflexes, and thereby remove this facilitation toward the generation of augmented breaths. While this specific hypothesis remains to be tested, an interaction between central and peripheral chemoreflexes has already been demonstrated by several other investigators. It has been found that central hypocapnia, or alkalosis, results in an augmented respiratory response to peripheral chemoreceptor stimulation (5, 17, 66). In other words, when hypoxia is accompanied by central hypocapnia, ventilation is greater than it is when central isocapnia is maintained. A similar effect on the mechanism that regulates the prevalence of augmented breaths may well exist. Specifically, when the carotid chemoreceptors are stimulated by hypoxia and the central chemoreceptors are exposed to hypocapnia, the prevalence of augmented breaths may become unusually high.

It is also important to consider that both hypoxia and hypocapnia can independently affect airway smooth muscle tone via alterations in vagal efferent motor outflow through chemoreceptor activation (36, 55, 56). This effect of hypoxia and CO₂ on airway caliber is known to be mediated by peripheral and central chemoreceptors, respectively (55). It is, therefore, an interesting possibility that any “disparity” in the central and peripheral inputs to the neural control of airway caliber, as is the case during hypocapnic hypoxia, might facilitate the generation of augmented breaths. Such a mechanism might involve differential alteration in regional airway resistances in the upper and lower pulmonary tract, which could result in an increased stimulation of a lung vagal afferents during the breath cycle.
Brain blood flow. Hypocapnic hypoxia, and hypoxia in the absence of hypocapnia, present the respiratory centers with two distinctly different conditions in terms of blood flow compensation. Brain stem blood flow is highly sensitive to changes in the partial pressures of O₂ and CO₂. Both hypoxia and increased CO₂ increase brain stem blood flow in an attempt to maintain adequate oxygen delivery and tissue pH (7, 52). On the other hand, hypocapnia has the opposite effect on brain stem blood flow; it is decreased in an attempt to slow the removal of CO₂ from the tissues and thereby maintain normal tissue pH (16, 34). Hypocapnic hypoxia, therefore, represents a specific condition wherein competing mechanisms are simultaneously acting to increase (hypoxia) and decrease (hypocapnia) brain blood flow, resulting in an adjustment that is ideal for neither the regulation of oxygen delivery nor tissue pH. These changes in blood flow in different respiratory backgrounds are regional differences with regard to the changes in perfusion that occur in brain stem regions containing neuronal populations that are involved in respiratory rhythm generation and sensory integration. For example, recent evidence shows that during hypoxic challenge, the ventral respiratory group receives a more pronounced increase in perfusion than does the nucleus tractus solitarius (NTS) (7). The NTS is, therefore, more likely to be affected by hypoxic episodes, and further by any hypocapnia-induced attenuation of blood flow that might also occur. The NTS is known to be important in the integration of sensory information, including afferent feedback from the carotid bodies. It is possible that deprivation of adequate blood flow, particularly during hypocapnic hypoxia may leave the NTS particularly susceptible to changes in oxygen delivery and CO₂/pH regulation, which might, in turn, increase the prevalence of augmented breaths. While this is an interesting possibility, the role of the NTS in the generation of augmented breaths is not known.

Neuronal excitability. Hypoxia-induced hypocapnia results in acute respiratory alkalosis, which is associated with increased neuronal excitability (68). Indeed, the ability of respiratory centers to control extracellular CO₂ and pH has been proposed to be an important mechanism through which the brain is capable of regulating the excitability of neuronal structures. The mechanism involved in this modulation of neuronal excitability may involve a pH-dependent modulation of adenosine and ATP at the cellular level (21). It is an interesting possibility that the respiratory alkalosis associated with hypocapnic hypoxia causes an increase in the excitability of the respiratory neuronal elements involved in the generation of augmented breaths, such that the propensity of the respiratory system to generate them is greatly increased.

Implications of the prevalence of augmented breaths during hypocapnic-hypoxia. Augmented breaths are known to precipitate instability in the breathing cycle (4, 23, 28, 29, 60, 81). Therefore, the unusually high prevalence of augmented breaths during hypocapnia predisposes the respiratory system to transient disturbances and instability more frequently than is the case in other respiratory backgrounds. We propose that the propensity of the respiratory system to generate augmented breaths in hypoxia may exacerbate sleep-disordered breathing when hypocapnia is present.

For example, periodic breathing or Cheyne-Stokes respiration is observed in most persons upon arrival at altitude above 2500 m, despite them having no indication of any disordered breathing at sea level (6, 27, 42, 51, 73). Although no study has specifically examined the rate of occurrence of augmented breaths at altitude or in hypobaric hypoxia in humans, they have been specifically implicated in destabilizing breathing (6). Moreover, CO₂ has been reported to stabilize breathing during sleep at simulated altitude by preventing arterial PCO₂ from decreasing below an “apnoeic threshold” (6). This apnoeic threshold is likely related to but should not be confused with the “CO₂ chemoreflex threshold”. The former is a more conceptual threshold that is not observed in awake humans (13, 14), while the latter can be measured during wakefulness or sleep by modified Read CO₂ rebreathing tests (20). Nevertheless, our results suggest that the stabilizing effect of CO₂ in hypoxia may involve the suppression of respiratory disturbances in the form of augmented breaths. This intriguing possibility requires further studies in humans at altitude, where the propensity to generate augmented breaths is observed and the stabilizing effect of CO₂ can be examined.

Our results may also be especially relevant to patients with chronic heart failure, a large clinical population wherein ~40% of patients exhibit Cheyne-Stokes breathing during sleep (37). Sleep-disordered breathing in these patients is known to be related to the chronic hypocapnia, which is a hallmark of heart failure (31, 43, 57). The observation that supplemental CO₂ stabilizes breathing in Cheyne-Stokes heart failure patients (32, 40, 64, 69) suggests that the respiratory status of hypocapnia precipitates their breathing instability.

Our results lead us to speculate that Cheyne-Stokes heart failure patients exhibit an unusual prevalence of destabilizing augmented breaths during sleep due to their respiratory status of hypocapnia. We further predict that supplemental CO₂ reduces the prevalence of augmented breaths and that this may help to explain how CO₂ acts to stabilize breathing in these patients. Nevertheless, the prevalence of augmented breaths in the breathing pattern of heart failure patients with Cheyne-Stokes breathing during sleep has not been investigated, so this possibility remains speculative.

While the prevalence of augmented breaths has not yet been investigated in heart failure patients or in persons at altitude, there is another clinical condition typically associated with hypocapnia wherein the prevalence of augmented breaths has been studied. Patients with panic disorder are commonly found to hyperventilate at rest resulting in a respiratory status of hypocapnia (59). It has also been documented that these patients exhibit an abnormally high prevalence of augmented breaths (1, 75–77). Interestingly, patients with panic disorder also exhibit a large degree of respiratory irregularity during resting breathing, during wakefulness (1), and sleep (49, 70). While the significance of the increased prevalence of augmented breaths in panic disorder patients remains a complex topic of debate, these observations in humans do corroborate our finding that hypocapnia increases the prevalence of augmented breaths.

Limitations. The following caveats regarding our observations and interpretations deserve mention. As our data shown in Fig. 3A shows, four of the five rats that we studied had a small increase in the prevalence of augmented breaths when 5% CO₂ was added to room air; in one rat there was a small decrease in number. Our statistical analysis could not resolve this difference as being significant, but this may reflect a type 2 error. Such an error could be related to the relatively small
number of animals used in the study, the relatively short 5-min sampling period, and the possibility that the relatively low incidence of augmented breaths may have allowed us to detect only relatively large effects, such as the potent increase in the prevalence of augmented breaths in hypocapnic hypoxia.

The effect of metabolic rate on outcome measures becomes an important consideration when studying small animals, such as rats. This is because smaller mammals have a higher metabolic rate than larger mammals (24). We are not aware of any study to date that has specifically examined the effect of metabolic rate per se on the incidence of augmented breaths. However, it is known that larger mammals are known to express less frequent augmented breaths compared with small mammals (46, 47). Indeed, one of the benefits of using rats to study mechanisms involved in the generation of augmented breaths is that they express them relatively frequently, several times per hour in normal conditions. Because of this inversely proportional relationship between body mass and prevalence of augmented breaths, we cannot exclude the possibility that the relatively higher metabolic rate in rats (vs. humans) may facilitate their rate of occurrence through as of yet undescribed mechanisms.

Metabolic rate also becomes an important issue to consider because of the phenomenon of metabolic depression, which is often observed in small mammals in response to hypoxia (53, 54). However, metabolic depression is unlikely to be mechanistically involved in the increased prevalence of augmented breaths that we observed during hypcapnic hypoxia. While small rats (<100 g) do have the capacity to decrease their metabolism in response to hypoxia, this ability is somehow largely lost as the animals become older and larger (>250 g) (54). In the present study, we confirmed that larger rats (in our case ~500 g) do not exhibit the phenomenon of hypoxia-induced metabolic depression. If anything, we found an apparent increase in the rate of CO2 elimination during exposure to hypcapnic hypoxia, compared with room air conditions. Since \( V_{\text{O}_2} \) did not change significantly across any condition, it is likely that the relative hyperventilation that occurred during exposure to hypcapnic hypoxia, as well as the specific cardiovascular and respiratory kinetics involved, resulted in an apparent increase in the rate of CO2 elimination over the time interval during which we monitored respiratory variables, due to a decrease in body stores of CO2. This explanation is corroborated by our observation that \( V_{\text{CO}_2} \) did not increase when the animals were exposed to hypoxia with 5% CO2 added.

In the rats we studied, augmented breaths caused noticeable instability in subsequent respiratory cycles that included a prolonged postexpiratory pause, alterations in tidal volume and breathing frequency and often resulted in prolonged apneas lasting for several respiratory cycles. However, we did not perform a focused assessment of the effect of the different respiratory backgrounds on any defined measures of breathing instability, which resulted from augmented breaths, as this was beyond the purpose of this report. We would like to note, however, that some respiratory backgrounds appear to be more prone to different forms of instability after an augmented breath occurs. Specifically, while augmented breaths occurring in hypcapnic hypoxia were far more frequent than in any other background we studied, there seemed to be less propensity for any one of them to result in a prolonged apnea. This is not to say instability did not result from augmented breaths in this background; indeed, breath parameters of subsequent respiratory cycles were affected and apneas did occur. Nevertheless, whether or not any one augmented breath is more likely to result in prolonged periods of instability, as well as the nature of the instability which is likely to be triggered in a given respiratory background, remain important and outstanding issues.

Perspectives and Significance

Under normal conditions in healthy adults, the respiratory control system is remarkably robust to transient disturbances such as augmented breaths, and it can rapidly recover to generate a stable breathing rhythm, even during sleep. However, under specific conditions such as early infancy, cardiac insufficiency, or an exposure to altitude, the respiratory system becomes prone to prolonged periods of instability that can be triggered by transient respiratory disturbances. It is therefore important to understand those factors that regulate the prevalence of augmented breaths, since they have been implicated in sleep-disordered breathing. In this study performed in adult rats, we have shown that hypcapnia resulting from hypoxia-induced hyperventilation leads to a dramatic increase in the rate of occurrence of augmented breaths, any one of which represents a potentially destabilizing event. While supplemental CO2 is known to provide a stabilizing influence on breathing in the vulnerable conditions previously mentioned, the mechanism of action is still not agreed upon. If our results are applicable to humans, then they suggest that supplemental CO2 may act to stabilize breathing in conditions normally associated with hypcapnia, at least in part through suppressing the elaboration of augmented breaths.

REFERENCES


