Modulation of the contractile responses of guinea pig isolated tracheal rings after chronic intermittent hypobaric hypoxia with and without cold exposure

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Submitted 19 November 2004; accepted in final form 25 April 2005

Chakrabarty, Kaveri, and M. Fahim. Modulation of the contractile responses of guinea pig isolated tracheal rings after chronic intermittent hypobaric hypoxia with and without cold exposure. J Appl Physiol 99: 1006–1011, 2005; doi:10.1152/japplphysiol.01304.2004.—Previous studies have documented that repetitive exposure to intermittent hypoxia, such as that encountered in preparation to high-altitude ascent, influences breathing. However, the impact of intermittent hypoxia on airway smooth muscle has not been explored. Ascents to high altitude, in addition to hypoxia, expose individuals to cold air. The objective of the present study is to examine the effect of chronic intermittent hypobaric hypoxia (CIH) and CIH combined with cold exposure (CIHC) on tracheal smooth muscle responses to various contractile and relaxant agonists. Experiments were performed on tracheal rings harvested from adult guinea pigs exposed either to CIH or CIHC [14 days (6 h/day) at barometric pressure of 350 mm Hg and without cold exposure of 5°C] or to room air (normoxia). CIH and CIHC attenuated maximum contractile responses to ACh compared with normoxia. The maximum contractile response to histamine decreased with CIH, whereas CIHC restored the response back to normoxia. Both CIH and CIHC attenuated maximum contractile responses to 5-HT. Altered contractile responses after CIH and CIHC were independent of epithelium. Isoproterenol-induced relaxation was not altered by CIH, whereas it was enhanced after CIHC, and these responses were independent of the epithelium. The data demonstrate that intermittent exposure to hypoxia profoundly influences contractile response of tracheal smooth muscle, and cold exposure can further modulate the response, implying the importance of cold at high altitude.

Various patterns of intermittent hypoxia have been used in several studies, depending on the duration and intensity of hypoxia (19). Our focus has been on the chronic intermittent hypobaric hypoxia (CIH) that is often encountered when repeated adjournment to high altitude is followed by return to the sea level. During CIH, an individual is exposed to repeated cycles of hypoxia and reoxgenation. CIH such as that encountered with high-altitude adjournment can evoke many beneficial adaptive responses to hypoxia (10, 19, 20). CIH due to repeated adjournment to high altitude can enhance hypoxic ventilatory response (21). Intermittent exposure to high altitude has been used to “optimize” acclimatization (18) and even for the treatment of bronchial asthma and chronic lung disease (23). Studies have been carried out on airway responsiveness to cholinergic agonists after exposure to chronic hypoxia (1, 4, 22). It has been reported that chronic hypoxia increased the sensitivity of tracheal smooth muscle to cholinergic agonists without altering maximum response (1) or with decreased maximum response (22). Furthermore, it has been reported that chronic hypoxia decreased the contractile responses of tracheal rings to lower concentrations of methacholine without altering maximum contractile response ($T_{\text{max}}$) (4). However, no study, to our knowledge, has yet investigated the effect of CIH on airway responsiveness to contractile and relaxant agonists. Furthermore, periodic exposure to high altitude also involves periodic cold exposure, which may potentially influence the responses to CIH. The present investigation was, therefore, designed to examine the responsiveness of tracheal smooth muscle to contractile agonists (ACh, histamine, and 5-HT) and to a $\beta_2$-adrenoceptor agonist (isoproterenol) in vitro after exposure to CIH and CIH combined with cold exposure (CIHC), respectively. The exogenous contractile agonists were chosen, keeping in view that these agents are present endogenously to control the activity of tracheal smooth muscle. It has been suggested that release of nitric oxide from airway epithelium caused attenuation of the contractile responses of tracheal smooth muscle to methacholine after exposure to chronic hypoxia (4). It may be possible that, similar to chronic hypoxia, release of relaxant substance(s) from airway epithelium may modify the contractile responses of tracheal smooth muscle to agonists after exposure to CIH and CIHC. We, therefore, examined the involvement of airway epithelium under these conditions. Guinea pigs were selected for this study, as it is well known that airway smooth muscle of guinea pigs closely resembles that of human beings (25). The present investigation demonstrated enhanced/attenuated contractile responses of guinea pig airway smooth muscles to contractile and relaxant agonists after exposure to CIH and CIHC.

MATERIALS AND METHODS

Experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The Institutional Animal Ethical Committee, Vallabhbhai Patel Chest Institute, University of Delhi, India, approved this study.

**CIH.** Male guinea pigs, weighing between 500 and 700 g, were exposed in a hypobaric chamber at barometric pressure of 350 mm Hg, corresponding to an altitude of ~20,000 ft. The temperature of the chamber was kept at 28°C. The animals were kept at this barometric pressure for 6 h daily and for a period of 2 wk. After the 2-wk exposure period, the animals were killed immediately after the last day’s exposure, and the tracheal smooth muscle activity to the contractile agonists (ACh, histamine, and 5-HT) and to a relaxant agonist (isoproterenol) was studied in both epithelium-intact and epithelium-denuded tracheal rings.

**CIHC.** A similar procedure as described above was carried out, but in this set the animals were exposed to cold air (5°C) along with hypobaric hypoxia (350 mm Hg).

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Control. Animals were maintained in the animal house under normobaria and at 28°C. After 2 wk, tracheal smooth muscle activity to agonists was studied.

In vitro experiments. Animals were anesthetized with pentobarbitone sodium (50 mg/kg ip). Trachea was carefully dissected and placed immediately in ice-cold Krebs-Henseleit solution bubbled with 95% O₂ and 5% CO₂. The trachea was cleaned of adhering connective tissue and then cut into rings of equal width (~4 mm width). Because both tracheal ring preparations (epithelium-intact and epithelium-denuded) were required, a portion of trachea was selected and divided into two rings. In one of the rings, the epithelium was left intact, and, in the adjacent ring, the epithelium was removed gently by rubbing the luminal surface of the tracheal rings. The tracheal rings were mounted between metallic hooks in a 10-ml organ bath filled with buffer and bubbled with 95% O₂ and 5% CO₂. During the experiment, the temperature and pH of the buffer were maintained at 37 ± 0.5°C, whereas the pH of the buffer was maintained at 7.4 ± 0.04. The tracheal rings were equilibrated for 2–3 h under initial tension of 2 g, and the buffer was changed every 15 min for stabilization of baseline tension. Changes in isometric tension were measured by using force displacement transducers (Grass FT-03c) connected to a polygraph (Lectromed) through strain-gauge amplifiers (Lectromed, model 5220). When baseline tension was stabilized, responses of ACh administered in a cumulative manner were recorded. The next higher concentration of drug was added only after a stable plateau was reached. Cumulative concentration–response curve (CCRC) for ACh was constructed. The tissues were washed four to five times until the baseline tension was achieved. After stabilization, similar procedures were carried out to obtain CCRCs for histamine and 5-HT. After sustained contraction was attained with ACh (10⁻⁶ M), isoproterenol was added in a cumulative manner to the bath to obtain CCRC. At the end of each experiment, the tracheal rings were blotted and weighed (wet weight). The removal of epithelium was confirmed by histological examination of the tissues.

Drugs and solution. The Krebs-Henseleit solution was of the following composition (in mM): 118 NaCl, 4.8 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, and 11 glucose. The pH of buffer was 7.4 aerated with 95% O₂ and 5% CO₂ at 37°C. ACh bromide, histamine dihydrochloride, 5-HT hydrochloride, and isoproterenol hydrochloride were purchased from Sigma Chemical. The dilutions of the drugs were prepared in double-distilled water on the day of the experiment.

Statistical analysis. Data are presented as means ± SE. One-way ANOVA followed by Bonferroni post hoc test for all pairs of columns was performed. Contractile responses are expressed as absolute values in milligrams per milligram wet weight and as percentage of maximum response. The number of observations is indicated by n. Results are expressed as Tₘₐₓ (mg/mg wet wt) and −log EC₅₀ (M) (the concentration of the drug producing 50% of the maximum response, reflecting sensitivity of the tissue to the agonists). −Log EC₅₀ were determined from concentration-response curves and calculated by using nonlinear regression analysis. P < 0.05 was considered statistically significant.

RESULTS

Contractile responses of tracheal rings to ACh. A rightward shift of the CCRCs with differences of −log EC₅₀ indicated that both CIH and CIHC evoked a small but statistically significant decrease in the sensitivity of tracheal rings to ACh in the presence and absence of epithelium (Fig. 1, A and C, and Table 1). CIH and CIHC significantly attenuated Tₘₐₓ in both the preparations (Fig. 1, B and D). Tₘₐₓ values of epithelium-intact rings were 38.7 ± 2.5 mg/mg wet wt after CIH and 51.2 ± 5.1 mg/mg wet wt after CIHC. Both values were less than the normoxic value (73.8 ± 3.3 mg/mg wet wt, P < 0.05). Tₘₐₓ values of epithelium-denuded rings were 37.7 ± 2.3 mg/mg wet wt after CIH and 56.8 ± 6.1 mg/mg wet wt after CIHC, which were also less than normoxia (81.3 ± 3.7 mg/mg wet wt, P < 0.05). The only difference between epithelium-intact and epithelium-denuded preparations was that there was

![Graph A](http://jap.physiology.org/)

![Graph B](http://jap.physiology.org/)

![Graph C](http://jap.physiology.org/)

![Graph D](http://jap.physiology.org/)

*Fig. 1. Effects of normoxia, chronic intermittent hypobaric hypoxia (CIH), and CIH combined with cold exposure (CIHC) on the responsiveness of epithelium-intact (A and B; n = 15) and epithelium-denuded (C and D; n = 12) tracheal rings. A and C: cumulative concentration-response curves for ACh. Contractile responses are expressed as percentage of maximum contraction to 10⁻⁴ M ACh. B and D: maximum contractile response (Tₘₐₓ) to 10⁻⁴ M ACh. Values are means ± SE. *P < 0.05 between normoxia and CIH. †P < 0.05 between normoxia and CIH. ‡P < 0.05 between CIH and CIHC.

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a small, statistically significant increase in $T_{\text{max}}$ of denuded preparations induced by CIHC (56.8 ± 6.1 mg/mg wet wt) compared with CIH (37.7 ± 2.3 mg/mg wet wt, $P < 0.05$) (Fig. 1D).

**Contractile responses of tracheal rings to histamine.** CIH caused rightward shift of the CCRCs with differences of $-\log EC_{50}$ values from normoxia in intact and denuded preparations, indicating a small but statistically significant decrease in the sensitivity of rings to histamine. There was no difference in CCRCs and $-\log EC_{50}$ values between normoxia and CIH in both of the preparations (Fig. 2, A and C, and Table 1). CIH decreased $T_{\text{max}}$ of epithelium-intact rings to histamine (34.8 ± 2.9 mg/mg wet wt) compared with normoxia (70.7 ± 3.6 mg/mg wet wt; $P < 0.05$). In contrast, $T_{\text{max}}$ to histamine after CIHC (65.7 ± 6.4 mg/mg wet wt) enhanced from CIH ($P < 0.05$) but matched the value of normoxic group in epithelium-intact rings ($P > 0.05$) (Fig. 2B). Similar differences of $T_{\text{max}}$ between the groups were observed in the denuded preparations (Fig. 2D).

**Contractile responses of tracheal rings to 5-HT.** CIH shifted CCRC to the left from normoxia and CIH in epithelium-intact rings. However, $-\log EC_{50}$ values indicated that CIH produced a small, statistically significant increase in the sensitivity of epithelium-intact rings to 5-HT, compared with normoxia and CIHC. No differences of CCRCs and $-\log EC_{50}$ values between normoxia and CIHC were observed after epithelial denudation. This indicated that the increased sensitivity observed after exposure to CIH in the intact preparations was normalized in the denuded preparations. The CCRCs and $-\log EC_{50}$ values were unaltered in both of the preparations after exposure to CIHC (Fig. 3, A and C, and Table 1). $T_{\text{max}}$ values of both of the preparations were significantly attenuated after exposure to both CIH and CIHC (Fig. 3, B and D). In epithelium-intact rings, $T_{\text{max}}$ values were 17.8 ± 2.3 mg/mg wet wt after CIH and 20.2 ± 1.5 mg/mg wet wt after CIHC. Both values were less than normoxic value (51.4 ± 3.4 mg/mg wet wt, $P < 0.05$). Similar differences of $T_{\text{max}}$ between the groups were observed in the denuded preparations.

**Relaxant responses of tracheal rings to isoproterenol.** The CCRCs for isoproterenol were shifted to the left with a significant shift of $-\log EC_{50}$ values by CIHC in both of the preparations from normoxia and CIH (Fig. 4, A and C, Table 1). Maximum relaxation values were not affected by CIH and CIHC in both of the preparations of rings (Fig. 4, B and D).

**DISCUSSION**

Previous studies with chronic hypoxia showed increased sensitivity of tracheal smooth muscle to cholinergic agonists (1, 22). However, the effect of chronic hypoxia on $T_{\text{max}}$ to

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**Table 1. Effects of CIH and CIHC on the $-\log EC_{50}$ values of tracheal smooth muscle to different agonists in epithelium-intact and epithelium-denuded tracheal rings**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>CIH (mg/mg wet wt)</th>
<th>CIHC (mg/mg wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Histamine</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>5-HT</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.15</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SE; $n$, no. of observations. CIH, chronic intermittent hypobaric hypoxia; CIHC, CIH combined with cold exposure; +Ep, epithelium intact; −Ep, epithelium denuded. $-\log EC_{50}$ (M) values were plotted from respective cumulative concentration response curves. *$P < 0.05$ between normoxia and CIH; †$P < 0.05$ between normoxia and CIHC; and ‡$P < 0.05$ between CIH and CIHC.

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**Fig. 2. Effects of normoxia, CIH, and CIHC on the responsiveness of epithelium-intact (A and B; $n = 16$) and epithelium-denuded (C and D; $n = 10$) tracheal rings. A and C: cumulative concentration-response curves for histamine. Contractile responses are expressed as percentage of maximum contraction to $10^{-6}$ M histamine. B and D: $T_{\text{max}}$ to $10^{-6}$ M histamine. Values are means ± SE. *$P < 0.05$ between normoxia and CIH. †$P < 0.05$ between CIH and CIHC.**
cholinergic agonists is not clear. \( T_{\text{max}} \) of tracheal rings to carbachol was found to be attenuated after exposure to chronic hypoxia (22). Conversely, other reports indicated that chronic hypoxia did not alter \( T_{\text{max}} \) of tracheal rings to ACh/methacholine (1, 4). However, chronic hypoxia could decrease the contractile responses of tracheal rings to lower concentrations of methacholine (4). The first finding of the present study was the attenuation of \( T_{\text{max}} \) of tracheal smooth muscle to ACh after exposures to CIH and CIHC. A small, statistically significant attenuation of the sensitivity of tracheal smooth muscle to ACh was observed after exposures to CIH and CIHC. It is clear from this study that hypersensitivity to cholinergic agonists induced
by chronic hypoxia was modified by CIH and CIHC. However, similar to chronic hypoxia (22), we observed decreased maximum contraction to ACh induced by both CIH and CIHC. Airway hyporesponsiveness to ACh induced by CIH and CIHC, in contrast with chronic hypoxia (1), may evoke a beneficial effect due to repeated ascents to high altitude. In an earlier study, it has been reported that either epithelium removal or administration of N^G-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, potentiated the attenuated contractile responses to methacholine after exposure to chronic hypoxia and suggested that release of nitric oxide from airway epithelium caused attenuation of contractile responses of tracheal smooth muscle under the same condition (4). However, we observed that removal of epithelium had no effect, either on T_{max} or on the sensitivity of tracheal rings to ACh following CIH or CIHC. This is thus unlikely that attenuation of contractile responses to ACh induced by CIH and CIHC was due to the release of nitric oxide from airway epithelium. It has been reported that different patterns of CIH (in terms of duration) caused downregulation of nitric oxide synthase (20) and decreased production of nitric oxide (24).

The cellular mechanisms underlying airway/vascular smooth muscle responsiveness after exposure to chronic hypoxia have been investigated. Roux et al. (22) suggested that airway hyporeactivity to carbachol induced by chronic hypoxia was due to decreased Ca^{2+} sensitivity of the contractile apparatus of smooth muscle cells. Previously, it has been reported that attenuated vascular reactivity under chronic hypoxia was due to decreased Ca^{2+} sensitivity of the contractile apparatus (2) and not due to the release of endothelium-derived relaxing factor (26). Impairment of receptor-mediated pharmacomechanical coupling of inositol 1,4,5-triphosphate was reported in vascular smooth muscle cells after exposure to chronic hypoxia (8, 29). At present, we can suggest from the effects of chronic hypoxia on airway and vascular smooth muscles that suppression of pharmacomechanical coupling or decreased Ca^{2+} sensitivity of the contractile apparatus may be responsible for the attenuation of contractile responses to ACh after exposure of CIH and CIHC. Whether CIH/CIHC alters Ca^{2+} signaling pathway of airway smooth muscle cells needs further investigation.

The tracheal temperature decreases by ~23°C due to cooling of the trachea by cold air during hyperventilation in humans (16). Evaporation that occurs during hyperventilation cools the airways. Inhalation of cold air and hyperventilation cause narrowing of the airways (6, 17). It is likely that repeated hyperventilation with inhalation of cold air at high altitude may also cause narrowing of the airways. In fact, it has been reported that cooling the trachea enhanced muscarinic agonist-induced contraction (11). Moreover, hypoxia itself could enhance reflex cholinergic contraction (15). Present results demonstrate that, in contrast to cooling (6, 11, 17) and hypoxia (15), CIH and CIHC attenuated the maximum contraction as well as sensitivity of tracheal smooth muscle to ACh and thus could evoke adaptive response by minimizing hypoxia- and cooling-induced airway constriction at high altitude.

As regards histamine, the responses of the tracheal smooth muscle following CIH were similar to that of ACh, but different from CIHC. CIHC enhanced the T_{max} of tracheal rings to histamine compared with CIH. A small, statistically significant increase in the sensitivity was also induced by CIHC compared with CIH. This epithelium-independent T_{max} and sensitivity of tracheal rings to histamine after CIHC matched the values of the normoxic group. It has been reported that acute cooling increased tension of guinea pig tracheal smooth muscle to histamine (9). In view of the present observation, we can suggest that release of histamine during cold exposure (3) might prevent the attenuation of contractile response of tracheal smooth muscle to histamine induced by CIHC, and thus cold exposure might prevent the effect of hypoxia. Another possibility is that upregulation of histamine receptors might be induced by chronic cold exposure. At present, it is not known whether these differential effects are the result of interaction between hypoxia and cold or whether these variables are due to their independent effects. The present study also demonstrated that the T_{max} of tracheal smooth muscle to 5-HT after exposure of CIH and CIHC resembled that of ACh. The decreased T_{max} may attenuate airway responsiveness to 5-HT. CIH evoked a small but statistically significant increase in the sensitivity to 5-HT. Neuroepithelial cells are the hypoxia-sensitive chemoreceptors of airways and, when stimulated by hypoxia, release 5-HT (12). The small increase in the sensitivity of tracheal smooth muscle to 5-HT induced by CIH might be due to the release of 5-HT from neuroepithelial cells activated by CIH. The normalization of sensitivity after epithelium removal might be due to the loss of neuroepithelial cells. Further studies are needed to understand whether the release of histamine under CIHC and the release of 5-HT under CIH can modulate smooth muscle contractility.

The mechanism by which CIH enhanced epithelium-independent relaxation of tracheal smooth muscle to isoproterenol is beyond the scope of the present study. We can only speculate that CIH might have upregulated β2-receptors. It has also been reported that acute cold exposure increases the sensitivity of guinea pig tracheal smooth muscle to isoproterenol (7). It has been suggested that the mechanisms by which hypoxia and cooling cause airway constriction might play a role in the enhanced airway hyperresponsiveness of asthmatic patients at high altitude (13). Conversely, it has been reported that asthmatic patients may have airway hyperresponsiveness at high altitude (5, 27). Asthmatic patients may do well at high altitude due to the following possible mechanisms: 1) expiration at low atmospheric pressure is easier compared with atmospheric pressure at sea level; 2) absence of allergen and pollutants; and 3) increased level of cortisol (5) and increased activity of the sympathoadrenal system, leading to liberation of catecholamines (14, 28). The present study indicated that the attenuated maximum contraction and sensitivity of tracheal smooth muscle to ACh may be one of the mechanisms reducing the airway hyperresponsiveness of asthmatic patients after chronic intermittent exposure to high altitude, as ACh is one of the main contractile mediators responsible for airway hyperresponsiveness in asthmatic patients (25).

In conclusion, we observed that the two types of experimental conditions, i.e., CIH and CIHC, are not the same and may be having effects on tracheal smooth muscle to histamine, 5-HT, and isoproterenol through different mechanisms. Logically, addition of intermittent cold exposure to intermittent hypobaric hypoxia closely replicates high-altitude conditions. Chronic intermittent exposure to simulate high-altitude conditions by exposing the animals chronically in a hypobaric chamber without cold exposure may not forecast the exact
picture of physiological responses at high altitude. The airway hyporesponsiveness to ACh induced by both CIH and CIHC may have a role in reducing airway hyperresponsiveness of asthmatic subjects following intermittent exposure to high altitude. The therapeutic beneficial effect of β2-adrenoceptor agonist after intermittent exposure to high altitude should be examined.

ACKNOWLEDGMENTS

The authors are grateful to the Director, Defense Institute of Physiology and Allied Sciences, Delhi, for kind permission to carry out experiments using the hypobaric chamber. The authors thank Dr. Nanduri R. Prabhakar for valuable suggestions in the manuscript.

GRANTS

This study was supported by Council of Scientific and Industrial Research (CSIR) New Delhi by awarding a Senior Research Fellowship to K. Chakrabarty. Financial assistance from CSIR is thankfully acknowledged.

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