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14. ABSTRACT Based upon the results obtained from these studies, we can draw the following conclusions: 1) Airway hyperresponsiveness developed in Ova-sensitized mice was less pronounced in TRPV1-null mice, indicating an important role of TRPV1. 2) An increase in airway temperature within the normal physiological range triggered bronchoconstriction in sensitized rats, but not in control rats. Chronic airway inflammation in sensitized animals is likely a major contributing factor in causing this response. 3) transient increase in airway resistance was generated immediately after hyperventilation with warm humid air in patients with mild asthma, but the same warm humid air challenge failed to cause any bronchoconstriction in healthy subjects. Furthermore, this bronchoconstriction is likely generated by the increase in airway temperature because hyperventilation with humidified air at room temperature did not generate any change in airway resistance in the same patients. These studies, once completed, should provide important and novel information for: 1) documenting the pulmonary stresses induced by hyperthermia in healthy individuals and in patients with sensitized airways; 2) understanding the mechanism underlying the hyperthermia-induced pulmonary dysfunction; and 3) detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity such as in airway allergy or mild asthma.					
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INTRODUCTION

The working hypothesis of this TATRC project is that the expression of the transient receptor potential vanilloid type 1 (TRPV1) channel is up-regulated in the airway mucosa of patients with mild asthma, allergic rhinitis and upper respiratory infection, which makes these patients more susceptible to the bronchoconstriction and other respiratory dysfunctions induced by thermal stress.

There are three specific aims for the second year of this translational project: 1) To investigate the role of the TRPV1 channel in triggering the bronchoconstriction caused by airway hyperthermia, and to determine whether this acute bronchoconstrictive effect was amplified by chronic airway allergic reaction. 2) To determine if the hyperthermia-induced airway responses were mediated through the cholinergic mechanism. 3) To determine if thermal stress generated various airway dysfunctions (airway constriction, cough, etc) in healthy volunteers, and in patients with mild asthma and allergic rhinitis.

BODY

In the last twelve months, we have made major research progresses in this project. Two study series have been completed, and three other study series are still in progress; the summaries of these studies are presented as follows:

Tasks 1-1 and 1-2: Bronchoconstriction induced by hyperventilation of humidified hot air in an animal model of asthma (ovalbumin-sensitized Brown Norway rats)

A full manuscript describing the study of the Task 1-2 is under preparation and will be submitted for publication in early 2013; results obtained from the study of Task 1-1 will also be presented in an abstract in the 2013 Experimental Biology annual meeting.

Summary: In the first-year annual report (October 2011), we have reported the rationale of choosing ovalbumin-sensitized Brown Norway rats as the animal model of asthma (replacing mice) in this study. We also reported the preliminary finding that hyperventilation of humidified hot air (HA) induced a pronounced increase in R_L in ovalbumin-sensitized rats, but not in control rats. This study is now completed, and a summary of the results is as follows: 1) In rats actively sensitized by chronic inhalation of ovalbumin (Ova) aerosol, isocapnic hyperventilation of humidified hot air (HA) for 2 min induced an increase in tracheal temperature (T_{tr}) to a peak of $40.6 \pm 0.3^\circ\text{C}$ and an immediate and sustained (> 10 min) increase in R_L (from 0.12 ± 0.01 to 0.21 ± 0.02 $\text{cmH}_2\text{O}/\text{ml}/\text{s}$; $n=7$, $P<0.01$). In sharp contrast, the HA challenge produced the same increase in T_{tr} , but did not generate any increase in R_L in matching control rats ($n=7$, $P>0.05$). 2) The responses in R_L were reproducible in both groups when the same HA challenge was repeated 60-90 min later ($n=4$). 3) This bronchoconstrictive effect was temperature dependent; a smaller increase in peak T_{tr} ($39.9 \pm 0.2^\circ\text{C}$) generated a smaller, but significant increase in R_L in sensitized rats: from 0.12 ± 0.01 to 0.19 ± 0.03 $\text{cmH}_2\text{O}/\text{ml}/\text{s}$ ($n=3$, $P<0.05$). In conclusion, an

increase in airway temperature within the normal physiological range triggered bronchoconstriction in sensitized rats, but not in control rats. Chronic airway inflammation in sensitized animals is likely a major contributing factor in causing this response.

Background and Rationale:

Hyperthermia can occur in lungs and airways under both normal and pathophysiological conditions. It has been reported that body core temperature can increase to near 42°C in humans and near 43°C in rats during strenuous exercise (1, 2). Furthermore, a recent study reported a significant increase in the exhaled air temperature ($\Delta \approx 2.7^\circ\text{C}$) in asthmatic patients, suggesting that the tracheal temperature may be elevated during inflammatory reaction (3). A previous study has shown that hyperventilation of humidified hot air induced an immediate, reversible bronchoconstriction in asthmatic patients, but the underlying mechanism was not known (4). In light of the background information, we used an animal model of allergic asthma to determine the airway response to an increase in tracheal temperature within the physiological range and to investigate the mechanisms involved in eliciting the response.

Methods:

Animal sensitization: Pathogen-free male Brown-Norway rats were actively sensitized by an initial intraperitoneal injection of a suspension containing 2 mg Ova in 1 ml Inject Alum as adjuvant. Three days after the initial intraperitoneal injection, rats were exposed to Ova aerosol for 15 min each time, three times per week for 3 weeks. Control rats received an intraperitoneal injection and aerosol inhalation of the vehicle (isotonic saline) following the identical procedures.

Measurement of lung mechanics: After 3 weeks of Ova or saline exposure, rats were anesthetized with an intraperitoneal injection of α -chloralose and urethane. Rats were ventilated mechanically via a short tracheal cannula inserted just below the larynx after a tracheotomy. A catheter for measuring intrapleural pressure was inserted into the right intrapleural cavity. Transpulmonary pressure was measured as the difference between the tracheal pressure and intrapleural pressure. Airflow was measured and integrated to give tidal volume. These signals were recorded continuously, and analyzed by computer software: total pulmonary resistance (R_L), dynamic lung compliance, arterial blood pressure, heart rate and tracheal temperature (T_{tr}).

Humidified hot air (HA) challenge: HA 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). HA was then delivered directly into the lung via the tracheal tube. Humidified room air (RA) was delivered in the same manner except that the water bath was not heated.

Summary of Results:

1. Hyperventilation of HA induced an increase in peak T_{tr} from $33.4 \pm 0.6^\circ\text{C}$ to $40.6 \pm 0.1^\circ\text{C}$, which was followed by a pronounced increase in R_L in Ova-sensitized rats, but not in control rats; the response occurred immediately after the HA challenge and lasted for > 10 min. This effect was dependent upon the increase in tracheal temperature because hyperventilation of RA failed to do so.
2. This increase in R_L was indeed temperature dependent; an intermediate increase in tracheal temperature (peak $T_{tr}=39.6 \pm 0.2^\circ\text{C}$) generated a smaller, but significant increase in R_L in sensitized rats.
3. The bronchoconstrictive effect was not caused by the humidity in HA alone because the same water content delivered by aerosol did not generate any increase in R_L (Fig. 1).
4. These bronchoconstrictive effects were reproducible when the second HA challenge was repeated 60-90 min later (Fig. 2A). Furthermore, there were no significant differences between the responses to the HA challenge generated from distilled water and isotonic saline in the same group of animals (Fig. 2B).
5. Pretreatment with either a combination of antagonists of neurokinin-1 and -2 receptors, or formoterol, a β_2 -agonist, completely abolished the peak increase in R_L induced by HA challenge in sensitized animals (Figs. 3 and 4); the latter but not the former, also abolished the delayed (at 10 min) increase in R_L (Figs. 3 and 4).

Conclusion:

In conclusion, an increase in tracheal temperature within the normal physiological range triggered a sustained but reversible increase in airway resistance in sensitized rats. In sharp contrast, this bronchoconstrictive effect of airway hyperthermia was not found in control rats. This effect was probably caused by airway smooth muscle contraction and/or protein extravasation in the airway mucosa.

Tasks 2-1, 2-2, 3-1 & 3-2: Bronchoconstriction induced by hyperventilation of humidified hot air in patients with mild stable asthma: role of cholinergic mechanism

Results obtained from these study series have been published in the American Journal of Respiratory and Critical Care Medicine in 2012 (Volume 185, pages 1190-6). The full paper (reprint) is submitted in the Appendix, and therefore only a short summary is presented here.

Summary: Hyperventilation of warm humid air induces transient bronchoconstriction in asthmatics, and the underlying mechanism is not known (4). Recent studies showed that an increase in temperature activates vagal bronchopulmonary C-fiber sensory nerves (5), which upon activation can elicit reflex bronchoconstriction. This study was designed to test the hypothesis that the bronchoconstriction induced by increasing airway temperature in asthmatics is mediated through cholinergic reflex resulting from activation of these airway sensory nerves.

Specific airway resistance (SR_{aw}) and pulmonary function test were measured to determine the airway responses to isocapnic hyperventilation of humidified air at warm (49°C; HA) and room temperature (20-22°C; RA) for 4 minutes in 6 patients with mild asthma and 6 healthy subjects. A double-blind design was used to compare between the effects of pretreatments with ipratropium bromide and placebo aerosols on the airway responses to HA challenge in these patients. Our results showed: SR_{aw} increased by 112% immediately after hyperventilation of HA, and only 38% after RA in asthmatic patients. Breathing HA, but not RA, also triggered coughs in these patients. In sharp contrast, the increases in SR_{aw} to the same challenges were distinctly smaller in healthy subjects, and there was no difference between the responses to HA and RA challenges. More importantly, pretreatment with ipratropium completely prevented the WA-induced bronchoconstriction in asthmatics. In conclusion, bronchoconstriction induced by increasing airway temperature in asthmatics is mediated through the cholinergic reflex pathway. The concomitant increase in cough response further indicates an involvement of airway sensory nerves, presumably the thermosensitive bronchopulmonary C-fiber afferents.

Tasks 2-3 & 3-3: Airway stress induced by hyperventilation of humidified hot air in patients with allergic rhinitis.

This study series is currently in progress. Preliminary results obtained from these studies will be presented in an abstract in the 2013 American Thoracic Society International Conference.

Background and Rationale:

Our study described above (Task 2-1, 2-2, 3-1 & 3-2) has demonstrated that hyperventilation of hot humid air triggered coughs and a reflex bronchoconstriction in patients with mild asthma, but not in healthy individuals (6). The bronchoconstriction is mediated through the cholinergic reflex, suggesting that the airway sensory nerves, presumably the TRPV1-expressing C-fiber sensory nerves innervating the lung and airways, are activated by an increase in airway tissue temperature in these patients (6). It is well documented that airway hypersensitivity is also present in patients with allergic rhinitis (7, 8). We hypothesize that TRPV1 is over-expressed in the airway sensory nerves in patients with allergic rhinitis resulting from chronic airway inflammation in the upper airways. Thus, an increase in airway temperature generated by hyperventilation of hot humid air can activate the TRPV1-expressing sensory nerves and trigger exaggerated airway responses in these patients.

Methods:

Four patients with chronic allergic rhinitis were recruited, and a screening test was performed in each patient after informed consent was obtained. Patients were asked to stop therapeutic medications for 2 weeks prior to beginning the study.

A humidified gas mixture of 4.5% CO₂ balance air at either hot (HA; 49°C and 75-80% relative humidity) or room temperature (RA: 20-22°C and 65-75% relative humidity) was delivered by a

device constructed by the University of Kentucky Center for Manufacturing. During the hyperventilation challenge, the subject breathed via a mouthpiece into this free stream of humidified gas mixture at ~40% of maximal voluntary ventilation for four minutes; CO₂ was added to maintain an isocapnic condition during hyperventilation. Humidity was generated from sterile isotonic saline by an ultrasonic atomizer.

Airway resistance (R_{aw}) and thoracic gas volume (V_{tg}) were measured by a whole-body constant-volume plethysmography (SensorMedics) for 6 minutes before and 16 minutes immediately after the hyperventilation challenge. Spirometry test was also performed along with the measurements of other physiological variables (body temperature, heart rate, arterial blood pressure, and oxygen saturation) before and after the challenge. The responses to HA and RA hyperventilation challenges were compared in these patients

Summary of Results:

1. Hyperventilation of humidified HA also triggered vigorous cough responses in all 4 patients with allergic rhinitis; the cough frequency increased from $0.02 \pm 0.02 \text{ min}^{-1}$ at the baseline to $1.03 \pm 0.48 \text{ min}^{-1}$ during the HA challenge (Fig. 5). In contrast, hyperventilation of RA did not cause any significant cough responses in the same patients. In healthy individuals, neither HA nor RA hyperventilation elicited any detectable cough responses.

2. After hyperventilation of humidified HA, R_{aw} increased immediately after hyperventilation and declined slowly toward baseline in allergic rhinitis patients; the average R_{aw} increased significantly from a baseline of $2.03 \pm 0.16 \text{ cmH}_2\text{O/liter/sec}$ to a peak of $2.72 \pm 0.35 \text{ cmH}_2\text{O/liter/sec}$ after the HA challenge ($n=4$, $P<0.05$; Fig. 6). In the same patients, hyperventilation of humidified RA gas mixture did not cause a significant increase in SR_{aw} .

Conclusion:

In summary, this study showed that hyperventilation of hot humid air triggered coughs and bronchoconstriction in patients with allergic rhinitis, but not in healthy individuals. These airway responses occurred rapidly after the onset of hyperventilation of humidified HA, and recovered in less than 16 minutes after the challenge.

Our observation that increasing airway temperature consistently triggered coughs in patients with chronic allergic rhinitis, but not in healthy individuals, further supports our hypothesis that the sensitivity and/or expression of TRPV1 is up-regulated in the airway sensory nerves of these patients.

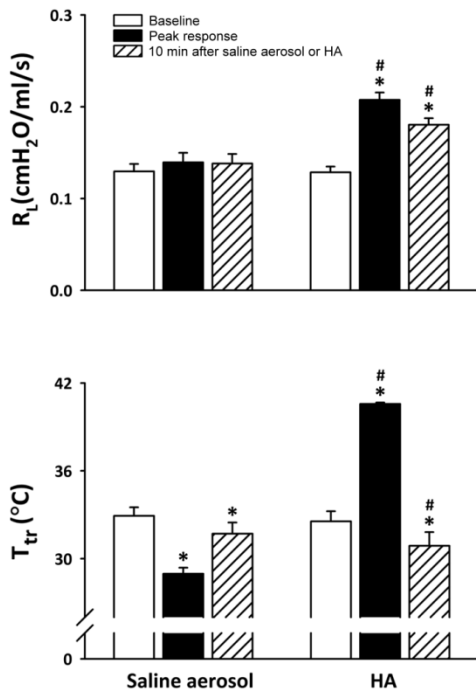


Fig. 1. Comparison of responses of R_L and tracheal temperature (T_{tr}) to hyperventilation with HA and with saline aerosol in the Ova-sensitized animals. The same amount of water content (77.6 ± 5.9 mg/L of air) was delivered by aerosol and HA in the same manner in the same animals, but the saline aerosol was generated and delivered at the room temperature ($\sim 23^\circ\text{C}$). Data were means \pm SE of 6 animals. *Significantly different from baseline ($P < 0.05$); #significant difference when corresponding data between HA and saline aerosol were compared ($P < 0.05$).

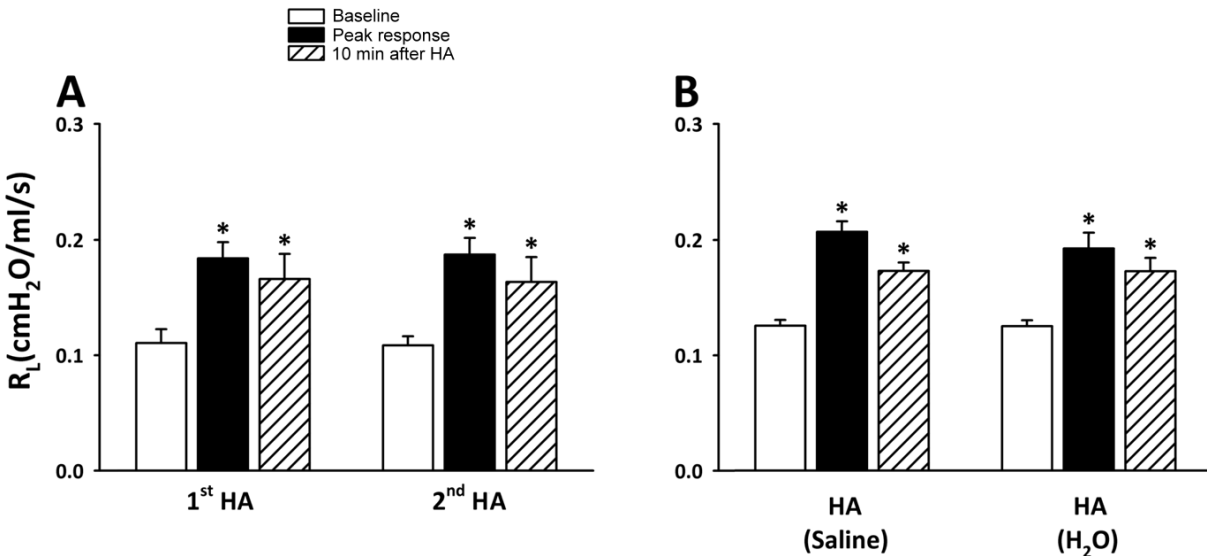


Fig. 2. A: reproducibility of the responses of R_L to two consecutive HA challenges in the same groups of sensitized rats. B: responses of R_L to HA were compared in the same group of sensitized rats when humidity was generated from distilled water and isotonic saline. Data were means \pm SE of $n=5$ in A and $n=6$ in B. *Significantly different from baseline ($P < 0.05$).

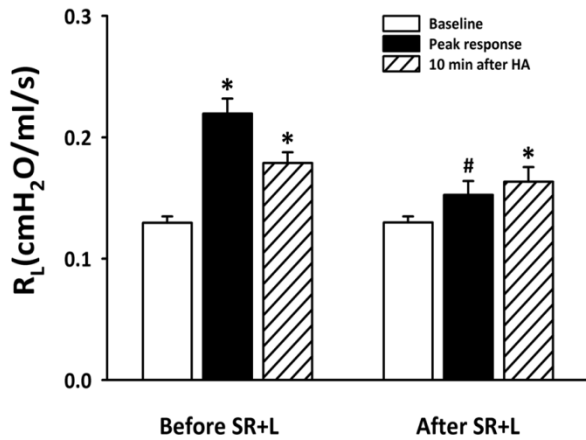


Fig. 3. Comparison of the responses of R_L to hyperventilation with HA before and after pretreatment with a combination of SR-48968 (SR; 0.3 to 1 mg/kg iv) and L-732138 (L; 6 mg/kg iv), antagonists of neurokinin-1 and neurokinin-2 receptors, respectively, in the same group of sensitized rats. Data were means \pm SE of 6 animals. *Significantly different from baseline ($P < 0.05$); #significant difference when corresponding data between before and after pretreatment were compared ($P < 0.05$).

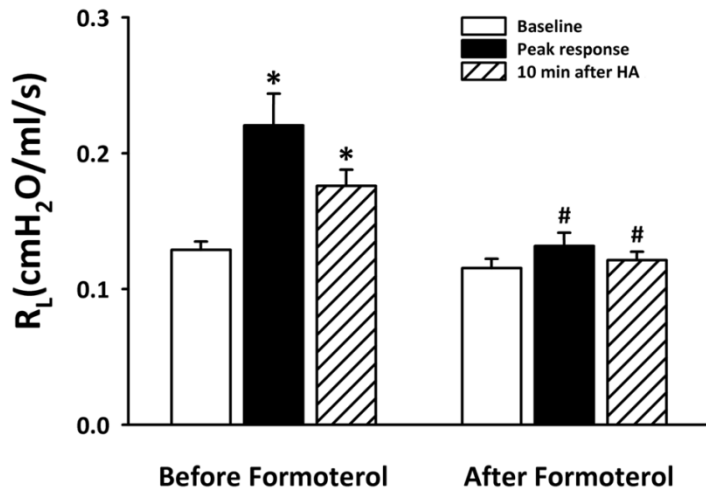


Fig. 4. Comparison of the responses of R_L to hyperventilation with HA before and after pretreatment with formoterol (10 μ g/kg iv), a β_2 -agonist, in the same group of sensitized rats. Data were means \pm SE of 6 animals. *Significantly different from baseline ($P < 0.05$); #significant difference when corresponding data between before and after pretreatment were compared ($P < 0.05$).

Allergic rhinitis

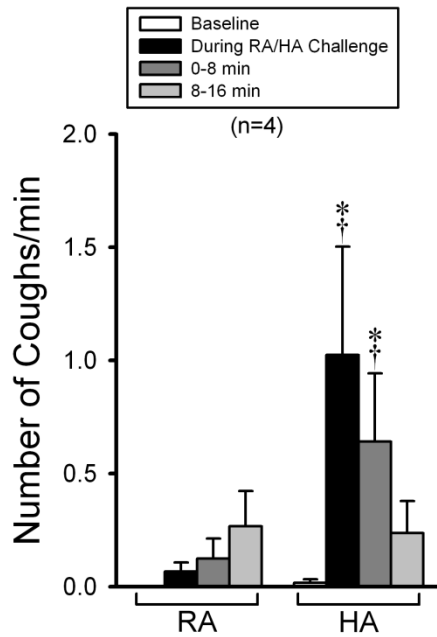


Fig. 5. Group data showing a comparison of the cough responses between hyperventilation of humidified room air and hot air in asthmatic patients (n=4). Cough frequencies were averaged in 8-min durations before and after hyperventilation challenge in each subject. Data are means \pm SEM. *, significantly different (P<0.05) from the baseline. †, significant difference (P<0.05) comparing the corresponding data between room air and hot air.

Allergic rhinitis

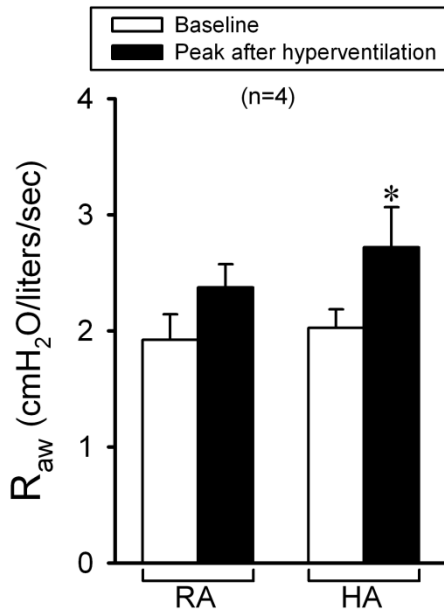


Fig. 6. Group data showing a comparison of the peak responses of airway resistance (Raw) between hyperventilation of humidified room air and hot air in patients with allergic rhinitis (n=4). Baseline and peak Raw were averaged over 8 and 4 consecutive breaths before and after hyperventilation challenge, respectively, in each subject. Data are means \pm SEM. *, significantly different (P<0.05) from the baseline.

KEY RESEARCH ACCOMPLISHMENTS

These proposed studies have established important information for:

- 1) documenting the pulmonary stresses induced by hyperthermia in healthy individuals and in patients with mild asthma and allergic rhinitis;
- 2) demonstrating the distinct difference in these airway responses to thermal stress between healthy individuals and patients with sensitized airways;
- 3) characterizing the critical role of the cholinergic mechanism underlying the hyperthermia-induced reflex bronchoconstriction and cough hypersensitivity;
- 4) indicating the involvement of TRPV1-sensory nerves in eliciting these airway response to thermal stress;
- 5) detecting the susceptibility to heat stress in soldiers with underestimated or overlooked airway hypersensitivity, such as in individuals with mild asthma or allergic rhinitis.

REPORTABLE OUTCOMES

1. Publications

The following publications reporting the findings obtained in the studies supported by this TATRC project have been either published or submitted for publication:

Hayes, D., P.B. Collins, M. Khosravi, and L.Y. Lee. Bronchoconstriction triggered by breathing hot humid air in asthmatics: role of cholinergic reflex. Am. J. Resp. Crit Care Med. 185: 1190-6, 2012. (Reprint is submitted in the Appendix)

Lin, R.L., Y. J. Lin, M. J. Geer and L.-Y. Lee. Pulmonary Chemoreflex Responses Are Potentiated by Tumor Necrosis Factor Alpha in Mice. J. Appl. Physiol. (Full manuscript submitted for publication in Oct. 2012)

Lee, L.Y. and D. Hayes. Airway constriction and cough induced by breathing warm humidified air in asthmatics: role of cholinergic mechanisms. Clin. Auton. Neurosci. 21: 303, 2012

Hsu, C.-C., C.B. Martin and L.-Y. Lee. Bronchoconstriction induced by hyperventilation of humidified hot air in ovalbumin-sensitized Brown Norway rats. FASEB J. 2012 (Abstract)

Lin Y. J. R. Tapia and L.Y. Lee. Hypersensitivity of vagal pulmonary C-fibers induced by hyperventilation of hot humid air in ovalbumin-sensitized Brown Norway rats. (Abstract to be presented in the Experimental Biology Annual Meeting, 2013)

Khosravi, M., P.B. Collins, and L.Y. Lee. Coughs and bronchoconstriction triggered by breathing hot humid air in patients with allergic rhinitis. (Abstract to be presented in the American Thoracic Society International Conference, 2013)

2. Employment Generated by this TATRC Contract

Salaries of the employees listed below are paid in part or in full with the funds provided by this research contract:

Lu-Yuan Lee, Ph.D., Principal Investigator (30% effort)

Mahdi Khosravi, M.D., Co-investigator (20% effort)

Paul B. Collins, B.S., RRT, Supervisor of Pulmonary Function Laboratory (10% effort).

Richard Kryscio, Ph.D., Co-investigator (3% effort), Consultant for Biostatistics (3% effort)

Marcus Geer, B.S., Lab Technician (100% effort; newly hired to work on this project)

Emy Lin, Ph.D., Postdoctoral Scholar (100% effort; newly hired to work on this project)

Robert Morton, Part-time Senior Research Analyst (20% effort; hired to work on this project)

Ruby Lin, B.S., Graduate Assistant (100% effort; newly hired to work on this project)

CONCLUSIONS

Based upon these results, we can draw the following conclusions:

1) An increase in tracheal temperature within the normal physiological range triggered a sustained but reversible increase in airway resistance in sensitized rats. In sharp contrast, this bronchoconstrictive effect of airway hyperthermia was not found in control rats. This effect was probably caused by airway smooth muscle contraction and/or protein extravasation in the airway mucosa.

2) Hyperventilation of hot humid air triggered an immediate and reversible increase in airway resistance in patients with mild asthma, but caused either only a very small or no response in healthy subjects. The bronchoconstriction in these patients was completely prevented by pretreatment with ipratropium aerosol, indicating an involvement of cholinergic reflex. Accompanying the bronchoconstriction, breathing hot humid air also triggered coughs consistently in these patients, suggesting an involvement of the airway sensory nerves, presumably the thermosensitive TRPV1-expressing C-fiber afferents.

3) Hyperventilation of hot humid air, but not room air, triggered coughs and bronchoconstriction in patients with allergic rhinitis, suggesting that the sensitivity and/or expression of TRPV1 is up-regulated in the airway sensory nerves of these patients.

4) Continuing studies will be required to determine whether the effect of hyperthermia is primarily mediated through an activation of the temperature-sensitive TRPV1 channel expressed on vagal bronchopulmonary C-fibers, and if TRPV1 expression is up-regulated in the airway mucosa of patients with chronic inflammation.

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APPENDIX

A full article of our paper published in *Am. J. Resp. Crit Care Med.* 185: 1190-6, 2012 can be downloaded from the following link:

<http://www.ncbi.nlm.nih.gov/pubmed/22505744>

or

<http://ajrccm.atsjournals.org/content/185/11/1190.long>