Bronchoconstriction induced by hyperventilation with humidified hot air:
role of TRPV1-expressing airway afferents

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Short Running Title: Bronchoconstriction induced by hot humidified air

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ABSTRACT

A recent study in our laboratory has shown that an increase in intrathoracic temperature activates vagal pulmonary C-fibers. Because these afferents are known to elicit reflex bronchoconstriction upon stimulation, this study was carried out to investigate if increasing airway temperature within the physiological range alters the bronchomotor tone. Adult guinea pigs were anesthetized and mechanically ventilated via a tracheal tube. After the lung was hyperventilated with humidified hot air (HHA) for 4 min, the tracheal temperature was elevated from 36.4 to 40.5°C, which induced an immediate bronchoconstriction, increasing total pulmonary resistance (R_L) to 177 ± 10% and decreasing dynamic lung compliance to 81 ± 6% of their respective baselines. The increase in R_L returned spontaneously toward the baseline in <10 min, and was reproducible in the same animals. There was no difference in the response whether the humidity was generated from distilled water or isotonic saline. In contrast, hyperventilation with humidified air at room temperature did not cause any increase in R_L. The increase in R_L caused by HHA (ΔR_L) was attenuated by 65.9% after a pretreatment with atropine alone, and by 72.0% after a pretreatment with a combination of atropine and neurokinin receptor type-1 and -2 antagonists. In addition, capsazepine, a selective transient receptor potential vanilloid type 1 (TRPV1) antagonist, reduced the HHA-induced increase in R_L by 64.1%, but did not abolish it. However, a pretreatment with formoterol, a β2 agonist, completely prevented the HHA-induced bronchoconstriction. These results indicate that increasing airway temperature induced a transient airway constriction in guinea pigs. Approximately two-third of the increase in bronchomotor tone was mediated through the cholinergic reflex that was probably elicited by activation of TRPV1-expressing airway afferents. The remaining bronchoconstriction was caused by other yet unidentified factors.
**Keywords:** vagus nerves; hyperthermia; exercise; asthma; transient receptor potential vanilloid type 1.
INTRODUCTION

Tissue temperature increases when the rate of heat production is elevated or the heat dissipation is diminished. Hyperthermia can occur under both normal and pathophysiological conditions. The most common cause of hyperthermia is an increase in metabolic rate such as during vigorous exercise. Body core temperature exceeding 41 °C has been reported during exertional exercise in healthy man (33, 43) and in animals (6). Hyperthermia (> 40 °C) also occurs frequently under pathophysiological conditions caused by endogenous pyrogens or infection, such as in patients suffering from severe fever. Moreover, tissue inflammation is known to lead to local hyperemia and an increase in temperature in the inflamed area (17, 42). This was further confirmed in the respiratory tract by a recent report that the airway temperature was significantly higher in asthmatics than healthy individuals (40). Coincidently, an earlier study has reported that hyperventilation with hot humidified air induced transient but pronounced bronchoconstriction in asthmatic patients, but the underlying mechanism was not known (1).

A recent study in our laboratory has demonstrated that an increase in intrathoracic temperature to above a threshold of ~39.2 °C activated vagal pulmonary C-fiber endings in anesthetized rats (44). Although the mechanism involved in generating this stimulatory effect of hyperthermia is not fully understood, more recent studies have shown a similar stimulatory effect of increasing temperature in isolated vagal pulmonary sensory neurons, and suggest an involvement of the temperature-sensitive transient receptor potential vanilloid type 1 (TRPV1) channel expressed in these neurons (36). More importantly, these pulmonary afferents upon stimulation are known to elicit bronchoconstriction mediated through both cholinergic reflex pathways and local release of tachykinins (10, 27, 29, 30). Whether an increase in airway temperature induces bronchoconstriction is not known.
In light of the background information and these important but unanswered questions, this study was carried out to determine the bronchomotor response to an increase in airway temperature within the physiological range and to investigate the mechanisms involved in eliciting the response.
MATERIALS AND METHODS

The procedures described below were performed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, and were also approved by the University of Kentucky Institutional Animal Care & Use Committee.

**Measurements of Lung Mechanics:** Young, male, pathogen-free Hartley guinea pigs were anesthetized with chloralose (100 mg/kg, i.p.) and urethane (500 mg/kg, i.p.), and supplemental doses of the same anesthetics were administered whenever necessary to maintain abolition of the corneal and withdrawal reflexes. The trachea was cannulated just below the larynx via a tracheotomy. Guinea pigs were placed in a supine position, and ventilated with a respirator (Harvard model 683) at a constant frequency (f) of 60 breaths/min and a tidal volume (VT) of ~8 ml/kg; the latter was adjusted in each animal to maintain the end-tidal CO2 concentration (Novametrix model 1260) between 4.6% and 5.0%. The right jugular vein and the right carotid artery were cannulated for intravenous (i.v.) injections and for arterial blood pressure (ABP) measurement, respectively. A catheter for measuring intrapleural pressure (P_{ip}) was inserted into the right intrapleural cavity via a surgical incision between the fifth and sixth ribs; this incision was subsequently sutured and further sealed air-tight with silicone jelly. Pneumothorax was then corrected by briefly opening the intrapleural catheter to ambient air during a held hyperinflation (3\times VT). During the experiment, the animals were paralyzed with pancuronium bromide (30 μg/kg, i.v.) whenever necessary. A heating pad was placed under the animal to maintain body temperature at ~36°C.

Transpulmonary pressure was measured as the difference between the tracheal pressure (P_t) and P_{ip} with a differential pressure transducer (Validyne MP 45-28). Respiratory flow was
measured with a heated pneumotachograph and a differential pressure transducer (Validyne MP 45-14). All signals were recorded on a chart recorder (Grass model 7); total pulmonary resistance ($R_L$) and dynamic lung compliance ($C_{dyn}$) were analyzed continuously by an on-line computer on a breath-by-breath basis. Results obtained from the computer were routinely checked by hand calculation for accuracy.

**Humidified Hot Air Challenge:** Humidified hot air (HHA) was generated by connecting the outlet of the respirator inspiratory line to an air stone and immersing it in isotonic saline (or distilled water) contained in a bottle that was placed in a heated water bath (Fig. 1A). HHA was then delivered directly into the lung via the tracheal tube. In one study series, humidified room air (HRA) was delivered in the same manner, except that the water bath was not heated. During either HHA or HRA challenge, minute ventilation was increased to ~375% of the baseline ($V_T$ and $f$ at 12 ml/kg and 150 breaths/min, respectively) for 4 min. To prevent arterial hypocapnia and alkalosis, a gas mixture containing 3.5-4.0% of CO$_2$, 21% O$_2$, balance N$_2$ was administered via the respirator during hyperventilation. In some of the experiments ($n=9$), we inserted a miniature thermistor (Physitemp Inc. model IT-18; time constant: 0.1 s) through the tracheal tube and positioned it near the thoracic entry (~0.5 cm distal to the tip of tracheal tube) to measure continuously the temperature in the tracheal lumen ($T_{tr}$; Fig. 1A) before, during and after the HHA and HRA challenges. We have determined in our pilot experiments that $T_{tr}$ can be elevated to ~40°C by maintaining the heated water bath temperature at 75°C (Fig. 1B). The amounts of water content in HHA and HRA measured in this study were 74.3 mg and 11.4 mg per liter of air, respectively. Only less than one third of the total water content (<70 mg) delivered by the HHA during the 4-min hyperventilation was retained in the lung, and the remainder was either exhaled or deposited along the breathing circuit.
**Experimental Protocols:** Six series of experiments were carried out in this study. **Series 1** was aimed to determine if HHA-hyperventilation induced bronchoconstriction, and if so, whether the effect was generated by the increase in temperature. In each animal, $R_L$ and $C_{dyn}$ were measured continuously on a breath-by-breath basis for 5 min at the baseline, and for 20 min immediately after the 4-min hyperventilation. To determine the effect of high temperature, the responses to hyperventilation with HHA and HRA were compared in the same animals; the sequence of these two challenges was alternated between animals to achieve a balanced design, and at least 60 min elapsed between two tests for recovery. The lung was hyperinflated ($3 \times V_T$) twice both at 5 min before and every 10 min after the hyperventilation to maintain a constant volume history of the lung. In **Series 2**, to determine whether the HHA-induced responses were reproducible, the same HHA challenge was repeated after >60 min in the same animals. In **Series 3**, the responses to HHA challenges were compared in the same animals when the humidity in HHA was generated from isotonic saline and distilled water. In **Series 4**, to determine the relative contributions of cholinergic mechanism and endogenous tachykinins, the HHA-induced responses were tested before and after pretreatments with atropine alone and a combination of atropine and neurokinin (NK) receptor type-1 and -2 antagonists, L-732138 and SR-48968, respectively. **Series 5** was designed to test a possible role of TRPV1, the responses to HHA were compared between before and after a pretreatment with capsazepine, a selective TRPV1 antagonist. **Series 6** was carried out to determine if the effect of HHA was caused by smooth muscle contraction. The responses to HHA challenges were compared between before and after a pretreatment with formoterol, a selective β₂ agonist.

**Statistical analysis:** In each experiment, the baseline $R_L$ and $C_{dyn}$ were averaged over 5 min before the HHA challenge; the responses to HHA were averaged over the 5 min
immediately after the challenge. Unless mentioned otherwise, a two-way analysis of variance (ANOVA) was used for the statistical analysis; for example, in Series 4 one factor was the effect of HHA challenge, and the other factor was the treatment effect of atropine. When the two-way ANOVA showed a significant interaction, pair-wise comparisons were made with a post hoc analysis (Fisher's least significant difference). Data are reported as means ± SEM. A $P$ value < 0.05 was considered significant.

**Materials:** Atropine sulfate (Sigma-Aldrich) was diluted in isotonic saline. Both capsazepine (Sigma-Aldrich) and formoterol (Sigma-Aldrich) were first dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at concentrations of 5 mg/ml and 1 mg/ml, and then diluted with saline to a final concentration of 1.2 mg/ml and 0.04 mg/ml, respectively. L-732138 (Tocris) was dissolved in DMSO at a final concentration of 3.7 mg/ml. SR-48968 (Sanofi Recherche) was first dissolved in polyethylene glycol (average mol. wt: 200; Sigma-Aldrich) and then diluted in saline at a 1:1 ratio to a final concentration of 0.67 mg/ml.
RESULTS

This study was carried out in a total of 36 guinea pigs with an average body weight of 396 ± 11 g. Some of the animals were used in more than one series of experiments. Tracheal temperature (Ttr; Fig. 1A) was measured in 9 animals (7 in Series 1 and 2 in Series 2). Hyperventilation with HHA led to a rapid and continuous rise in Ttr from 36.4 ± 0.4 °C to 40.5 ± 0.5 °C, which stabilized after ~3 min (Fig. 1B). In contrast, hyperventilation with humidified air at room temperature (HRA) caused a slight decrease in Ttr (35.2 ± 0.3 °C). During the 4-min HHA hyperventilation, body temperature did not change significantly. In 11 animals, the arterial blood PO2, PCO2 and pH were 69.1 ± 4.5 mmHg, 28.8 ± 2.8 mmHg and 7.45 ± 0.02, respectively, at baseline, and 80.9 ± 9.7 mmHg, 26.9 ± 1.9 mmHg and 7.47 ± 0.02, respectively, during the last 30 s of hyperventilation with HHA (3.5-4.0% CO2, 21% O2, balance N2); there was no significant change in either PO2 (P>0.3; paired t-test), PCO2 (P>0.4) or pH (P>0.4) generated by the HHA-hyperventilation in the same animals.

Series 1: Hyperventilation with HHA induced pronounced increase in RL and decrease in Cdyn (Fig. 2). These changes occurred immediately following the HHA, and the increase in RL gradually declined and returned toward baseline in <10 min. Although the HHA-induced decrease in Cdyn sustained, it was completely reversed when the lung was hyperinflated at the end of 20-min recording period, suggesting that the reduced Cdyn was, at least partially, caused by lung atelectasis. When these responses were averaged over the 5-min durations before and immediately following HHA, RL increased from a baseline of 0.12 ± 0.02 cmH2O/ml/s to 0.22 ± 0.04 cmH2O/ml/s after HHA (n=7, P<0.01), and Cdyn decreased from a baseline of 0.54 ± 0.08 ml/cmH2O to 0.41 ± 0.05 ml/cmH2O after HHA (n=7, P<0.01) (Fig. 3). In distinct contrast, hyperventilation with HRA of the same gas mixture did not generate any detectable change in...
either $R_L$ or $C_{dy}$ (Fig. 2 & 3). After termination of hyperventilation with either HHA or HRA, there was an initial slight decrease, followed by a gradual increase in ABP. However, there was no difference in ABP between HHA and HRA, averaged over 5 min after the challenges ($P>0.05$; $n=7$). During the same time period, heart rate averaged over 5 min after HHA (289 ± 13 beats/min) was significantly higher than that after HRA (263 ± 13 beats/min; $P<0.05$; $n=7$).

**Series 2:** When the same HHA challenge was repeated in the same animals ~60 min later, the changes in both $R_L$ and $C_{dy}$ were reproducible (Fig. 4A), and no significant difference was found between their responses to the first and second HHA challenges ($P>0.1$; $n=10$).

**Series 3:** In this study series, effects of hyperventilation with HHA on both $R_L$ and $C_{dy}$ were compared in the same animals when humidity was generated from distilled water and isotonic saline. The sequence of these two tests were alternated between animals, and >60 min elapsed between tests for recovery. The HHA generated from distilled water and isotonic saline both induced bronchoconstriction, and there was no significant difference in either $\Delta R_L$ ($P>0.5$, $n=5$) or $\Delta C_{dy}$ ($P>0.1$, $n=5$) between the two different sources of humidity of HHA (Fig. 4B).

**Series 4:** To determine if the HHA-induced bronchoconstriction was mediated through cholinergic reflex, the response to HHA was tested both before and 20 min after a pretreatment with atropine sulfate (0.1 mg/kg, i.v.). Heart rate increased from a baseline of 288 ± 13 beats/min to 301 ± 15 beats/min after atropine. The HHA-induced increase in $R_L$ was significantly reduced from $0.075 \pm 0.006$ cmH$_2$O/ml/s before to $0.023 \pm 0.009$ cmH$_2$O/ml/s ($P<0.01$, $n=6$) after atropine. However, the bronchoconstriction was not completely abolished; after the atropine pretreatment, $R_L$ increased from a baseline of $0.106 \pm 0.008$ cmH$_2$O/ml/s to $0.129 \pm 0.012$ cmH$_2$O/ml/s after HHA ($P<0.05$; Fig. 5A).
To further test a possible involvement of endogenous tachykinins, the response was tested both before and at 20 min after a pretreatment with a combination of atropine and L-732138 (3 mg/kg, i.v.) and SR-48968 (0.3 mg/kg, i.v.), selective NK-1 and NK-2 antagonists, respectively, in a separate group of animals. Once again, the HHA-induced increase in $R_L$ was significantly reduced from $0.091 \pm 0.012 \text{ cmH}_2\text{O/ml/s}$ before to $0.027 \pm 0.013 \text{ cmH}_2\text{O/ml/s}$ ($P<0.01, n = 7$) after the pretreatment with these antagonists (Fig. 5B). However, HHA still caused a significant bronchoconstriction ($P<0.05$) even after the pretreatment (Fig. 5B). Furthermore, a pretreatment with the same doses of L-732138 and SR-48968 alone (without atropine) did not significantly reduce the HHA-induced increase in $R_L$ tested in a separate group of animals ($P>0.05, n=3$). In contrast, the same pretreatment with these NK-1 and NK-2 antagonists completely prevented the bronchoconstriction ($\Delta R_L = 0.088 \pm 0.011 \text{ cmH}_2\text{O/ml/s}, n = 3$) caused by a bolus injection of capsaicin (1.5 $\mu$g/kg, i.v.) in the same group of guinea pigs after bilateral vagotomy, indicating the effectiveness of these antagonists in blocking the non-cholinergic bronchoconstriction mediated through C-fiber stimulation in these animals.

**Series 5:** To investigate the role of the temperature-sensitive TRPV1 channel in the HHA-induced bronchoconstriction, the response to HHA was determined before and immediately after a pretreatment with capsazepine (CPZ, 0.5 mg/kg/min, i.v., for 4 min both before and during the 4-min HHA challenge), a selective TRPV1 antagonist. The HHA-induced increase in $R_L$ was significantly reduced from $0.101 \pm 0.014 \text{ cmH}_2\text{O/ml/s}$ before to $0.031 \pm 0.006 \text{ cmH}_2\text{O/ml/s}$ ($P<0.01, n = 6$) after the pretreatment. However, CPZ did not completely eliminate the HHA-induced bronchoconstriction (Fig. 6A).

**Series 6:** To determine whether airway smooth muscle contraction was responsible for the HHA-induced increase in $R_L$, we tested the effect of a pretreatment with formoterol (10
μg/kg, i.v.), a selective β2 agonist. Formoterol effectively prevented the bronchoconstriction; 60 min after the formoterol pretreatment, HHA hyperventilation no longer caused any significant change in either R_l (P>0.05, n=6) or C_{dyn} (P>0.05, n=6) from their respective baselines (Fig. 6B).
DISCUSSION

In this study, a transient increase in airway resistance was consistently found immediately after hyperventilation with humidified hot air in anesthetized guinea pigs. The effect was caused by the increase in airway temperature because hyperventilation with humidified air at room temperature failed to generate any change in airway resistance in the same animals. The increase in airway resistance resulted from an increase in airway smooth muscle contraction as it could be completely prevented by a pretreatment with formoterol. Furthermore, atropine pretreatment abrogated approximately two-third of the increase in bronchomotor tone induced by HHA, indicating that it was primarily mediated through the cholinergic reflex. Although the type(s) of sensory nerves mediating this reflexogenic response was not identified in this study, the bronchopulmonary afferents expressing the TRPV1 channel were most likely responsible since the HHA-induced bronchoconstriction was attenuated to a similar degree after a pretreatment with capsazepine.

Reversible bronchoconstriction induced by breathing HHA air has been previously reported in asthmatic patients by Aitken et al. (1). In their study, after asthmatic patients hyperventilated with air of varying temperature and relative humidity for three minutes, the most intense bronchoconstriction that occurred immediately was generated by breathing warm humid air. The reduction in specific airway conductance was almost two fold of that generated by breathing cold dry air at the same time point in the same subjects (1). Although it was not tested in their study, the rapidity of response and recovery appeared to suggest a possible involvement of neural reflexes. Our finding of an involvement of the cholinergic reflex in the present study provides a strong support of such a possibility. In contrast, airway constriction developed slowly after
hyperventilation with cold dry air in the same patients, reaching a peak after 5-10 minutes (2, 34). Indeed, cold dry air-induced bronchoconstriction (or exercise-induced bronchoconstriction) has been extensively investigated and documented in the literature; it is generally recognized that the primary cause of cold air-induced bronchoconstriction is the injury of airway mucosa, resulting the release of various inflammatory mediators such as leukotrienes and histamine, which in turn trigger bronchoconstriction (2, 20, 34). In comparison, the bronchoconstriction induced by breathing humidified hot air in this study reversed spontaneously in less than 10 minutes and was reproducible in the same animals, suggesting that damage or injury of airway mucosa is not a primary causal factor.

Sensory signals arising from the lung and airways are conducted almost exclusively in vagus nerves and their branches (9, 30, 45). Morphological studies have shown that ~75% of the vagal pulmonary afferent fibers are unmyelinated (C-) fibers (23). It is well documented that these C-fiber sensory nerves exhibit polymodal sensitivity and play an important role in protecting the lungs under various physiological and pathophysiological conditions (10, 29). A recent study in our laboratory has shown that an increase in intrathoracic temperature stimulates pulmonary C-fibers (44). Stimulation of these afferents can elicit powerful centrally-mediated reflex responses mediated through autonomic nervous system, including bronchoconstriction (10, 29). Our results in this study show that the atropine pretreatment abolished two-third of the bronchomotor response to HHA, and suggest a major role of the cholinergic mechanism and a possible involvement of C-fiber activation. The involvement of cholinergic reflex was further confirmed by the observation that bilateral vagotomy also attenuated ~63% of the HHA-induced bronchoconstriction in a separate group of guinea pigs (n =3; unpublished data). Approximately one-third of the HHA-induced bronchoconstriction persisted even after atropine. This was not due to an insufficient dose of
atropine because in a previous study (22) the same dose of atropine completely prevented a much more intense bronchoconstriction generated by acetylcholine (10 μg/kg, i.v.) that increased R_L by 828% and decreased C_\text{dyn} by 68% in anesthetized guinea pigs (n=3), prepared in the same manner as in this study. In addition, activation of these afferents is known to trigger the release of tachykinins from the sensory terminals, which can act on airway smooth muscles and cause contraction (27, 32). However, a lack of additional blocking effect of NK-1 and NK-2 antagonists on the HHA-induced bronchoconstriction is somewhat surprising because tachykinins are known to play a dominant role in the bronchoconstriction triggered by C-fiber stimulation in guinea pigs (22, 32). The lack of blocking effect of these NK-1 and NK-2 antagonists was not due to insufficient doses because their effectiveness in preventing the bronchoconstriction caused by endogenous tachykinins was verified in this study when the pretreatment with these compounds completely blocked the bronchoconstrictive response to capsaicin in vagotomized animals.

It has been reported that inhalation of distilled water or hypotonic saline aerosols induced a fall in \text{FEV}_1 in asthma patients, but not in normal subjects (3, 46). Since the response was attenuated by atropine (46), a major component of the bronchoconstriction was probably mediated through cholinergic reflex resulting from activation of C-fiber afferents and rapidly adapting receptors in the airways by the low chloride ion concentration in aerosol solution 14, 39. However, the possible effect caused by of distilled water-induced cholinergic reflex can be ruled out in the present study because the HHA-induced bronchoconstriction was also present when the humidity was generated from isotonic saline.

TRPVs are a subfamily of the TRP super-family of ion channel proteins containing six trans-membrane domains that form non-selective, non-voltage-gated cationic channels (8, 38). The subtypes of TRPV channels, TRPV1-4, are generally considered as the primary temperature sensors
in mammalian species, and each type of TRPVs is activated in a different temperature range (4, 11). In addition to its role as a thermal sensor, the function of TRPV1 as a polymodal transducer for various nociceptive stimuli in primary sensory neurons has been well documented (8, 38). Recent studies have presented compelling evidence of an important role of TRPV1 in the manifestation of various symptoms of airway hypersensitivity associated with airway inflammation (16, 24, 28); for example, over-expression of TRPV1 is found in the biopsies of bronchial tissue from patients with chronic cough (18, 35). Furthermore, cough sensitivity to TRPV1 activators, capsaicin or citric acid aerosol, is markedly elevated in patients with asthma or airway inflammation (12, 39).

In healthy lung, TRPV1 is predominantly expressed in bronchopulmonary C-fiber afferents. This is especially evident in the observations made in the electrophysiological experiments: capsaicin, the selective activator of TRPV1, is a selective and potent stimulant of C-fiber afferents, but rarely activates myelinated afferents in rat lung (21). A recent study in our laboratory has demonstrated that the baseline activity and sensitivities of vagal pulmonary C-fiber endings were elevated when the temperature in the isolated perfused thoracic chamber was raised to a threshold of ~39.2 °C (44). Although the mechanism underlying this sensitizing effect was not fully understood, one distinct possibility is the activation by hyperthermia of certain temperature-sensitive ion channels, particularly the TRPV1 channel, expressed in the sensory terminals. Indeed, more recent studies in isolated pulmonary sensory neurons have yielded strong evidence in support of this possibility (36, 37). Our results obtained from the capsazepine pretreatment suggest that TRPV1 play an important role in eliciting the HHA-induced bronchoconstriction. In a recent study, Gavva et al. reported that a treatment with the TRPV1 antagonist can cause an increase in body temperature in various animal species, suggesting that the tonic activity of TRPV1 is involved in regulating the normal core temperature (15). Although it seems very unlikely, we cannot completely
rule out the possibility that the effect of capsazepine in attenuating the HHA-induced bronchoconstriction may be partially due to an elevated baseline body temperature and therefore a smaller increase in airway temperature during HHA.

Neither atropine nor capsazepine was able to completely prevent the bronchoconstriction induced by the HHA hyperventilation, the remaining increase in bronchomotor tone resulted from other causal factors that have not yet been identified. One of such possibilities is the local effect of HHA on smooth muscles. Sudden increase in temperature has been shown to act directly on isolated guinea pig airway smooth muscles and cause contraction (47). Hyperthermia is also known to trigger the release of certain chemical mediators (e.g., prostaglandin F$_{2\alpha}$, leukotriene B$_4$, etc.) and pro-inflammatory cytokines (e.g. tumor necrosis factor $\alpha$, etc.) (5, 7, 26). Some of these endogenous substances can act directly or indirectly on airway smooth muscles and cause contraction if they are released locally in the airways and lung tissue during HHA hyperventilation.

Water content is believed to be a critical factor for delivering the “heat load” in the airways and “respiratory heat exchange” during the HHA challenge (1), which was confirmed in our preliminary study; hyperventilation with heated dry air (heated by electrical heater to the same inspired air temperature as that in HHA) did not generate a significant increase in tracheal temperature, and consequently no detectable increase in airway resistance (unpublished observation). Similarly, hyperventilation is also required to deliver the heat load into the lung; normal ventilation with the same humidified hot air failed to elevate either airway temperature or airway resistance. After HHA, water vapor condensation and/or deposition in the airway epithelium could result in congestion and partial obstruction of the airway lumen, which may then lead to an
increase in airway resistance. Judging from the effectiveness of formoterol in preventing the HHA-induced bronchoconstriction, we can rule out the possibility of airway congestion by water content as a major contributing factor. However, it is possible that an increase in the water deposition and/or condensation in the airways may decrease the osmolarity of airway surface fluid. The TRPV4 channel is known to be an “osmotic sensor” and activated by hypotonicity (31), and its expression has been detected in bronchial epithelial cells and airway smooth muscles (13, 25). It is known that TRPV4 activation triggers Ca\(^{2+}\) influx (19, 31, 48) and smooth muscle contraction in guinea pig airways (25). Therefore, a potential involvement of TRPV4 activation in the remaining mild bronchoconstriction that was not blocked by either atropine or capsazepine pretreatment in this study (Fig. 5 & 6) cannot be dismissed.

The elevated airway temperature (40.5 °C) in this study is certainly within the physiological range. Although we could not measure the temperature in the lung periphery, it was presumably lower than that measured in the tracheal lumen because there was no detectable increase in body temperature during the HHA challenge in this study. An increase in airway temperature can occur under both normal and pathophysiological conditions. For example, body temperature higher than 40.5 ° occurs frequently in patients suffering from severe fever or heatstroke (5). Further, tissue inflammation is known to lead to an increase in temperature in the inflamed area (17, 42). Indeed, a recent study has reported that the end-expiratory temperature plateau (an indirect measurement of the lung temperature) is 2.7 °C higher in asthmatics than healthy individuals (40). More importantly, the body core temperature exceeding 41 °C has been reported in healthy humans and animals during exertional exercise (6, 33, 43). It is expected that even higher temperatures in the airways and lung will be reached during heavy exercise in a warm and humid environment because exercise not only elevates body temperature but also induces hyperventilation. In addition, other changes in airway
functions associated with activation of these pulmonary afferents (e.g., chest tightness, dyspneic sensation, cough, etc.) may occur concurrently with bronchoconstriction as airway temperature is elevated under those conditions. Furthermore, recent studies have reported an over-expression of TRPV1 in pulmonary sensory neurons in animals with chronic allergic airway inflammation (49, 50) and in patients with chronic cough (18, 35). Presumably, the bronchoconstrictive response to the HHA challenge can be further augmented in those hypersensitive airways, which remains yet to be investigated.
ACKNOWLEDGEMENTS

This study was supported in part by grants from the National Institute of Health (HL-58686 & HL-67379).
REFERENCES


Figure Legends

**Fig. 1.** A: Schematic drawing of the experimental setup for delivery of humidified hot air into the trachea of anesthetized guinea pigs. T\(_{tr}\), tracheal temperature. B: Change in T\(_{tr}\) during the 4-min hyperventilation with humidified hot air (closed circles) and humidified room air (water bath kept at room temperature 23°C; open circles). Data are means ± SEM in 9 animals.

**Fig. 2.** Effect of hyperventilation with humidified hot air on total pulmonary resistance (R\(_L\)) and dynamic lung compliance (C\(_{dyn}\)) in anesthetized guinea pigs. Open circles: responses obtained form hyperventilation with humidified air at room temperature; closed circles: humidified hot air in the same animals. Responses were not recorded during hyperventilation (arrow), which was administered between 0-4 min. Data before time zero represent the baseline values. At least 60 min elapsed between the two tests, and the sequence of tests was alternated between animals to achieve a balanced design. Data are means ± SEM obtained from 7 animals; in each animal, each data point was measured as the average of 10 consecutive breaths.

**Fig. 3.** Comparison of the responses of total pulmonary resistance (R\(_L\)) and dynamic lung compliance (C\(_{dyn}\)) to hyperventilation with humidified hot air (HHA) and humidified room air (HRA) in anesthetized guinea pigs. At least >60 min elapsed between tests for recovery, and the sequence of these two tests were alternated between animals. Open bars represent the baseline (BL) data averaged over 5 min before, and closed bars represent the responses averaged over 5 min immediately after the HHA or HRA challenge. Data are means ± SEM of 7 animals. *

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significantly different (P<0.05) from the baseline. † significant difference (P<0.05) comparing the corresponding data between HHA and HRA.

**Fig. 4.** A: Reproducibility of the responses of $R_L$ and $C_{dyn}$ to 2 consecutive HHA challenges in the same groups of anesthetized guinea pigs. At least 60 min elapsed between the two tests so that both $R_L$ and $C_{dyn}$ had returned to baselines before the second challenge. B: The responses of $R_L$ and $C_{dyn}$ to HHA were compared in the same animals when humidity was generated from distilled water and isotonic saline. The sequence of these two tests were alternated between animals, and >60 min elapsed between tests for recovery. Open bars represent the baseline (BL) data averaged over 5 min before, and closed bars represent the responses averaged over 5 min immediately after the HHA challenge. Data are means ± SEM (n=10 in A; n=5 in B). *, significantly different (P<0.05) from the baseline.

**Fig. 5.** A: Comparison of the responses of $R_L$ and $C_{dyn}$ to hyperventilation with HHA before (left panel) and after a pretreatment with atropine sulfate (1 mg/kg, i.v.; right panel). B: Comparison of the responses to hyperventilation with HHA before (left panel) and after a pretreatment with a combination of atropine (1 mg/kg, i.v.), SR-48968 (0.3 mg/kg, i.v.) and L-732138 (3 mg/kg, i.v.) (right panel). At least 60 min elapsed between two HHA challenges for recovery. Open bars represent the baseline (BL) data averaged over 5 min before, and closed bars represent the responses averaged over 5 min immediately after the HHA challenge. Data are means ± SEM (n=6 in A; n=7 in B). *, significantly different (P<0.05) from the baseline. † significant difference (P<0.05) comparing the corresponding data between before and after the pretreatment.
Fig. 6. A: Comparison of the responses of $R_L$ and $C_{dyne}$ to hyperventilation with HHA before (left panel) and after a pretreatment with capsazepine (CZP; 0.5 mg/kg/min for 4 min, i.v.; right panel). B: Comparison of the responses to hyperventilation with HHA before (left panel) and after a pretreatment with formoterol (10 µg/kg, i.v.; right panel). At least 60 min elapsed between two HHA challenges for recovery. Open bars represent the baseline (BL) data averaged over 5 min before, and closed bars represent the responses averaged over 5 min immediately after the HHA challenge. Data are means ± SEM (n=6 in A; n=6 in B). *, significantly different (P<0.05) from the baseline. † significant difference (P<0.05) comparing the corresponding data between before and after the pretreatment.
Fig. 1

A

Ventilator

Room air (~23°C) → Humidified hot air

→ Expired air

Heated water bath (~75°C)

B

Tracheal temperature (°C)

0 1 2 3 4

Time (min)

Humidified room air
Humidified hot air
Fig. 2

- **RL (cmH₂O/ml/s)**
  - Humidified room air
  - Humidified hot air

- **Cdyn (ml/cmH₂O)**

Time (min)
Fig. 5

A

Atropine

$R_L$ (cmH$_2$O/ml/s)

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B

Atropine +SR+L

$R_L$ (cmH$_2$O/ml/s)

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C$_{dyn}$ (ml/cmH$_2$O)

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* Significant difference
Fig. 6

A

\[ R_L \text{ (cmH}_2\text{O/ml/s)} \]

B

\[ R_L \text{ (cmH}_2\text{O/ml/s)} \]

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\[ C_{dyn} \text{ (ml/cmH}_2\text{O)} \]