Inflammation-Induced Airway Hypersensitivity: From Ion Channels to Patients

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BACKGROUND AND HYPOTHESES

My research interest focuses on the neurogenic mechanisms involved in the bronchial hyperreactivity, and ongoing projects are currently conducted in the Airway Sensory Neurobiology Laboratory at the University of Kentucky to study this in animal models and in human subjects. Airway hypersensitivity is a characteristic feature of airway inflammatory diseases such as asthma. When airway hypersensitivity develops during airway mucosal inflammation, the excitability of chemosensitive nerve endings innervating the airways is drastically enhanced.

In healthy lungs, these tachykinin-containing sensory terminals located superficially in the airway mucosa (Fig. 1) play an important role in protecting the airways against inhaled irritants. Stimulation of these sensory endings elicits extensive cardiopulmonary reflex responses such as cough, bronchospasm, hypersecretion of mucus, etc. However, when these nerve endings become hypersensitive as a result of inflammation or injury of airway mucosa, a given level of stimulus will then evoke more sustained and intense stimulation. Thus, greater intensities of the reflex reactions as well as the neurogenic inflammation mediated through local release of tachykinins can lead to the development of bronchial hyperreactivity.

Function of TRPV1-expressing sensory nerves and their interaction with other cell types in airway mucosa. EO, eosinophil; LO, lipoxygenase; PGE₂, prostaglandin E₂; BK, bradykinin; NGF, nerve growth factor; MBP, eosinophil major basic proteins; TKs, tachykinins; CGRP, calcitonin gene-related peptide. (From: Lee & Gu, 2009)
LONG-TERM OBJECTIVES

Our long-term objectives are to answer the following questions:

• What are the physiological and pharmacological properties of the chemosensitive nerve endings in the lung?
• What are the roles of these sensory nerves in regulating cardiopulmonary functions under normal and various pathophysiological conditions of the airways?
• What are the endogenous and exogenous chemical substances that can alter the sensitivity of these sensory endings?
• What are the cellular mechanisms underlying the hypersensitivity of these sensory nerves caused by inflammation of airway mucosa, such as during airway injury or allergic reaction?
• What is the role of the transient receptor potential vanilloid type 1 (TRPV) ion channels in the airway hypersensitivity (exaggerated cough and bronchoconstrictive responses to inhaled irritants) developed during airway inflammatory reaction?

ONGOING PROJECTS

Several projects are currently conducted in our lab aimed specifically to:

• Uncover the mechanism of hypersensitivity of bronchopulmonary C neurons caused by cationic proteins, that are released from inflammatory cells (e.g., eosinophil, neutrophil, etc.) during airway anaphylaxis or injury.
• Identify the specific ion channels and the intracellular signal transduction pathways involved in the hypersensitivity of pulmonary sensory neurons induced by certain endogenous chemical mediators (e.g., prostaglandin E2, adenosine, hydrogen ions, etc.) and pro-inflammatory cytokines (e.g., tumor necrosis factor-α)
• Determine the temperature-sensitive properties of bronchopulmonary C neurons, and investigate their roles in regulating the cardiopulmonary functions in responses to an acute increase in airway temperature (e.g., during airway inflammation, exercise, etc.)
• Understand the underlying mechanisms of airway hypersensitivity in patients with mild asthma, allergic rhinitis, laryngopharyngeal reflux, and post-viral infection
• Investigate the possible involvement of the TRPV1 channel in the manifestation of bronchoconstriction, cough and dyspneic sensation in these patients.

REPRESENTATIVE RECENT STUDIES

Sensitization of Vagal Pulmonary Sensory Neurons by Human Eosinophil-Derived Cationic Proteins

Rationale and Hypothesis: During airway inflammatory reaction (e.g., in asthma), a number of low molecular weight, highly cationic proteins, such as major basic protein (MBP) and eosinophil cationic protein, are secreted by eosinophils that infiltrate into the airways. It is well documented that the release of these proteins can induce mucosal injury and airway hyperresponsiveness. However, the mechanism underlying both the initial stimulatory and the sustained sensitizing effects of cationic proteins on pulmonary sensory nerves is poorly understood.
**Summary of Results:** Our studies have established the first evidence demonstrating an intense and sustained effect of eosinophil granule-derived proteins on vagal bronchopulmonary C-fiber endings, which is dependent on their cationic charge. Our results also demonstrate that MBP potentiates the capsaicin-evoked inward current in isolated pulmonary sensory neurons and up-regulates the excitability of these neurons to electrical stimulation. Our data further demonstrate that MBP significantly inhibits both $I_{K_{dr}}$ and $I_{K_a}$ in these neurons. Our studies therefore suggest that the direct and long-lasting sensitizing effect of these cationic proteins on pulmonary sensory nerves may play an important part in the manifestation of airway hyperresponsiveness associated with eosinophil infiltration in the airways.

**Hypersensitivity of TRPV1 Induced by Activation of Protease-activated Receptor-2**

**Rationale and Hypothesis:** Protease-activated Receptor-2 (PAR$_2$) belongs to a family of G-protein-coupled, seven-transmembrane-domain receptors named PARs that are uniquely activated by proteolysis. Compelling evidence indicates that PAR$_2$ plays a critical role in the pathogenesis of airway inflammation and airway hyperresponsiveness. The primary objective of this study is to uncover the mechanism involved in the interaction between PAR$_2$ and C-fiber sensory nerves in the lung/airways.
**Summary of Results:** In anesthetized spontaneously breathing rats, airway exposure to trypsin, an endogenous agonist of PAR$_2$, significantly amplifies the capsaicin-induced pulmonary chemoreflex responses. In isolated rat vagal pulmonary sensory neurons, pretreatment with trypsin potentiated the capsaicin-induced whole-cell inward current; the effect is mimicked by PAR$_2$-activating peptide (PAR$_2$-AP) in a concentration-dependent manner. Activation of PAR$_2$ up-regulates single-channel activities of TRPV1 and the effect is mediated through the protein kinase C-dependent transduction pathway.

**An increase in capsaicin sensitivity and TRPV1 expression of pulmonary myelinated afferents in ovalbumin-sensitized rats**

**Rationale and Hypothesis:** Increasing evidence indicates that the TRPV1 receptor selectively expressed in vagal bronchopulmonary C-fibers plays a pivotal role in the manifestation of airway hypersensitivity, a prominent pathophysiological feature of airway inflammatory diseases. This study was carried out to investigate whether the sensitivity vagal afferents and expression of TRPV1 in these nerves are altered when chronic airway inflammation is induced by ovalbumin (Ova) sensitization in an animal model of asthma.
Summary of Results: This study showed that allergen sensitization markedly elevates the baseline activity and excitability of pulmonary C-fibers. Interestingly, vagal bronchopulmonary myelinated afferents also exhibited capsaicin sensitivity in sensitized rats. Furthermore, the immunohistochemistry experiments showed that there was a significant increase in the proportion of TRPV1-expressing pulmonary neurons in nodose ganglia of sensitized rats, particularly in neurofilament-positive (myelinated) neurons.

Hyperthermia Increases Sensitivity of Pulmonary C-fiber Neurons: Role of TRPV channels

Rationale and Hypothesis: When body temperature increases during strenuous exercise or fever, the lung tissues including the sensory endings residing within the lung structures are subjected to hyperthermia. The TRPV channels expressed in the sensory terminals of bronchopulmonary C fibers are known to function as thermal transducers, with a different activation temperature threshold in each subtype of the TRPV channels. This study was, therefore, carried out to answer the following specific questions: 1) Are vagal pulmonary C-fibers sensitive to an increase in the temperature within the normal physiological range, and if so, can hyperthermia also directly activate isolated vagal pulmonary sensory neurons? 2) Is the response of these neurons to increase in temperature mediated through activation of TRPV channels?

Fig. 7

Fig. 7. Effect of increasing temperature on vagal pulmonary sensory neurons in voltage-clamp (A) and current-clamp mode (B). C: the temperature-current relationships obtained from the two hyperthermia challenges in the same neuron (data taken from A).

Fig. 8

Fig. 8. Blocking effects of capsazepine (CPZ, 10 µM) and ruthenium red (RR, 3 µM) on the hyperthermia-induced current in rat vagal pulmonary sensory neurons. C: group data (n=11) showing the effects of different treatments on cell response to hyperthermia.
Hyperthermia-induced bronchoconstriction and cough in patients with mild asthma: a translational study

Rationale and Hypothesis: Our recent studies have demonstrated that an activation of TRPV1 is primarily responsible for the stimulatory effect of hyperthermia on vagal bronchopulmonary C-fibers. We reasoned that a stimulation of these afferents will elicit bronchoconstriction via cholinergic reflex mechanism. Further, we hypothesized that the temperature threshold for activating these afferents will be reduced in patients with mild asthma. Hence, breathing warm, humid air can activate TRPV1 and elicit reflex bronchoconstriction and other airway dysfunction (e.g., cough, dyspnea, etc.).
Summary of Results: This pilot study showed that breathing warm humidified air triggered cough and acute bronchoconstriction in mild asthmatic patients; the latter could be prevented by a pretreatment with ipratropium (atropine). These preliminary results indicated the involvement of airway sensory nerves and cholinergic mechanisms in the manifestation of various symptoms evoked by airway hyperthermia in these patients, which further suggests the potential involvement of TRPV1 over-expression in asthmatic airways as a possible contributing factor.

Fig. 10. Effect of pretreatment with ipratropium aerosol or placebo on the response of airway resistance to warm humid air inhalation challenge air in six asthmatic patients.

Fig. 11. Bronchomotor and cough responses were measured in asthmatic patients (UK Pulmonary Function Testing Laboratory and Clinical Research Development & Operations Center)

Fig. 12. Experimental records illustrating a comparison of the flow-volume curves between baseline (blue) and that after termination of hyperventilation with humidified air (red) at room (RA; panel A) or high temperature (HA; panels B,C & D) on four different days in a patient with mild asthma.
REPRESENTATIVE RECENT PUBLICATIONS (2008-2013):


