ELECTROLYTE SECRETION FROM AIRWAY SUBMUCOSAL GLAND ACINAR CELLS

TSUKASA SASAKI, MD
The First Department of Internal Medicine, Tohoku University
School of Medicine, Sendai, Japan

Introduction

Airway epithelial cells and submucosal gland serous and mucous cells secrete and release materials, including antibacterial agents (such as lysozyme), antiproteases, mucins, ions and water to control the properties of the mucus gel that lines the airway. Bronchial obstruction by hypersecretion of mucus is the most common pathological failure of chronic obstructive pulmonary diseases, bronchial asthma and cystic fibrosis. In humans the glandular volume is estimated to be 40 times of that of mucus cells present in the airway surface epithelium (L. Reid, 1960), indicating the submucosal gland as a major source of the physiological airway secretion. Studies on composition of human airway surface liquid revealed that 90% or more of the total volume of the secretion is water (L.W. Matthews et al., 1963) which is associated with Cl^- secretion. Transepithelial Cl^- secretion is thus critical for the production of properly hydrated secretions in epithelial tissues including airways. Airway Cl^- originates physiologically from both the superficial epithelial layer and the submucosal glands. Although a significant fraction of Cl^- appears to be derived from the submucosal glands (P.M. Quinton, 1979), available data concerning the mechanism of Cl^- secretion from the gland are sparse. In addition, almost all the reports studying this issue employed cultured cells as the materials. I here present results obtained from the freshly isolated submucosal gland acinar cells of mammals including human, using

patch clamp technique and intracellular Ca^{2+} measurement. The main result is that the Cl^- secretion from the submucosal gland is triggered not by agents that raise cytosolic cAMP but by intracellular Ca^{2+}-mobilizing agents such as the cholinergic and α_1-adrenergic agonists. We also found that intracellular Ca^{2+} had a pivotal role in cholinergically-induced Cl^--current in freshly isolated human and feline tracheal submucosal gland acinar cells. Inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+}-relase is shown to play an important role in the acetylcholine (ACh)-evoked current response. However, the blockade of the IP_3-mediated pathway by a mAb against IP_3-receptors could not totally abolish the ACh-induced current in airway glands, indicating an additional Ca^{2+}-mobilizing mechanism unrelated to the IP_3-induced intracellular release of Ca^{2+} in airway gland acinar cells.

Methods

Submucosal glands were isolated either from the tracheae of anesthetized cats or those of human surgical specimens from patients of laryngeal cancer (see ref. 23 and 24, for detailed description about the isoltion technique). The isolated glands were further dispersed enzymatically into single or clustered acinar cells by sequential treatment with collagenase (200 U/ml, 30 min) and trypsin (2 mg/ml, 30 s) at 37°C. After dispersion and washing the cells were resuspended in a standard extracellular solution (see below). For patch clamp study, a List LM-EPC7 (List Electronics, Darmstadt, Germany) patch clamp amplifier was used for measurements of whole-cell currents of acinar cells. The solutions employed were of the following compositions (in mM): *extracellular (bath) solution;* 120 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, and 10 Hepes; *intracellular (pipette) solution;* 120 KCl, 1.13 $MgCl_2$, 0.5 EGTA, 1 Na_2ATP, 10 glucose, and 10 Hepes. All solutions were at pH 7.2, and all experiments were carried out at room temperature (22-25°C).

Results

a. *Basic electrophysiological characteristics of tracheal gland acinar cells*

In respond to acetylcholine (ACh, a cholinergic neurotransmitter) and phenylephrine (Phenyl, an α–adrenergic agonist), tracheal submucosal gland acinar cells exhibited oscillatory Cl^-- and K^+-currents with whole-cell configurations. At a membrane holding potential of -80mV, at which potential we can see Cl^- current without contamination of K^+ current, the frequency of Cl^--current oscillations was dependent on concentrations of agonists used. For example, 2, 6 and 31 min^{-1} in the presence of 4, 5, 6 nM of ACh, respectively. Higher concentrations of agonists showed a sustained current response and 1μM or more of ACh exhibited a marked desensitization, that is, the triggered currents were attenuated spontaneously within a few minutes. On the contrary, administration of isoproterenol (a β-adrenergic agonist), dibutyryl cAMP, cpt cAMP combined with IBMX had no effect on the basic electrical profiles.

In membrane pattches excised from clustered acinar cells, probably on a basolateral aspect of the cells, we found a large conductanse K^+ channel (164+/-8.2pS, n=5) sensitive to cytosolic Ca^{2+} concentration, and a nonselectiva cation channel (18.4+/-1.1 pS, n=10) was also present. In order to specify the Cl^--pathway on the apical side of the cell, we used a primary cultures of human submucosal gland (from Dr. Yamaya at Tohoku University). The surface membrane oriented to the culture fluid in confluent cells contained four types of channels. They include a very small conductance (4.2+/-0.3 pS, n=8) Cl^- channel with linear I-V relation and Ca^{2+} sensitivity, an intermediate conductance Cl^- channel that was insensitive to Ca^{2+}, outwardly rectified- and voltage-activated Cl^- channel (17.4 pS at 0 mV and 74.3 pS around 100 mV) and a nonselective cation channel with quite similar characteristics of those obtained on basolateral membranes.

b. *Inositol 1,4,5-trisphosphate (IP3)-mediated electrolyte sevretion*

We examined the role of IP3 receptor in ACh-induced Cl^- current in acinar cells of human and feline airway submucosal glands using whole-cell patch-clamp analysis. ACh (10nM-1μM) induced an initial Cl^- current followed by a K^+ current, which was mimicked by the application of IP3 (10μM) intracellularly via the patch pipette. Caffeine (20-50 mM) and intracellular ryanodine (1-100 μM) induced a K^+-current alone without Cl^- current. Most of the experiments using monoclonal antibodies to the IP3 receptor abolished both ACh-induced K^+ and Cl^- currents, but in some instance, ACh caused an activation of K^+ cuurent alone. Immunohistochemical analysis revealed the localization of IP3 receptors on both the cytosol and some regions of the endoplasmic reticulum beneath the apical membrane of acinar cells. These results indicate that apically localized IP3 receptors control Cl^- secretion from airway submucosal gland cells.

c. *A possible involvement of cADPR in electrolyte secretion from submucosal gland*

Cyclic ADP-ribose (cADPR), a newly identified Ca^{2+}-mobilizing second messenger, has been reported to operate in several mammalian cells. In order to investigate whether cADPR is involved in electrolyte secretion from airway gland acinar cells, we have used patch clamp technique, measurement of microsomal Ca^{2+} release, and reversed transcriptase-PCR (RT-PCR) of CD38 mRNA in human and feline tracheal glands. cADPR, introduced into the cell interior via patch pipette, caused current activations dependent on intracellularly released Ca^{2+}. The current evoked by cADPR (4μM) was tetraethyl ammonium chloride (TEA)-sensitive K^+ current, which had the same characteristics as that induced by intracellular ryanodine in these cells. Microsome fractions derived from the isolated glands released Ca^{2+} in response to both inositol 1,4,5-trisphosphate (IP_3) and cADPR. The cADPR-sensitive Ca^{2+}-store was distinct from that sensitive to IP_3 because microsomes

desensitized with IP_3 or those treated with heparin did not affect the cADPR-induced Ca^{2+}-release. The mRNA of CD38, an enzyme protein involved in cADPR metabolism, was detected in human tissues including tracheal glands. We concluded that cADPR, in concert with IP_3, operates in airway gland acinar cells to mobilize Ca^{2+} resulting in Cl^- secretion.

References

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