UTP stimulates chloride secretion via CFTR and Ca^{2+}-activated chloride channels in cultured human middle ear epithelial cells

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All my life, my mother and father have believed in me, in us three daughters. We know without hesitation that no matter how direct or winding the paths we choose, their love and support will guide our steps. My degree is dedicated to my mother, Soon Wha Jung, for her love and dedication to us have no comparison. She has always shown us the wisdom of life through words and deeds. Knowing I have my mother's blessings is my courage and strength in every hardship I have faced so far and forevermore. You have shown us the love of God and we love you so much.

And as always, I thank God for His everlasting love and patience.

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<**ABSTRACT**>

UTP stimulates chloride secretion via CFTR and Ca^{2+}-activated chloride channels in cultured human middle ear epithelial cells

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OBJECTIVE Nucleotide binding to purinergic P2Y receptors contributes to the regulation of fluid and ion transport in middle ear epithelial cells. Here, we investigated the regulatory mechanism of the $P2Y_2$ receptor agonist uridine-5'-triphosphate (UTP) on Cl^- transport in cultured normal human middle ear (NHMEE) cells. **METHODS** Monolayers of cultured NHMEE cells were mounted in Ussing chambers to perform electrophysiological measurements. Molecular expression of Ca^{2+}-activated chloride channels (CACC) was examined by RT-PCR. **RESULTS** Apical addition of UTP in presence of amiloride evoked a transient rise and a sustained response in short circuit currents (I*sc*) due to Cl^- efflux. Application of different chloride channel blockers to the apical side of the tissues was able to significantly decrease UTP-induced I*sc*. Niflumic acid (NFA), a known blocker of CACC, and CFTRinh-172, a selective inhibitor of CFTR, partially inhibited the UTP-induced Cl^- secretion respectively. Calcium-chelating agent BAPTA-AM blocked the UTP-induced activation of CACC. Inhibition of PKC reduced UTP-dependent stimulation of Cl^- efflux. CACC mRNA expression was confirmed by RT-PCR. **DISCUSSION & CONCLUSION** Cl^- transport across airway epithelia plays a predominant role in regulating airway hydration. Nucleotides are known to stimulate anion secretion across airway epithelia by acting on P2Y receptors on the apical membrane. In this study, the $P2Y_2$ receptor agonist UTP is shown to increase both CACC and CFTR-dependent

Cl^- secretion in NHMEE cells, and that the PKC pathway is involved in CFTR activation in human middle ear epithelial cells.

Key words : UTP, chloride secretion, cystic fibrosis transmembrane conductance regulator, Ca^{2+}-activated chloride channels, middle ear

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I. INTRODUCTION

A fluid-free middle ear cavity is maintained by middle ear epithelium. The middle ear epithelial cells control the airway surface liquid (ASL) volume and composition tightly by regulating both fluid and electrolyte absorption and secretion. Otitis media with effusion (OME) results from retention of cellular exudates in the middle ear cavity following upper respiratory tract infection. OME is a very common childhood disease and about 80% of all children experience at least one episode of OME in preschool ages. The number of annual hospital visits for otitis media is over 130 thousand,[1] and the visits cost about 3 billion dollars in the USA.[2] In Korea, statistics report the prevalence of OME as over 2% in 1993 and rising.[3]

Inflammation in the middle ear mucosa, caused usually by bacterial and viral pathogens, is the primary event in the middle ear predisposing the development of OME. However, little is known about factors leading to retention of effusion. One important feature of OME involves goblet cell hyperplasia in the middle ear epithelia and resultant mucus hypersecretion. Goblet cell hyperplasia is especially more evident in mucous effusions that are usually refractory to medical therapy and require surgical intervention.[4-6] Since the middle ear mucosa is considered an extension of the respiratory tract epithelium and its surface is covered with a thin layer of mucus and airway surface liquid (ASL). The

volume and composition of ASL in lower airway is known to be meticulously maintained by various ion transport systems such as epithelial sodium channel (ENaC) and cystic fibrosis transmembrane conductance regulator (CFTR) to allow effective mucociliary clearance. Recent studies report similar ion transport systems in the middle ear epithelium and their role in the pathogenesis of OME.[7-11] Alterations in the function of these ion channels are proposed to result in increased secretion or reduced absorption of ASL and lead to development of OME.

Airway epithelia utilize active ion transport to modulate the ASL volume.[12] Chloride ion (Cl^-) transport is thought to play predominant roles in airway hydration. Electrolyte and fluid transport system of the middle ear epithelium is similar to that of lower airway epithelium. Inflammatory mediators can stimulate transepithelial Cl^- secretion in middle ear epithelial cells, and resulting fluid transport may be involved in the pathogenesis of OME.[8]

Cl^- enters polarized airway epithelial cells through basolateral Na^+-K^+-Cl^- Cotransporter (NKCC) and is secreted by two apical channels: CFTR and Ca^{2+}- activated Cl^- channel (CACC). CFTR is a well-known cAMP-regulated Cl^- conductance and plays a critical role of maintaining ASL volume under basal conditions.[13-14] CACC is a recently identified anion channel in the apical membrane which is stimulated via a $P2Y_2$ receptor-mediated increase in intracellular Ca^{2+} in both human and murine airways.[15] Contribution of CACC, as an alternative Cl^- channel, in fluid transport and thus ASL maintenance is considered even more important in cystic fibrosis patients who lack CFTR.

Luminal nucleotides are known to stimulate anion secretion in airway epithelia by activating P2 receptors in the apical cell membrane. In the respiratory system, $P1A_{2b}$, $P2Y_2$, $P2Y_6$, some P2X receptor subtypes are expressed and control mucociliary clearance, ion transport, ciliary beat frequency and mucin release. [16-19] The middle ear mucosa shows slightly different patterns of P2Y receptors: $P2Y_2$ and $P2Y_6$ receptors have been identified.[11] Also, UTP has been shown to induce mucin secretion in middle ear epithelial cells.[11] However, little is known about the role of UTP in the ion and fluid transport in the

middle ear mucosa. Since abnormal fluid retention and mucin hypersecretion are the main pathologic processes, the effect of luminal UTP on Cl^- secretion may be important in understanding the pathology of OME.

The aim of this study was to examine the effect of UTP on Cl^- transport system to elucidate the possible role of CACC in cultured normal human middle ear epithelial (NHMEE) cells.

II. MATERIALS AND METHODS

1. Solutions and chemicals

The HCO_3^- -buffered NaCl solution contained (in mM): 120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 D-glucose, 5 HEPES, and 25 $NaHCO_3$ at pH 7.4. The HCO_3^-buffered solutions was continuously gassed with 95% O_2 and 5% CO_2 to maintain solution pH. The osmolarity of all solutions was adjusted to 310 mosmol/kg with the major salt prior to use. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 1. Function of chemicals.

Chemicals	**Function**	**Concentration**	**Reference**
Amiloride	ENaC inhibitor	100 μM	Gary & Palmer(1997)[20]
TEA (tetraethylammonium)	Apical K^+ channel blocker	5 mM	Rechkemmer & Halm(1989)[21]
UTP (uridine-5'-triphosphate)	P2 receptor agonist	100 uM	von Kugelgen & Wetter(2000)[22]
CFTRinh172	CFTR inhibitor	100 μM	Ma *et al*. (2002)[23]
BAPTA-AM(2-bis 2-aminophenoxy ethane-N,N,N',N'-tetraacetic acid -acetoxy methyl ester)	Ca^{2+}-chelating agent	50 uM	Dormer (1984)[24]
Niflumic acid	CACC inhibitor	100 μM	Gruber *et al*. (1998)[25]
Calphostin C	PKC inhibitor	0.1 uM	Chen *et al*. (2001)[26]

2. Cell culture

Primary cultures of NHMEE cells were prepared as described previously.[27] All procedures were approved by the Institutional Review Board of Yonsei Medical Center. Passage-2 NHMEE cells were plated on a collagen-coated semi-permeable membrane with a pore size of 0.45 um (Transwell-clear, Costar Co., Cambridge, MA) at a density of 1.0 x 10^4 cells/cm^2. The cells were maintained in a 1:1 mixture of bronchial epithelial growth medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and all supplements.[28] Cultures were grown submerged for the first 9 days, at which time, the air-liquid interface was created by removing the apical medium and feeding the cultures from the basal compartment only and further cultured for 7 to 10 day for complete differentiation. Confluence of passage-2 NHMEE cells (8-9 days after seeding) was verified by measurement of transepithelial resistance (R_t; > 1000Ω/cm^2 at room temperature) using endohm meter.

3. Ussing chamber Study

Passage-2 NHMEE cells were grown at an air-liquid interface (ALI) on Snapwell permeable supports with a surface areas of 1.13 cm^2 (Costar Co., Cambridge, MA, USA) for 4 additional days after confluence until they formed a tight epithelium. The cells were then mounted in modified Ussing chambers (World Precision Instruments, Sarasota, FL, USA). The epithelium was bathed on both sides with 5 ml of warmed (37°C) regular bicarbonate solution circulated by gas lifts with 95% O_2-5% CO_2. Solution pH was maintained at 7.4. The epithelial culture was voltage clamped with an automatic voltage clamp and the short-circuit current (I*sc*) was measured. A 15-min equilibration was achieved to stabilize the transepithelial current. Then, to measure ENaC-dependent current, amiloride (100 μM) was added to the apical bath. Data were acquired and analyzed with Acquire and Analysis (version 1.2) software.

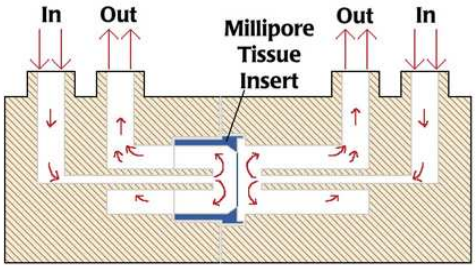


Figure 1. Ussing chamber to measure transepithelial Isc (short-circuit current)

4. RT-PCR

Gene-specific PCR primer set for hCLCA1 was designed to detect mRNA in cultured NHMEE cells (Table 1). Oligonucleotide amplimers for β2 microglobulin, which generated a 266 bp PCR fragment, were used as the control gene for RT-PCR Total RNAs were collected from cultured NHMEE cells using Trizol reagent according to user's manual (Gibco BRL, Rockville, MA, USA), and PCR was performed. RT-PCR

products were separated by electrophoresis on a 2% agarose gel containing 50 ng/ml ethidium bromide. Bands of the expected sizes were visualized under ultraviolet light and photographed with Polaroid Type 55 film. Negative controls were performed by omitting reverse transcriptase from the RT reactions to verify that the amplified products were from the mRNA and did not originate from genomic DNA contamination. No PCR products were observed in the absence of reverse transcriptase.

Table 2. PCR primer sequences specific to the target gene.

Primers	Sequences
hCLCA1	Sense: 5'- GCAAGGTGGCTTTGTAGTGG-3'
(471 bp)	Antisense: 5'- GGAATTTGCTGGTGTCCTTG-3'
β2M	Sense: 5'- CTCGCGCTACTCTCTCTTTCTGG -3'
(266 bp)	Antisense: 5'- GCTTACATCTCTCCATCCCACTTAA-3’

5. Immunocytochemistry

Cytospin slides were prepared. Immunostaining was performed with the horseradish-peroxidase labeled streptavidin-biotin technique (LSAB2, Dako, Germany) to assess hCLCA1 protein expression (anti-hCLCA1 antibody, Abnova, Taiwan). Dilution of antibody was 1:100.

6. Collection and preparation of middle ear effusion

To evaluate *in vivo* presence of ATP in the middle ear, middle ear effusions were collected after myringotomy in 6 patients with chronic otitis media with effusion undergoing ventilation tube insertion procedure. The samples were frozen immediately after removal at surgery and stored at -20°C.

7. Bioluminescence detection of ATP in middle ear effusion

ATP assay was performed according to previous studies.[29-30] Briefly, Standard curves of ATP (Sigma, St. Louis, MO, USA) at known concentrations were performed with 2 mg/ml luciferase-luciferin reagent in OptiMEM-I medium by serial dilution from a 0.5 M ATP stock (made fresh at the time of performing standard curves) to approximate the concentrations of ATP released from cells. The same mixture of luciferase-luciferin reagent was mixed in a 1:1 volume with the collected middle ear effusion aspirate, and luminescence was assayed. Luminescence was corrected for the total volume of the middle ear effusion, and the concentration of ATP in a given middle ear effusion sample was determined through comparison to the standard curve.

8. Statistical analysis

The results of at least four different experiments are presented as mean ± SD. Statistical analysis was performed by paired Student's *t*-test. A value of $p<0.05$ was considered statistically significant.

III. RESULTS

1. Apical UTP induced I*sc* (short-circuit current)

To examine the effect of apical UTP on ion transport in NHMEE cells, the short circuit current (I*sc*) was measured. The cells were pretreated: amiloride (100 uM) was applied to the apical side of the epithelia to block Na channels and tetraethylammonium (TEA, 5mM) was applied to the apical side of the epithelia to block apical K channels. Apical addition of UTP (100 uM) induced a significant and transient increase in I*sc* due to electrogenic Cl^- ion secretion. UTP-induced peak response was 3.92 ± 0.97 uA/cm^2) (**Fig.**

2A). The cells were exposed to different Cl^- channel inhibitors during plateau phase induced by UTP. CFTR-specific inhibitor CFTRinh172 (100 uM) reduced I*sc* by 44.6 ±8.3%, and CACC inhibitor niflumic acid (100 uM) reduced I*sc* by 46.7 ± 6.2 %. (**Fig. 2B&C**) Cotreatment with CFTRinh172 and niflumic acid reduced UTP-induced I*sc* to baseline. The findings suggest that the UTP increases Cl^- secretion through both CACC and CFTR in NHMEE cells.

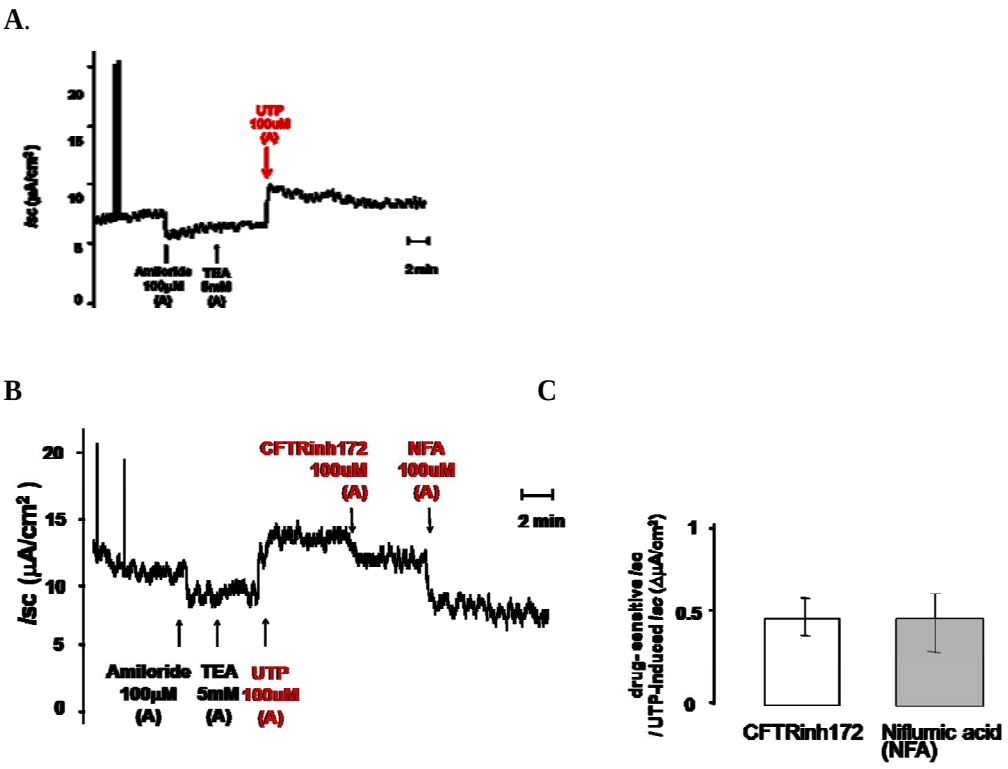


Figure 2. Apical UTP-induced Isc in NHMEE cells.

A. After pretreatment with amiloride (100uM) and tetraethylammonium (TEA, 100uM), apical addition of UTP (100uM) induced a significant increase of Isc.

B, C. UTP-induced I*sc* was inhibited by CFTRinh172 (100 uM) and niflumic acid (100uM). Drug-sensitive portion of UTP-induced currents are shown.

2. Effects of P2Y receptor antagonists on UTP-induced Isc

The cells were exposed to different P2Y receptor antagonists to confirm that UTP-induced Cl^- current was due to $P2Y_2$ receptor activation. Suramin acts as a strong antagonist to $P2Y_{1,2,11,12}$ and$_{13}$, and a much weaker antagonist to $P2Y_4$ and $P2Y_6$. When UTP was added to the apical solution, UTP-induced Isc was significantly decreased to 0.8±0.11 uA/cm^2 (**Fig. 3A&C**). However, the UTP-induced Isc was relatively unchanged after treatment with PPADS, which acts strongly on P2X and $P2Y_1$ and only weakly on $P2Y_2$, $P2Y_{11}$ and $P2Y_1$ **(Fig. 3B&C)**. The findings suggest that UTP activates $P2Y_2$ receptors.

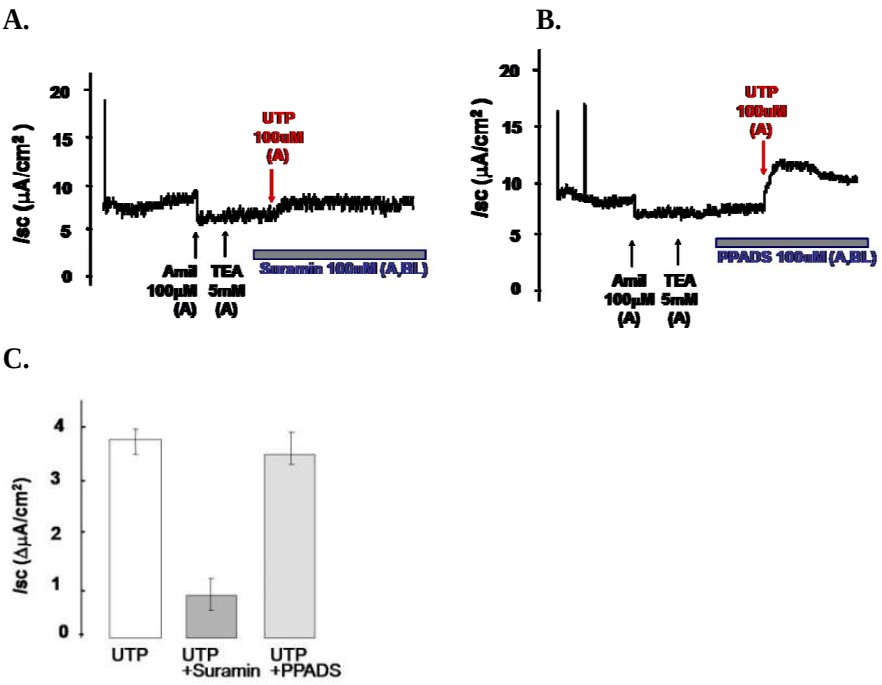
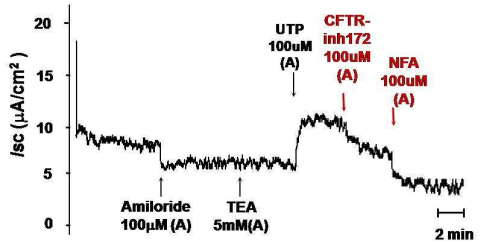


Figure 3. Effects of P2Y receptor antagonists on UTP-induced I*sc*

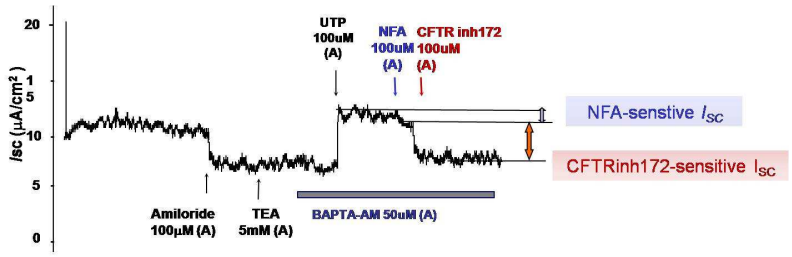
UTP-induced current was significantly decreased in presence of suramin (**A**), but remained unchanged in presence of PPADS (**B**). UTP-induced I*sc* in various conditions are shown in **C**.

3. Effect of Ca^{2+}-chelating agent on UTP-induced Isc

To examine whether the UTP-induced Cl^- current is dependent on intracellular Ca^{2+} concentration, the cells were pretreated with BAPTA-AM, a Ca^{2+} -chelating agent. UTP –induced current was decreased to 2.87 ±0.42 uA/cm^2. As expected CFTRinh172-sensitive portion of UTP-induced current remained relatively unchanged and niflumic acid-sensitive portion was significantly decreased (**Fig. 4**).



A.



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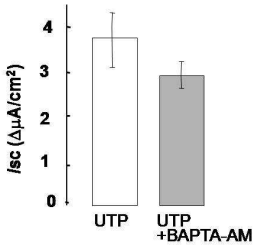

C.

Figure 4. Effect of Ca^{2+}-chelating agent on UTP-induced I*sc*

Representative tracings of Isc in conditions without and with BAPTA-AM pretreatment (**A**, **B**, respectively). UTP-induced Cl^- current was decreased when the cells were pretreated with a Ca^{2+}-chelating agent BAPTA-AM(**C**).

4. Role of PKC in UTP-induced Isc

Next, the cells were exposed to a PKC inhibitor calphostin C. UTP-induced Isc was decreased by about 50%, suggesting a role of PKC in the signal pathway of UTP activation (**Fig. 5**).

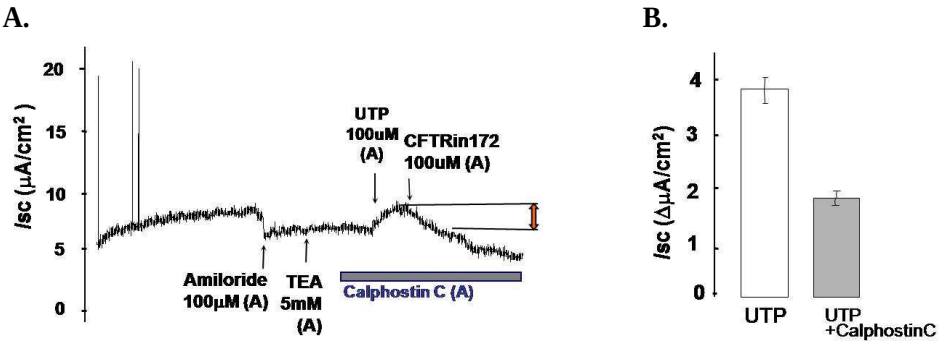

Figure 5. Role of PKC in UTP-induced I*sc*

A. Representative tracing of UTP-induced I*sc* in the cells exposed to PKC inhibitor. **B**. UTP-induced current was decreased in the presence of calphostin C (n=2)

5. Expression of hCLCA1 in NHMEE cells

Human CLCA1 (hCLCA1) protein is a member of a novel family of proteins that mediates of Ca^{2+}-activated conductance.[15,25] The molecular expression of hCLCA1 mRNA was confirmed by RT-PCR using a specific primer (**Fig. 6A**). Immunostaining with anti-hCLCA1 antibody was performed. Positively staining cells were scarce, but

when the cells were treated with IL-1β (10nM, 48 h), positive cells were increased (**Fig. 6A&B**), as expected when considering the current understanding of the role of hCLCA1 in mucin hypersecretion in inflammatory conditions.

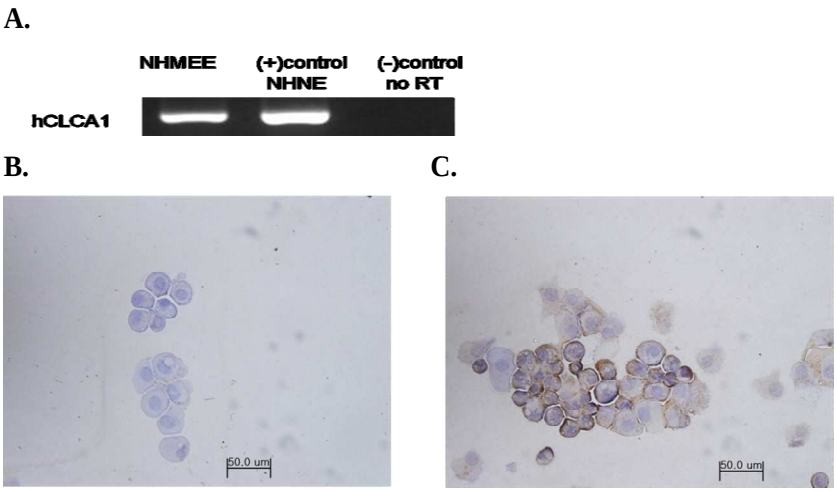

Figure 6. Expression of hCLCA1 in NHMEE cells.
A. Expression of mRNA of hCLCA1 in NHMEE cells were confirmed by RT-PCR. Cultured normal human nasal epithelial (NHNE) cells were used as the positive control. **B & C.** Immunostaining with anti-hCLCA1 antibody in cultured NHMEE cells was increased after treatment with IL-1β (10nM, 48 h) **(C)** compared to control **(B).**

6. Identification of ATP in middle ear effusion

One of the limitations of the Ussing chamber experiments is that UTP was added to the apical bathing solution to simulate the local effects of the purine agonist in the middle ear mucosa. To be able to extrapolate the physiological importance of our study in the clinical situation, the question whether extracellular nucleotides are present in adequate amounts in the ASL in the middle ear mucosa needs to be addressed. However, it is technically difficult to assess UTP and we investigated whether another nucleotide ATP is present the

middle ear in patients with chronic otitis media with effusion. The middle ear mucosa is normally covered with a thin layer of airway surface liquid which is difficult to collect for ATP assay. Therefore we collected middle ear effusions from 6 patients and bioluminescence assay of ATP was performed. ATP was detected in the middle ear aspirates in all six patients (**Fig. 7**). The concentrations varied over a wide range (mean 853.67 nM, range 97 pM – 3.75 nM). Our preceding experiments showed that an outward chloride currents were induced by both UTP and ATP in cultured NHMEE cells (data not shown).

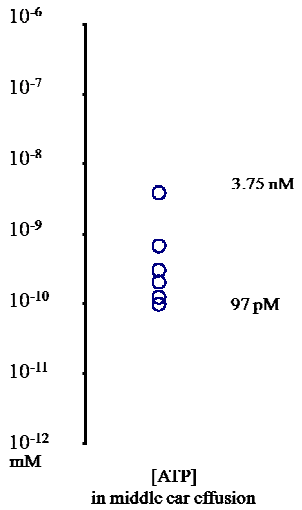

Figure 7. A middle ear effusion luminometry scatterplot of [ATP] detected in middle ear effusions.
Bioluminescence assay of ATP confirmed the presence of ATP in variable concentrations in middle ear effusions (mean 853.67 nM, range 97 pM – 3.75 nM).

IV. DISCUSSION

Purinergic receptors play a major role in regulating epithelial electrolyte and fluid transport, prerequisite for mucocilirary clearance and host defense.[26, 31-32] Major functions comprise upregulation of Cl^- and mucin secretion and ciliary motility.[34-36] $P2Y_2$ receptors are the dominant purinergic receptors in the airway. [33] Extracellular purinergic agonists ATP and UTP play significant regulatory roles in various biological responses by activating $P2Y_2$ receptors. Stimulation of $P2Y_2$ receptors in airway cells activates Ca^{2+} dependent Cl^- secretion and inhibits Na^+ absorption by the epithelial sodium channel ENaC. [37-38] As a part of respiratory tract, the middle ear mucosa also express $P2Y_2$ receptors[8]. UTP has been shown to upregulated mucin secretion via Ca^{2+} -dependent pathway in the middle ear mucosa.[8] The present study addresses the question whether UTP influences ion transport responses as well as intracellular Ca^{2+} signaling in the middle ear mucosa.

The experiments were designed to focuse on electrogenic Cl^- ion transport in the middle ear epithelium. The cultured cell monolayers were pretreated with amiloride and TEA in the apical solution to block Na^+ and K^+ channels. Purinergic agonists such as ATP and UTP are known to exert inhibitory effects on Na absorption in polarized epithelium by blocking epithelial sodium channels. Also, K^+ efflux through apical K^+ channels contributes to hyperpolarization of the cell potential, shifting towards Cl^- secretion. By blocking apical Na^+ and K^+ channels, the electrophysiological measurements reflect the isolated effect of UTP on chloride secretion across the middle ear epithelial cells. As shown in Figure 2, UTP induced a significant and transient increase in I*sc* attributed to an outward chloride current. Using specific inhibitors of different Cl^- channels, the suramin inhibitable UTP-induced current can be explained as CFTR- and CACC-dependent. Treatment with both CFTRinh172 and niflumic acid (a selective inhibitor of CACC) nearly abolished UTP-induced Cl^- current. As expected, the niflumic acid-sensitive portion of UTP-induced current was dependent on $[Ca^{2+}]i$, while the CFTRin172-sensitive portion remained unchanged. Present results suggest the CFTR-dependent portion of UTP-induced Cl^- current is partly sensitive to PKC inhibition. In various tissues, involvement of cAMP-dependent protein kinase (PKA) and PKC pathways in CFTR

regulation has been studied. Phosphorylation of the CFTR channel by the PKA, regulates CFTR, and Ca^{2+}-dependent and Ca^{2+}-independent isoforms of PKC activate a recombinant CFTR Cl^- channel.[13] Synergistic modulation of CFTR activity by both PKA and PKC has been identified in a heterologous expression system.[39] P2-receptor activation has been reported to stimulate both Ca^{2+}-dependent Cl^- channels and Ca^{2+}-insensitive, CFTR-like Cl^- channels in rat submandibular gland.[39-40] Further experiments are needed to understand the mechanism by which UTP stimulation of $P2Y_2$ receptor might modulate CFTR activity in the middle ear epithelia.[41]

The sources for extracellular nucleotides such as ATP and UTP are considered as the airway epithelial cells themselves. Respiratory epithelial cells release ATP and UTP both apically and basolaterally under basal conditions, and various stimuli induce acute increases in nucleotide release.[31,42] Particularly, membrane stretch which occurs during coughing leads to a transient accumulation of nucleotides within the thin airway surface liquid, which reaches sufficiently high concentrations locally liquid.[43] A similar scenario can be postulated in the middle ear mucosa. Although the presence of extracellular nucleotides in the middle ear cavity could not be identified in healthy ears, ATP was detected in significant concentrations in the middle ear effusion aspirates collected from all six patients with otitis media with effusion. Since disruption of ion/fluid transport leading to abnormal retention of fluid and inflammatory exudates contribute to the pathogenesis of otitis media with effusion, it can be presumed that nucleotides are involved in regulating epithelial responses crucial to maintaining effective mucociliary clearance. ATP concentrations in the middle ear effusion varied over a wide range. Different characteristics of the effusion samples collected may correlate with the relative abundance or lack of ATP detected, although no definite observation could be made due to the small number of samples. Retained effusion in the middle ear cavity has variable viscosity due to variable mucin and serous composition, which may also change over time during the disease course. ATP release can be expected to vary depending on the cellular response to various inflammatory stimuli.

Interestingly, CACC has been implicated in goblet cell hyperplasia and mucin hypersecretion in tracheobronchial epithelium. Abnormally increased mucin content in

the airway surface liquid can critically impair mucociliary clearance just as imbalance in the aqueous component. Overexpression of CLCA in asthmatic airways correlates with mucin hypersecretion due to increased mucin gene expression, mucin granule release in response to external stimuli and goblet cell hyperplasia. Consistently, the CACC inhibitor niflumic acid has been shown to be able to reduce mucin hypersecretion induced in by various inflammatory cytokines, extracellular nucleotides and cigarette smoke in airway epithelia. Mucin hypersecretion is considered one of the main mechanisms in the pathogenesis of chronic otitis media with effusion. The disease is characterized histologically by mucous hyperplasia of the middle ear mucosa as well as abnormally retained effusion.[44] Identification of hCLCA1 protein expression in inflammatory cytokine treated conditions in cultured NHMEE cells suggest that CACC may also be involved in the mucin secretion in the middle ear epithelia and it can be speculated that the inhibition of CACC may suppress mucin secretion and ion transport to interrupt the development of middle ear effusion. Further studies are required to clarify the relationship of CACC and mucin secretion in the middle ear mucosa.

V. CONCLUSION

In summary, the findings of the present investigation include: (1) a demonstration of that apical stimulation by UTP activates a Cl- current (I_{SC}), (2) a partial inhibition of UTP-induced I_{SC} by CFTRinh172, a partial inhibition of UTP-induced I_{SC} by niflumic acid, (3) an inhibition of UTP-induced I_{SC} by suramin, but not by PPADS, and (4) an inhibition of UTP-induced I_{SC} by PKC inhibitors. These results are believed to suggest that UTP-induced I_{SC} is due to the activation of CFTR and CACC via $P2Y_2$ receptor linked to PKC signaling pathway in cultured NHMEE cells.

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< ABSTRACT (IN KOREAN)>

사람중이점막세포에서 UTP 자극에 의한 CFTR과 Ca^{2+}-activated chloride channel을 통한 Chloride 분비 활성화

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목적: 중이점막에서 이온 채널은 수분의 흡수와 분비에 결정적인 역할을 한다. P2Y 퓨린수용체에 세포외 핵산이 결합하여 수분과 이온의 이동을 조절하는데 관여한다. 본 연구에서는 배양된 사람 중이점막상피세포에서 $P2Y_2$ 수용체 작용제인 uridine-5'-triphosphate (UTP)가 Chloride (Cl^-) 이온의 분비를 촉진시키는 기전에 대해 알아보고자 한다. **재료 및 방법**: 사람 중이점막상피세포를 채취하여 계대 배양하여 air-liquid interface에서 분화를 유도한 후, 경상피 이온 이동을 확인하기 위해 Ussing chamber에서 voltage clamp상태에서 단락 전류 (short-circuit current, I*sc*)를 측정하였다. Ca^{2+}-activated chloride channels (CACC) 의 발현을 확인하기 위해 RT-PCR을 시행하였다. **결과**: 사람 중이점막상피세포에서 amiloride를 처치한 후, 첨부 측 관류액에 UTP를 첨가하였을 경우 Cl^- 분비에 의해 I*sc* 가 일시적으로 급상승하여 지속되는 반응을 확인할 수 있었다. Cl^- 채널 길항제를 첨부 측 관류액에 추가하였을 경우 UTP에 의한 I*sc*가 억제되었다. CACC의 억제제로 알려진 niflumic acid와 CFTR의 선택적 억제제인 CFTRinh-172를 처치한 경우 각각 UTP에 의해 유도된 Isc가 부분적으로 억제되었다. 칼슘 킬레이트 제제인 BAPTA-AM 를 첨가할 경우 UTP에 의한 CACC 활성화가 억제되었고, PKC를 억제하면 Cl^- 분비에 의한 I*sc*가 억제되었다. CACC mRNA 발현을 확인할 수 있었다. **결론**: 기도 점막에서 Cl^- 이온의 이동은 수분 의 이동 조절에 중요하며, $P2Y_2$ 수용체 자극시 anion 분비가 촉진되는 것이 알려져 있다. 본 연구에서 사람 중이점

막상피세포에서 $P2Y_2$ 수용체 작용제인 UTP에 의해 Cl^- 이온 분비가 촉진되며, 이는 CACC와 CFTR에 의한 부분이 모두 증가되는 것으로 이해된다. 또한 UTP에 의해 유도된 Cl^- 이온 분비가 PKC와 연관되어 있다는 것을 알 수 있었다.

핵심되는 말 : 중이점막, UTP, Chloride 분비, cystic fibrosis transmembrane conductance regulator, Ca^{2+}-activated chloride channels,