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

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Lin R-L, Hayes D Jr, Lee L-Y. Bronchoconstriction induced by hyperventilation with humidified hot air: role of TRPV1-expressing airway afferents. J Appl Physiol 106: 1917–1924, 2009. First published March 19, 2009; doi:10.1152/japplphysiol.00065.2009.—A recent study by 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 an increase in airway temperature within the physiological range alters bronchomotor tone. Adult guinea pigs were anesthetized and mechanically ventilated via a tracheal tube. After the lung had been 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 (Rt) to 177 ± 10% and decreasing dynamic lung compliance to 81 ± 6% of their respective baselines. The increase in Rt returned spontaneously toward the baseline in <10 min and was reproducible in the same animals. There were no difference in the responses 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 Rt. The increase in Rt caused by HHA 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 Rt by 64.1% but did not abolish it. However, pretreatment with formoterol, a β2-agonist, completely prevented the HHA-induced bronchoconstriction. These results indicate that the increase in airway temperature induced transient airway constriction in guinea pigs. Approximately two-thirds of the increase in bronchomotor tone was mediated through the cholinergic reflex, which was probably elicited by the activation of TRPV1-expressing airway afferents. The remaining bronchoconstriction was caused by other, yet unidentified factors.

vagus nerves; hyperthermia; exercise; asthma; transient receptor potential vanilloid type 1

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 the metabolic rate, such as during vigorous exercise. A body core temperature exceeding 41°C has been reported during exertional exercise in healthy humans (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 hyperperemia and an increase in temperature in the inflamed area (17, 42). This was further confirmed in the respiratory tract by a recent report (40) showing that the airway temperature is significantly higher in asthmatics than in healthy individuals. Coincidentally, a previous study (1) has reported that hyperventilation with hot humidified air (HHA) induced transient but pronounced bronchoconstriction in asthmatic patients, but the underlying mechanism was not known.

A recent study (44) by 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. Although the mechanism involved in the generation of this stimulatory effect of hyperthermia was not fully understood, a more recent study (36) has shown a similar stimulatory effect of an increase in temperature in isolated vagal pulmonary sensory neurons and suggested the involvement of the temperature-sensitive transient receptor potential (TRP) vanilloid type 1 (TRPV1) channel expressed in these neurons. 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 recommendations from the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were also approved by the University of Kentucky Institutional Animal Care and Use Committee.

Measurements of lung mechanics. Young, male, pathogen-free Hartley guinea pigs were anesthetized with chloralose (100 mg/kg ip) and urethane (500 mg/kg ip), 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 (model 683, Harvard) at a constant frequency 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 (model 1260, Novametrix) between 4.6% and 5.0%. The right jugular vein and right carotid artery were cannulated for intravenous injections and for arterial blood pressure (ABP) measurements, respectively. A catheter for the measurement of intrapleural pressure (Pip) was inserted into the right pleural 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

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opening the intrapleural catheter to ambient air during a held hyperventilation (3 × Vt). During the experiment, animals were paralyzed with pancuronium bromide (30 μg/kg iv) whenever necessary. A heating pad was placed under the animal to maintain their body temperature at ~36°C.

Transpulmonary pressure was measured as the difference between the tracheal pressure (Pt) and Pp with a differential pressure transducer (MP 45-28, Validyne). Respiratory flow was measured with a heated pneumotachograph and a differential pressure transducer (MP 45-14, Validyne). All signals were recorded on a chart recorder (model 7, Grass); total pulmonary resistance (Rt) and large lung compliance (Cdyn) were analyzed continuously by an online computer on a breath by breath basis. Results obtained from the computer were routinely checked by hand calculation for accuracy.

HHA challenge. HHA was generated by connecting the outlet of the respiratory inspiratory line to an air stone and immersing it in isotonic saline (or distilled water) contained in a bottle that had been placed in a heated water bath (Fig. 1A). HHA was then delivered directly into the lung via the tracheal tube. In one series of the study, 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 (Vt and frequency 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% CO2, 21% O2, and balance N2 was administered via the respirator during hyperventilation. In some of the experiments (n = 9), we inserted a miniature thermistor (model IT-18, Physitemp, time constant: 0.1 s) through the tracheal tube and positioned it near the thoracic entry (−0.5 cm distal to the tip of the tracheal tube) to continuously measure the temperature in the tracheal lumen (Ttr; Fig. 1A) before, during, and after the HHA and HRA challenges. We have determined in our pilot experiments that Ttr can be elevated to ~40°C by maintaining the heated water bath temperature at 75°C (Fig. 1B). The amount of water content in HHA and HRA measured in this study was 74.3 and 11.4 mg/l 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, Rt and Cdyn 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 the two tests for recovery. The lung was hyperinflated (3 × Vt) 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, 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 the cholinergic mechanism and endogenous tachykinins, 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 (NK-1 and NK-2) antagonists (L-732138 and SR-48968, respectively). Series 5 was designed to test a possible role of TRPV1. Responses to HHA were compared before and after a pretreatment with capsazepine (CPZ), a selective TRPV1 antagonist. Series 6 was carried out to determine if the effect of HHA was caused by smooth muscle contraction. Responses to HHA challenges were compared between before and after a pretreatment with formoterol, a selective β2-agonist.

Statistical analysis. In each experiment, baseline Rt and Cdyn were averaged over 5 min before the HHA challenge; responses to HHA were averaged over the 5 min immediately after the challenge. Unless mentioned otherwise, two-way 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 two-way ANOVA showed a significant interaction, pair-wise comparisons were made with a post hoc analysis (Fisher’s least-significant difference test). Data are reported as means ± SE. P values of <0.05 were considered significant.

Materials. Atropine sulfate (Sigma-Aldrich) was diluted in isotonic saline. Both CPZ (Sigma-Aldrich) and formoterol (Sigma-Aldrich) were first dissolved in DMSO (Sigma-Aldrich) at concentrations of 5 and 1 mg/ml, respectively, and then diluted with saline to a final concentration of 0.2 and 0.04 mg/ml, respectively. L-732138 (Toceirs) 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 ratio of 1:1 to a final concentration of 0.67 mg/ml.

RESULTS

This study was carried out in a total of 39 guinea pigs with an average body weight of 391 ± 11 g. Some of the animals were used in more than one series of experiments. Ttr (Fig. 1A) was measured in 9 animals (7 animals in series 1 and 2 animals in series 2). Hyperventilation with HHA led to a rapid and continuous rise in Ttr from 36.4 ± 0.4 to 40.5 ± 0.5°C, which stabilized after ~3 min (Fig. 1B). In contrast, hyperventilation with 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, 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, and balance N2); there were no significant changes in either PO2 (P > 0.3 by 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 a pronounced increase in $R_L$ and decrease in $C_{dyn}$ (Fig. 2). These changes occurred immediately after HHA, and the increase in $R_L$ gradually declined and returned toward baseline in <10 min. Although the HHA-induced decrease in $C_{dyn}$ was sustained, it was completely reversed when the lung was hyperinflated at the end of 20-min recording period, suggesting that the reduced $C_{dyn}$ was, at least partially, caused by lung atelectasis. When these responses were averaged over the 5-min durations before and immediately after HHA, $R_L$ increased from a baseline of 0.12 ± 0.02 to 0.22 ± 0.04 cmH~2~O·ml⁻¹·s⁻¹ after HHA (n = 7, P < 0.01), and $C_{dyn}$ decreased from a baseline of 0.54 ± 0.08 to 0.41 ± 0.05 ml/cmH~2~O 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 changes in either $R_L$ or $C_{dyn}$ (Figs. 2 and 3). After the termination of hyperventilation with either HHA or HRA, there was an initial slight decrease followed by a gradual increase in ABP. However, there were no differences 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 $C_{dyn}$ were reproducible (Fig. 4A), and no significant differences were found between their responses to the first and second HHA challenges (P > 0.1, n = 10).

Series 3. In series 3, the effects of hyperventilation with HHA on both $R_L$ and $C_{dyn}$ to hyperventilation with HHA and HRA in anesthetized guinea pigs. At least 60 min elapsed between tests for recovery, and the sequence of these two tests was 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 ± SE of 7 animals. *Significantly different from BL (P < 0.05); †significant difference when corresponding data between HHA and HRA were compared (P < 0.05).

Series 4. To determine if the HHA-induced bronchoconstriction was mediated through the cholinergic reflex, the response to HHA was tested both before and 20 min after pretreatment with atropine sulfate (0.1 mg/kg iv). Heart rate increased from a baseline of 288 ± 13 to 301 ± 15 beats/min after atropine. The HHA-induced increase in $R_L$ was significantly reduced from 0.075 ± 0.006 cmH~2~O·ml⁻¹·s⁻¹ before atropine to 0.023 ± 0.009 cmH~2~O·ml⁻¹·s⁻¹ after atropine (P < 0.01, n = 6). However, the bronchoconstriction was not completely abolished; after the atropine pretreatment, $R_L$ still increased from a baseline of 0.106 ± 0.008 to 0.129 ± 0.012 cmH~2~O·ml⁻¹·s⁻¹ after HHA (P < 0.05; Fig. 5A).

To further test for the possible involvement of endogenous tachykinins, the response was tested both before and at 20 min after pretreatment with a combination of atropine and L-732138 (3 mg/kg iv) and SR-48968 (0.3 mg/kg iv), which are 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 ± 0.012 cmH~2~O·ml⁻¹·s⁻¹ before to 0.027 ± 0.013 cmH~2~O·ml⁻¹·s⁻¹ (P < 0.01, n = 7) after the pretreatment with these antagonists (Fig. 5B). However, HHA still caused significant bronchoconstriction (P <
series 5. To investigate the role of the temperature-sensitive TRPV1 channel in HHA-induced bronchoconstriction, the responses to HHA were determined before and immediately after a pretreatment with CPZ (0.5 mg·kg\(^{-1}\)·min\(^{-1}\) iv) 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 ± 0.014 cmH\(_2\)O·ml\(^{-1}\)·s\(^{-1}\) before to 0.031 ± 0.006 cmH\(_2\)O·ml\(^{-1}\)·s\(^{-1}\) (\(P < 0.01, n = 6\)) 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 pretreatment with formoterol (10 \(\mu\)g/kg iv), a selective \(\beta_2\)-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).
air-induced bronchoconstriction is the injury of airway mucosa; it is generally recognized that the primary cause of cold bronchoconstriction (or exercise-induced bronchoconstriction) peaks after 5–10 min (2, 34). Indeed, cold dry air-induced dilatation with cold dry air in the same patients and reached a contrast, airway constriction developed slowly after hyperventilation with cold dry air in the same subjects (1). Although it was not tested in their study, the bronchopulmonary afferents expressing the TRPV1 channel were most likely responsible since the HHA-induced bronchospasm was attenuated by a similar degree after a pretreatment with CPZ.

In this study, a transient increase in airway resistance was consistently found immediately after hyperventilation with HHA in anesthetized guinea pigs. The effect was caused by the increase in airway temperature because hyperventilation with HRA 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 pretreatment with formoterol. Furthermore, atropine pretreatment abrogated approximately two-thirds 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 were most likely responsible since the HHA-induced bronchoconstriction was attenuated to a similar degree after a pretreatment with CPZ.

Reversible bronchoconstriction induced by breathing HHA 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 3 min, the most intense bronchoconstriction that occurred immediately was generated by breathing warm humid air. The reduction in specific airway conductance was almost twofold 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 the 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 strong support for such a possibility. In contrast, airway constriction developed slowly after hyperventilation with cold dry air in the same patients and reached a peak after 5–10 min (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 in 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 HHA in this study reversed spontaneously in <10 min 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). A morphological study (23) has shown that ~75% of the vagal pulmonary afferent fibers are unmyelinated fibers (C-fibers). 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 (44) by our laboratory has shown that an increase in intrathoracic temperature stimulates pulmonary C-fibers. Stimulation of these afferents can elicit powerful centrally mediated reflex responses mediated through the autonomic nervous system, including bronchoconstriction (10, 29). Our results in the present study show that atropine pretreatment abolished two-thirds 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 the cholinergic reflex was further confirmed by the observation that a bilateral vagotomy also attenuated ~63% of the HHA-induced bronchoconstriction in a separate group of guinea pigs (n = 3; unpublished results). 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 iv), which increased $R_L$ by 828% and decreased $C_{dyn}$ by 68% in anesthetized guinea pigs (n = 3) prepared in the same manner as in this study. In addition, the 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). How-
ever, 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 the forced expiratory volume in first second 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 the cholinergic reflex, resulting from the 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 the 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.

TRPV channels are a subfamily of the TRP superfamily of ion channel proteins, which contain six transmembrane domains that form nonselective, nonvoltage-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 TRPV channel is activated in a different temperature range (4, 11). In addition to its role as a thermal sensor, the function of the TRPV1 channel as a polymodal transducer for various nociceptive stimuli in primary sensory neurons has been well documented (8, 38). Recent studies (16, 24, 28) have presented compelling evidence of an important role of the TRPV1 channel in the manifestation of various symptoms of airway hypersensitivity associated with airway inflammation; for example, overexpression of the TRPV1 channel is found in 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, the TRPV1 channel 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 the TRPV1 channel, is a selective and potent stimulant of C-fiber afferents but rarely activates myelinated afferents in the rat lung (21). A recent study (44) by our laboratory has demonstrated that the baseline activity and sensitivities of vagal pulmonary C-fiber endings are elevated when the temperature in the isolated perfused thoracic chamber is raised to a threshold of $\sim 39.2^\circ\text{C}$. Although the mechanism underlying this sensitizing effect was not fully understood, one distinct possibility is the activation by hyperthermia of certain temperaturesensitive ion channels, particularly the TRPV1 channel, expressed in the sensory terminals. Indeed, more recent studies (36, 37) in isolated pulmonary sensory neurons have yielded strong evidence in support of this possibility. Our results obtained from the CPZ pretreatment suggest that TRPV1 channels play an important role in eliciting HHA-induced bronchoconstriction. In a recent study, Gavva et al. (15) reported that treatment with a TRPV1 antagonist can cause an increase in body temperature in various animal species, suggesting that the tonic activity of the TRPV1 channel is involved in regulating the normal core temperature. Although it seems very unlikely, we cannot completely rule out the possibility that the effect of CPZ 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 CPZ was able to completely prevent the bronchoconstriction induced by 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. A 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., PGF$_2\alpha$, leukotriene B$_4$, etc.) and proinflammatory cytokines (e.g., TNF-\(\alpha\), 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 an 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 observations). Similarly, hyperventilation is also required to deliver the heat load into the lung; normal ventilation with the same HHA 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 CPZ pretreatment in this study (Figs. 5 and 6) cannot be dismissed.

The elevated airway temperature ($40.5^\circ\text{C}$) 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 pathophysiol-
ical conditions. For example, a body temperature higher than 40.5°C occurs frequently in patients suffering from severe fever or heatstroke (5). Furthermore, tissue inflammation is known to lead to an increase in temperature in the inflamed area (17, 42). Indeed, a recent study (40) has reported that the end-expiratory temperature plateau (an indirect measurement of the lung temperature) is 2.7°C higher in asthmatics than in healthy individuals. More importantly, a body core temperature exceeding 41°C has been reported in healthy humans and animals during exertional exercise (6, 33, 43). It is expected because exercise not only elevates body temperature but also induces hyperventilation. In addition, other changes in airway functions associated with the 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 overexpression of TRPV1 channels in pulmonary sensory neurons in animals (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.

GRANTS

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