Effect of Interleukin-1 β on epithelial sodium channel (ENaC) and fluid absorption in normal human middle ear epithelial cells

Yoon-Seok Choi

Department of Medicine

The Graduate School, Yonsei University

Effect of Interleukin-1β on epithelial sodium channel (ENaC) and fluid absorption in normal human middle ear epithelial cells

Directed by Professor Jeung-Gweon Lee

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Yoon-Seok Choi

December 2005

This certifies that the Doctoral Dissertation of Yoon-Seok Choi is approved.

Thesis Supervisor : Jeung-Gweon Lee

Joo-Heon Yoon

Man-Wook Hur

Min Goo Lee

Je-Kyeong Sung

The Graduate School
Yonsei University

December 2005

감사의 글

이제 새로운 첫 걸음을 내딛으려 합니다. 이 한 걸음을 위하여 지내왔던 순간순간들이 바로 어제 있었던 일들처럼 떠오릅니다. 그 중에서도 정신없던 전공의 시절, 처음으로 대학원에 입학하던 때가 가장 소중하게 느껴지는 건, 아마도 새로운 시작에 대한 설레임과 두려움 그리고, 미약하나마 진리탐구의 길을 계속 걸을 수 있다는 가벼운 흥분이 지금 대학원을 마치고 새로운 길을 가려는 현재의 제 모습과 너무나도 비슷했기 때문은 아닌가 합니다. 그 소중했던 첫 순간부터 언제나 곁에서 지켜봐 주시고 끊임없는 격려와 질책으로 지금까지 저를 이끌어 주신 수많은 분들께 이 글을 통해 감사의 마음을 조금이라도 전하고자 합니다.

먼저 지도교수님이신 이정권 선생님께 감사드립니다. 석사학위 때부터 저의 지도교수님을 맡아주시면서 제가 해이해 지려고 할 때마다 해주셨던 학문과 삶에 대한 선생님의 진심 어린 말씀 항상 잊지 않고 최선을 다해 살아가겠습니다.

윤주헌 선생님께 감사드립니다. 때로는 엄한 스승으로 때로는 따뜻한 아버지와 형님의 모습으로 학문하는 방법과 무엇이 값진 인생인가를 처음으로 가르치고 이끌어 주신 선생님의 은혜는 영원히 잊을 수 없을 것 같습니다. 제 인생의 지침이 된 선생님의 가르침 저도 누군가에게 전해줄 수 있는 사람이 되도록 하겠습니다.

허만욱 선생님과 이민구 선생님께 감사드립니다. 진정한 학자의 모습을 보여주신 선생님들이시며 연구와 강의로 바쁘신 중에도 저의 학위 논문의 심사를 흔쾌히 맡아 주시고 아낌없는 조언을 해주신 선생님들께 진심으로 감사드립니다.

서울대학교 성제경 선생님께 감사드립니다. 전공의 때 처음 봤던 진리탐구에 대한 선생님의 열정은 아무것도 모르던 저에게는 너무도 소중한

경험이었고 지금도 제 마음속에 남아있습니다. 선생님의 앞길에 큰 성과가 있으시길 기원하겠습니다.

김창훈 선배님과 최재영 선배님께도 감사드립니다. 지난 10여 년간 함께 울고 웃으며 무지한 후배를 이끌고 가르쳐 주신 선배님들의 사랑에 감사의 뜻을 전합니다.

오랜 친구인 진구와 종우에게 고마움을 전합니다. 이들의 따뜻한 관심과 애정은 힘들고 지칠 때마다 저에게는 큰 쉼터가 되어 주었습니다.

제게 생명 주신 하나님께 감사드리며 저를 믿고 격려해 주시는 부모님, 이제는 하늘에서 지켜보시는 어머님, 멀리 떨어진 곳에 살면서도 저를 항상 지지해주는 누님과 동생에게도 존경의 마음을 전합니다.

마지막으로 제 삶의 원동력이며 기쁠 때나 슬플 때나 항상 같이하며 좋은 친구이자 연인이 되어주는 아들 현민과 아내 진아에게도 마음속 깊은 고마움을 전합니다.

저자 씀

Table of contents

List of figures

Abstract

Effect of interleukin-1 β on epithelial sodium channel and fluid absorption in normal human middle ear epithelial cells

Yoon-Seok Choi

Department of Medicine

The Graduate School, Yonsei University

(*Directed by Professor* Jeung-*G*weon Lee)

Liquid secretion and absorption by airway epithelia are driven by active transport of ions across the epithelial barrier via ion channels. The middle ear epithelium, an extension of airway epithelia, has been found to be able to absorb and secrete electrolytes by various ion channels. An amiloride-sensitive epithelial sodium channel (ENaC) is the rate-limiting step for Na^+ transport in airway epithelial cells. Recently, accumulating evidences showed that cytokines inhibit fluid absorption by suppression of Na^+ channel in airway epithelial cells. Interleukin (IL)-1ß is one of the important cytokines in the inflammatory process

and increased levels of IL-1ß have been reported in the effusion of otitis.

In this study, we tried to investigate the role of the ENaC in fluid absorption in normal human middle ear epithelial (NHMEE) cells, the effects of IL-1ß on ENaC expression and the underlying signal pathway.

We measured remained volume after application of 100 μl of fluid to the luminal side of NHMEE cells. Amiloride-sensitive short-circuit current, was measured with/without IL-1ß and various inhibitors treatment using a Ussing chamber.

Addition of amiloride, a potent ENaC blocker, in luminal membrane of NHMEE cells decreased the fluid absorption in a dose-dependent manner. At 24 hrs after treatment of 100 μM of amiloride, 86± 2.3 μl of fluid remained. Transepithelial short circuit current (*I*te) decreased with IL-1ß treatment. Treatment for 48 hrs with 10 ng/ml IL-1ß reduced the *I*te from 14.8± 1.9 $\mu A/cm^2$ to 9.8± 0.9 $\mu A/cm^2$. However, when the NHMEE cells were pretreated with PLC inhibitor (U73122, 10 μM), PKC inhibitor (Calphostin C, 10 μM) and ERK inhibitor (PD98059, 10 μM) respectively, the amount of amiloride sensitive current was reversed to the control level. But both p38 and JNKs inhibitors did not affect.

These results suggest that ENaC has an important role in fluid absorption in NHMEE cells and IL-1ß suppresses ENaC-dependent fluid transport and PLC-PKC-ERK pathway are involved in IL-1ß induced ENaC suppression.

Key words: epithelial sodium channel, interleukin 1 beta, signal pathway

Effect of interleukin-1β on epithelial sodium channel and fluid absorption in normal human middle ear epithelial cells

Yoon-Seok Choi

Department of Medicine
The Graduate School, Yonsei University

(*Directed by Professor Jeung-Gweon Lee*)

I. INTRODUCTION

The physiology of the middle ear is primarily concerned with keeping the middle ear cavity air filled and fluid free, to allow transmission of the sound vibration from the ear drum to the inner ear. Middle ear epithelial cells are thought to play a key role in this process.[1] They actively absorb water to clear any fluid present in excess. The origin of the periciliary fluid is not known but has been proposed to originate via transepithelial osmosis as governed by mucosal ion transport mechanisms.[2]

Liquid secretion and absorption by airway epithelia are driven by active transport of ions across the epithelial barrier via ion channels.[3,4] In particular, sodium absorption by an amiloride-sensitive channel is the main driving force of lung liquid clearance at birth and lung edema clearance in adulthood. An amiloride-sensitive epithelial sodium channel (ENaC) is the rate-limiting step for Na^+ transport in airway epithelia.[5,6] This channel constitutes the main pathway for active Na^+ flow and periciliary fluid absorption. The middle ear epithelium, an extension of airway epithelia, has been found to be able to absorb and secrete electrolytes by various ion channels. Previous electrophysiological investigations have shown that amiloride blocks the short-circuit current in a middle ear cell line and gerbil epithelium.[1,7, 8]

Recently, accumulating evidences showed that cytokines inhibit fluid absorption by suppression of Na^+ channel in airway epithelial cells.[2,9] Interleukin (IL)-1ß is one of the important cytokines in the inflammatory process,[10] and increased levels of IL-1ß have been reported in the effusion of otitis.[11] Since the activity of ENaC is critical for Na^+-dependent fluid absorption, we hypothesized that IL-1ß may modulate ENaC in middle ear epithelial cells.

This study first examined the role of the ENaC in fluid absorption in normal human middle ear epithelial (NHMEE) cells. Secondly, the effects of IL-1ß on ENaC expression and function in middle ear epithelial cells were investigated. Thirdly, we tried to elucidate the underlying signal pathway of ENaC suppression by IL-1ß.

II. MATERIALS & METHODS

1. Chemicals and Solutions

All chemicals, including amiloride and interleukin, were acquired from Sigma-Aldrich (St. Louis, MO). 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. All HCO_3^--buffered solutions were 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.

2. Cell culture

Primary cultures of NHMEE cells were prepared as described previously by Yoon et al.[12,13] 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 μm Transwell-clear culture inserts, (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.[13]

3. RT-PCR

Total RNA was isolated from NHMEE cells using TRIzol (Invitrogen) and cDNA was synthesized with random hexamers (Perkin Elmer, Roche, Branchburg, NJ) using MMLV reverse transcriptase (RT; Perkin Elmer Life Sciences). Oligo-nucleotide primers for PCR were designed based on the Genebank ™ sequence of ENaC α subunit (5'primer GAGCCCATACCAGGTCTCAT; 3 ' primer ATGGTGGTGTTGTTGCAGAA), β subunit (5' primer GGGGTACTCGTGGATAA GCTT; 3' primer GAGACAAGACGTGGAAAATCC), γ subunit (5' primer ACCACCAGCCA TGGTCTAAG; 3' primer GTTCAGGTCCCGGATTTAT). The polymerase chain reaction (PCR) conditions comprised 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C (β ; 57°C) for 30 sec and polymerization at 72°C for 30 sec. The PCR products obtained were run on a 1.5% agarose gel and visualized with ethidium bromide under a transilluminator. To verify that the amplified products were from mRNA and did not result from genomic DNA contamination, negative control were produced by omitting RT from the RT-PCR. In the absence of RT, no PCR products were observed.

4. Western blot analysis

Cells were lysed with 2 x lysis buffer [250 mM Tris-HCL, pH 6.5, 2% sodium dodecyl sulfate (SDS), 4% β-mercaptoethanol, 0.02% bromphenol blue, 10% glycerol] . Equal amounts of whole cell lysates were resolved using 10% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Milipore, Bedford, MA). Membranes were blocked with 5% skimmed milk in Tris-buffered saline (TBS; 50mM Tris-HCL, pH 7.5, 150mM NaCl) for 2hrs at room temperature. This blot was

then incubated overnight with primary antibody in 0.5% Tween 20 in TBS (TTBS). After washing with TTBS, the blot was further incubated for 45 min at room temperature with anti-rabbit or anti-mouse antibody (Cell Signaling) in TTBS, and visualized using the enhanced chemiluminescence system (Amersham Biosciences).

5. Measurement of fluid absorption capacity

To evaluate the fluid transport activity across the NHMEE cells, we measured remained volume after application of 100 μl of fluid to the luminal side.[14] Briefly, NHMEE cells were cultured in a 12 well-sized Costar Transwell insert for at least 35 days as previously described (37°C, 5% CO_2 in a humidified atmosphere). The luminal surface at air-liquid interface of fully differentiated NHMEE cells was washed with PBS three times and the surface liquid was completely aspirated. Then, 100 μl of Krebs bicarbonate Ringer (KBR) solution containing 2% blue dextran (BD), a cell-impermeable fluid volume marker dye, was added. After 1, 4, 8, 12, 24 and 48 hrs incubations in the humidified chamber, microaliquots (2~5μl) of luminal liquid were sampled. BD concentration was measured optically and remaining fluid volume was calculated as previously described.[14]

6. Measurement of Bioelectric Properties of NHMEE cells

Primary, NHMEE cells grown at ALI on Snapwell (1.13 cm2 surface area) permeable supports with surface areas of 1.13 cm^2 (Costar Co., Cambridge,

MA) for 4 additional days after confluence until they formed a tight epithelium were mounted in modified Ussing chambers (World Precision Instruments, Sarasota, FL). 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 and 5% CO_2. Solution pH was maintained at 7.4. The epithelial culture was voltage clamped and short-circuit current (*I*sc), and transepithelial resistance (*R*te) were measured with an automatic voltage clamp (Physiological Instruments). After a 15 min equilibration, amiloride (100 μ*M*) was added to the luminal bath. Data were acquired and analyzed with Acquire and Analysis (version 1.2) software.

7. Statistical Analysis

The results of multiple experiments are presented as means ± S.E. Statistical analysis was carried out by analysis of variance or Student's *t* test as appropriate. *P* value < 0.05 was considered statistically significant.

III. RESULTS

1. Effect of IL-1β on ENaC gene expression in NHMEE cells

To investigate whether IL-1β affect ENaC gene expression in NHMEE cells, we carried out RT-PCR after treating IL-1β for 24hrs. The results showed that the levels of mRNA of ENaC subunit γ didn't changed, that of α decreased slightly by 16%, and that of β decreased significantly by about 41% (Fig. 1A and B).

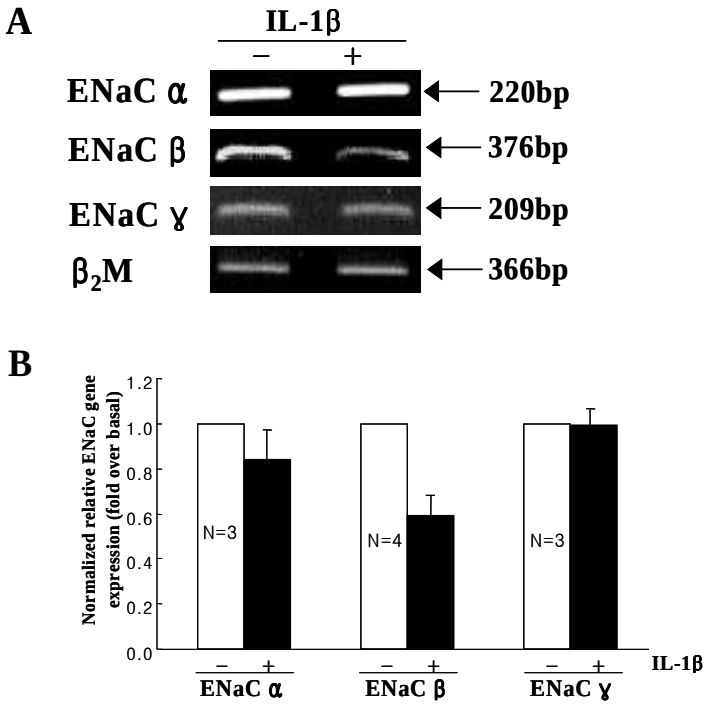

Fig. 1. ENaC mRNA expression in noramal human middle ear epithelial (NHMEE) cells. All the gene expressions of three ENaC subunits, α, β and γ, expressed significantly (A). After treating of IL-1β for 24 hrs, ENaC subunit β mRNA expression decreased significantly (A, B).

As a next step, to elucidate whether IL-1β induced ENaC β suppression occurs in protein level, we carried out Western blot analysis. ENaC β protein activation decreased by about 52% (Fig. 2A and B).

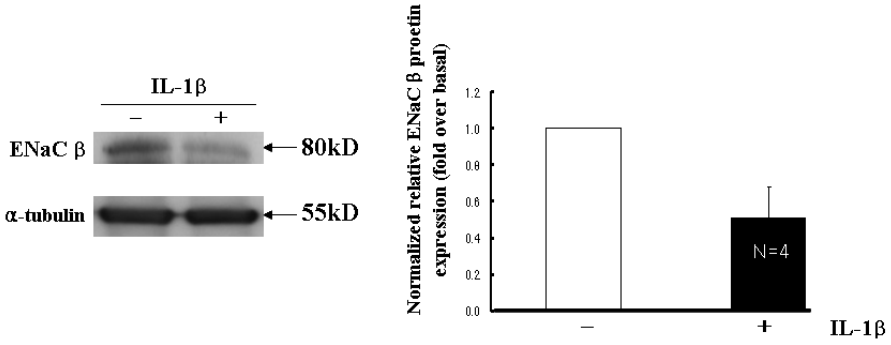

Fig. 2. Western blot analysis of ENaC subunit β. Activation of ENaC subunits β decreased significantly after treating of IL-1β for 24 hrs (A, B).

2. Role of ENaC in the fluid absorption

In order to identify the physiologic role of ENaC in the fluid absorption in NHMEE cells, we next measured fluid transport across the NHMEE cells. When 100 μl of KBR solution (0.1% DMSO) was applied on the luminal surface, cells absorbed the fluids with time and 65.8± 1.9 μl of fluids are remained at 24 h after the application. Interestingly, addition of amiloride in luminal membrane of NHMEE cells decreased the fluid absorption in a dose-dependent manner. At 24 h after treatment of 100 μM of amiloride, 86± 2.3 μl of fluid remained (Fig. 3). These results indicate that ENaC has an important role in fluid absorption in NHMEE cells.

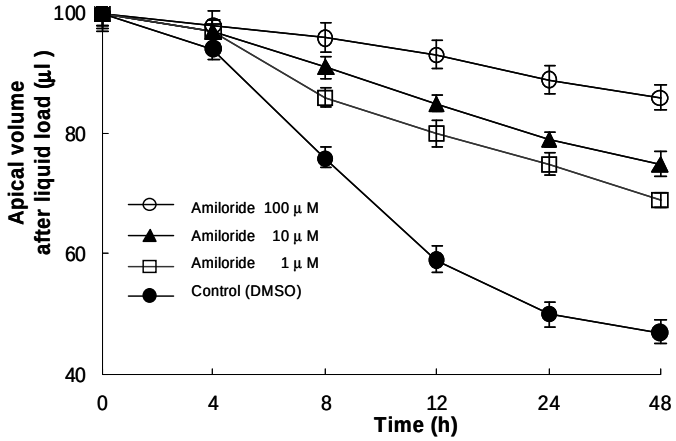

Fig. 3. Role of ENaC in fluid transport. The fluid absorption rate was measured after application of 100 µl of KRB solution on the luminal surface of NHMEE cells with/without amiloride. The fluid absorption rate was reduced by the amiloride in a dose-dependent manner.

3. Effect of IL-1β on transepithelial resistance and basal current

The bioelectric properties of NHMEE cells were tested under control conditions and IL-1β treatment. There were no significant differences in *R*te between control and IL-1β treated cells (control; 1670± 83 Ω), 48 h IL-1β treated; 1609± 69 Ω). However, transepithelial short circuit current decreased with IL-1β treatment. In unstimulated cells, an *I*te of 14.8± 1.9 µA/cm^2 was detected across the monolayer. Treatment for 48 h with 10 ng/ml IL-1β decreased the *I*te to 9.8± 0.9 µA/ cm^2 (Fig. 4).

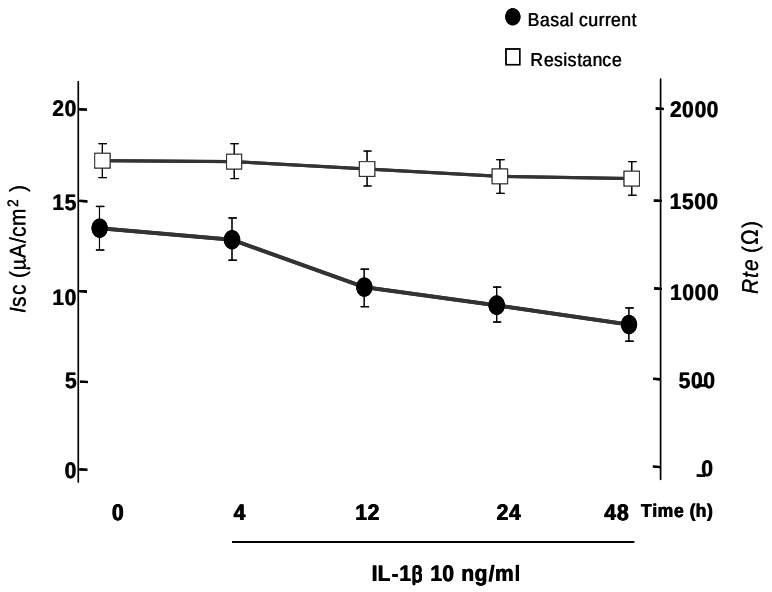

Fig. 4. Effect of IL-1β on transepithelial basal current and resistance. Transepithelial short circuit current (*Isc*) decreased with IL-1β treatment, but on the other hand resistance (*Rte*) between control and IL-1β treated cells didn't change.

4. Effect of IL-1β on ENaC function

In order to evaluate the effect of IL-1β on ENaC function, we measured amiloride sensitive short-circuit current in NHMEE cells. Figures 5B and A show typical *I*sc traces for determination of ENaC in NHMEE cells treated or not with IL-1β for 24 h, respectively. The luminal addition of amiloride (100 μ M) produced a rapid decrease of current due to the blockage of ENaC. The mean amplitude of the amiloride-sensitive current, which reflects ENaC activity, was 15.2± 1.3 μA/cm^2 in NHMEE cells, which decreased to 11.3± 1.4

μA/cm^2 after IL-1β treatment (Fig 5C).

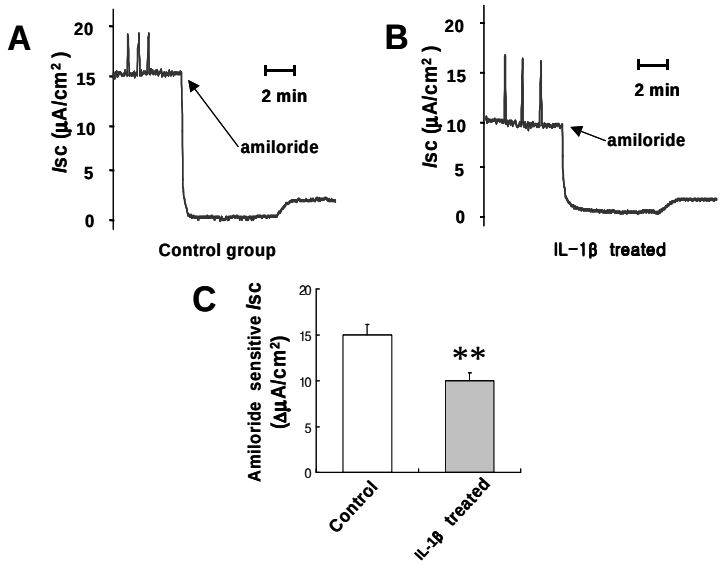

Fig. 5. Effect of IL-1β on amiloride sensitive short circuit current (Isc). This graph is a result from six different experiments. There is a high amplitute of amiloride sensitive current before IL-1β treatment (A). This current decreased significantly after 12 h IL-1 β treatment (B). Amiloride sensitive current decreased by about 30% after IL-1β treatment (C).

5. Inhibition of ENaC-mediated fluid absorption by IL-1β treatment

In order to identify the physiologic impact of IL-1β induced ENaC downregulation, we next measured fluid absorption across the NHMEE cells. In resting state, middle ear epithelia absorb fluids and 54.7± 1.4 μl of fluids are remained at 24 h after the application. When the cells were pretreated with IL-1

β luminally for 12 h (10 ng/ml), the transepithelial fluid absorption was significantly reduced and more fluid (66.8± 1.6 μl) remained at 24 h after the fluid application in the luminal cell surface (Fig. 6A).

Treatment of amiloride (100 μM) suppressed fluid absorption in both control and IL-1β treated sample. Interestingly, amiloride-sensitive fluid transport at 24 h after the fluid application decreased from 32.1± 1.8 μl to 23.1± 2.1 μl by IL-1 β treatment (Fig. 6B). These results indicate that IL-1β suppresses ENaC-dependent fluid transport in NHMEE cells.

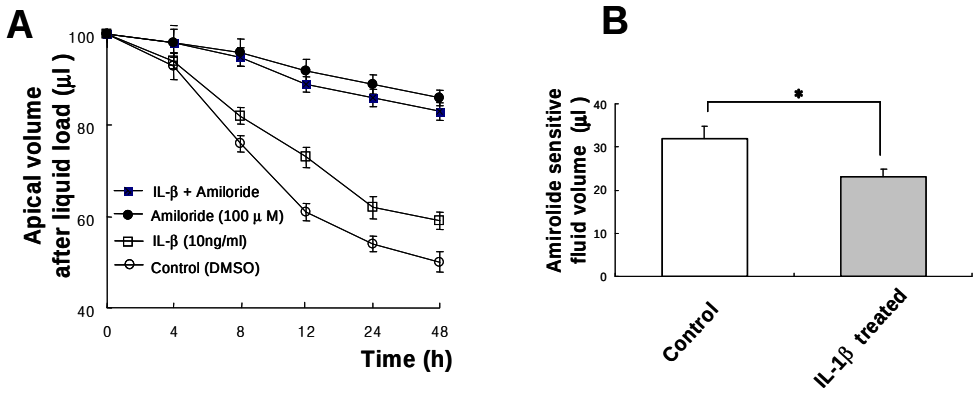

Fig. 6. Effect of IL-1β on fluid absorption by ENaC. In resting state, IL-1β*treatment decreased fluid absorption capacity about* 10% (A). Treatment of amiloride (100 μM) suppressed fluid absorption in both control and IL-1β treated sample. Interestingly, amiloride-sensitive fluid transport decreased by IL-1β treatment (B). (* $p<0.05$)

6. Inhibitor Studies of signaling pathways involved in IL-1β induced ENaC suppression

To determine which signaling pathway is involved in IL-1ß induced ENaC

suppression, various inhibitors were used in this study. The first task was to determine whether the phospholipase (PLC) and protein kinase (PKC) are involved. When the cells were pretreated with PLC inhibitor, U73122 (10 μM) and PKC inhibitor, calphostin C (10 μM) respectively, the amount of amiloride sensitive current was reversed to the control level. We also evaluated which MAP kinase is involved downstream. Inhibition of ERK by PD98059 reversed the amiloride sensitive current to control level, but both p38 and JNKs inhibitors did not affect. Our data indicated that PLC-PKC-ERK pathways are probably involved in IL-1ß induced ENaC suppression (Fig. 7).

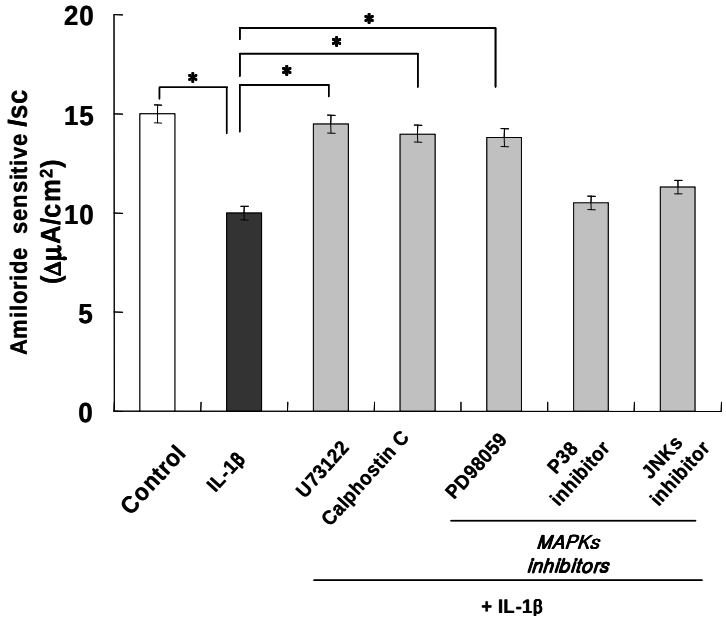

Fig. 7. Inhibitor studies to determine which signaling pathway is involved in IL-1β induced ENaC supression. Only when the cells were pretreated with PLC inhibitor, U73122 (10 μM), PKC inhibitor, calphostin (10 μM) and ERK inhibitor, PD98059 (10 μM), respectively, the amount of amiloride sensitive current was reversed to the control level. ($* p<0.05$)

IV. DISCUSSION

The amiloride-sensitive epithelial sodium channel (ENaC) is important for transepithelial liquid movement in various tissue.[15] The channel consists of 3 subunits, α, β and γ, of which the α subunitis prerequisite for appreciable channel function but β and γ play an important role also.[16] Experiments in several animal models indicate that the function of ENaC is vital for lung fluid clearance at birth. Instillation of amiloride, a Na^+ channel blocker, into the trachea of newborn guinea pigs results in impediment of lung liquid absorption. Amiloride-sensitive apical Na^+ channels have been demonstrated to be the major determinant of baseline electrogenic ion transport in Mongolian gerbil middle ear epithelium.[17,18] Since the middle ear epithelium is an extension of the airway epithelia, it is appropriate to suggest that ENaC has an important role also in the middle ear**.** However, it has been unclear whether ENaC is essential for the fluid transport in NHMEE cells.

In this study we also demonstrated that ENaC is functionally expressed in human middle ear epithelial cells (Fig. 1A). Our data showed that amiloride strongly suppressed fluid absorption in NHMEE cells in a dose-dependent manner, which strongly suggested that ENaC plays an important role in fluid absorption in NHMEE cells (Fig. 3).

It is possible that transepithelial fluid transport is actively modulated during inflammation. IL-1ß is one of the important cytokines in the inflammatory process which is produced by macrophages.[10] Recently, there has been an

increasing body of evidences supporting the important role of cytokines in the modulation of ion channels and transporters.[2,9,19] It has been shown that IL-1β altered Na^+absorption and fluid transport in the airway epithelia.[9] Moreover, previous reports demonstrated that the effusion of otitis media contains high levels of IL-1ß.[10] These findings promote us to hypothesize that liquid transport are regulated in response to inflammatory stimuli and test this hypothesis by investigating the effects of the pleiotypic, early-response cytokine, IL-1β on cultured NHMEE cells.

In this study, we could see that IL-1β supress ENaC mRNA expression (especially subunit β), and activation in protein level (Fig. 1 and 2). So, it might be postulated that various inflammatory mediators, including IL-1β, in chronic inflammatory conditions reduce ENaC mRNA expression resulting in decrease of ENaC function. And its main target is probably ENaC subunit β.

Figure 4 demonstrated that transepithelial short circuit current is decreased by IL-1β treatment. In figure 5A and B, we can see more obviously that basal transepithelial current decreased after IL-1β treatment and the luminal addition of amiloride (100 μM) produced a steep decrease of current to the same current level. It means that IL-1β suppresses ENaC function because the amiloride sensitive short circuit current represents ENaC function. Our data is consistent with several lines of evidences, which suggest that bacterial and viral infections modulate the amiloride-sensitive Na^+ transport in lung epithelial cells.[20]

Futhermore we demonstrated physiologically that IL-1β suppresses ENaC

dependent fluid absorption in NHMEE cells. As shown in figure 6A and B, addition of amiloride (100 μM) decreased the fluid absorption in both control and IL-1β pretreated NHMEE cells. However, in IL-1β pretreated cells, the decreased absorption volume was smaller than that of control cells. Because decreased ENaC function due to IL-1β caused decreased effect of amiloride on ENaC. These results indicate that IL-1β suppresses ENaC-dependent fluid transport in NHMEE cells.

If we understood the underlying signal pathway of ENaC suppression by IL-1ß, we might be able to inhibit that suppression. Mitogen-activated protein kinases (MAPKs) pathways are thought to be most important in transmitting inflammatory signals from cell surface to the nucleus. MAPKs may be subdivided into extracellular signal-regulated kinase (ERKs), p38 and c-Jun amino-terminal kinases (JNKs).[21] ERKs control cell division and proliferation. p38 MAPKs are activated by inflammatory cytokines and environmental stresses, and JNKs are important for controlling apoptosis.[21]

In these inhibition studies, we could inhibit the ENaC suppression by IL-1ß with PLC, PKC and ERK inhibitors. It indicated that PLC-PKC-ERK pathways are probably involved in IL-1β induced ENaC suppression. Although the signaling pathway of IL-1β induced ENaC suppression may depend on various conditions, and more exact molecular mechanisms need to be determined in future studies, however, such a understanding of signaling pathway as this would provide deeper insights into the management of fluid control in otitis media with effusion (OME).

In this study we showed that ENaC is essential for the fluid absorption in NHMEE cell. We also showed that IL-1β suppresses amiloride sensitive current and fluid absorption in NHMEE cells. Our data suggest that fluid overcollection by suppression of ENaC-dependent fluid absorption could be one of the pathogeneses of OME.

V. CONCULSION

ENaC was found to be expressed in NHMEE cells, and play an important role in fliud absorption. PLC-PKC-ERK pathways are involved in IL-1β induced ENaC suppression mainly via suppression of subunit β. Fluid overcollection by suppression of ENaC-dependent fluid absorption could be one of the pathogeneses of OME.

REFERENCES

1. **Portier F, Kania R, Planes C, Hsu WC, Couette S, Huy P, Herman P.** Enhanced sodium absorption in middle ear epithelial cells cultured at air-liquid interface. *Acta Otolaryngol* 2005125:16-22.

2. **Barmeyer C, Amasheh S, Tavalali S, Mankertz J, Zeitz M, Fromm M, et al.** IL-1beta and TNF alpha regulate sodium absorption in rat distal colon. *Biochem Biophys Res Commun* 2004;317:500-507.

3. **Boucher RC.** Human airway ion transport. Part one. *Am J Respir Crit Care Med* 1994;150: 271-281.

4. **Widdicombe JH, Widdicombe JG.** Regulation of human airway surface liquid. *Respir Physiol* 1995;99:3-12.

5. **Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, et al.** Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. *Nature* 1994367:463-467.

6. **Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC.** Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med* 2004;10:487-493.

7. **Herman P, Friedlander G, Tran Ba Huy P, Amiel C.** Ion transport by primary cultures of Mongolian gerbil middle ear epithelium. *Am J Physiol* 1992;262:373-380.

8. **Portier F, Van den Abbeele T, Lecain E, Sauvaget E, Escoubet B, Tran Ba Huy P, et al.** Oxygen modulates Na^+ absorption in middle ear epithelium. *Am J Physiol*

1999;276:312-317.

9. **Dagenais A, Frechette R, Yamagata Y, Yamagata T, Carmel JF, Clermont ME, et al.** Downregulation of ENaC activity and expression by TNF-alpha in alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2004;286:301-311.

10. **Monton C, Torres A, El-Ebiary M, Filella X, Xaubet A, de la Bellacasa J.** Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. *Crit Care Med* 1999;27**:**1745-1753.

11. **Yellon RF, Leonard G, Marucha PT, Craven R, Carpenter RJ, Lehmann WB**. Characterization of cytokines present in middle ear effusions. *Laryngoscope* 1991;101:165-169.

12. **Choi JY, Kim CH, Lee WS, Kim HN, Song KS, Yoon JH.**Ciliary and secretory differentiation of normal human middle ear epithelial cells. *Acta Otolaryngol* 2002;122:270-275.

13. **Yoon JH, Kim KS, Kim SS, Lee JG, Park IY.** Secretory differentiation of serially passaged normal human nasal epithelial cells by retinoic acid: expression of mucin and lysozyme. *Ann Otol Rhinol Laryngol* 2000;109:594-601.

14. **Pilewski JM, Frizzell RA.** Role of CFTR in airway disease. *Physiol Rev Suppl* 1999;79:215-255.

15. **Kellenberger S, Schild L.** Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev*2002;82:735-767.

16. **Kelly G, Yanbin D, Giuseppe AS.**Regulation of the epithelial sodium channel by accessory proteins. *Biochem J* 2003;371:1-14.

17. **DeSerres LM, Van Scott MR, Pillsbury HC, Prazma J.** Bioelectric properties of gerbil middle ear epithelium. *Arch OtolaryngolHead Neck Surg*

1991;117:416-421.

18. **Herman P, Cassingena R, Friedlander G, Soler P, GrodetA, Tran Ba Huy P, et al.** Middle ear cell line that maintains vectorial electrolyte transport. *J Cell Physiol* 1993;154:615-622.

19. **Atherton H**, **Mesher J**, **Poll CT**, **Danahay H**. Preliminary pharmacological characterisation of an interleukin-13-enhanced calcium-activated chloride conductance in the human airway epithelium. *Naunyn Schmiedebergs Arch Pharmacol* 2003; 367:214-217.

20. **Chen XJ, Seth S, Yue G, Kamat P, Compans RW, Guidot D, et al.** Influenza virus inhibits ENaC and lung fluid clearance. *Am J Physiol Lung Cell Mol Physiol*2004;287:366-373.

21. **Johnson GL, Lapadat R.** Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 2002;298:1911-1912.

Abstract (in Korean)

인체중이점막 상피세포에서 interleukin-1β가 Na^+ 이온통로와 수분흡수에 미치는 영향

<지도교수 이 정권>

연세대학교 대학원 의학과

최 윤석

호흡기점막 상피세포에서의 수분의 이동은 체내 이온 이동에 의존한다. 호흡기점막 상피 세포의 연장인 중이 점막에서의 수분 이동 역시 여러 가지 이온 통로에 의해 조절된다. A*milori*d*e*-s*ensiti*v*e* e*pithelial* N*a*$^+$ *cha*nne*ls* (EN*a*C)은 호흡기점막 상피세포에서 N*a*$^+$ 이온수송에 있어 중요한 역할을 한다. 최근 들어 *cyt*okine에 의해 호흡기점막 상피세포의 N*a*$^+$ 이온 통로가 억제된다는 보고가 늘고 있다. 그 중 IL-1β는 염증 반응에 있어 중요한 *cyt*okine으로서 삼출성 중이염환자의 중이강 내에서 증가한다고 알려져 있다.

저자들은 인체중이점막 상피세포에서 EN*a*C의 역할과 IL-1β가 EN*a*C에 미치는 영향을 그 작용기전과 함께 알아보고자 하였다.

먼저 100 μl의 배양액을 인체중이점막 상피세포에 공급하고 그 흡수 정도를 측정하였고, IL-1β나 여러 가지 signal pathway의 inhibitor를 가감하며 Ussing chamber를 이용한 Amiloride-sensitive short circuit current를 측정하였다.

강력한 ENaC blocker인 amiloride를 첨가 할수록 인체중이점막 상피세포의 수분 흡수는 감소되었다. 100 μM의 amiloride를 첨가하고 24시간 후에 86± 2.3 μl가 남아 있었다. Transepithelial short circuit current(Ite) 역시 IL-1β에 의해 감소되었는데 48시간 동안 10 ng/ml IL-1β 로 인체중이점막 상피세포를 처치한 결과 Ite 가 14.8± 1.9 μA/cm^2 에서 9.8± 0.9 μA/cm^2로 감소되었다. 그러나 PLC inhibitor (U73122, 10 μM), PKC inhibitor (Calphostin C, 10 μM) 또는 ERK inhibitor (PD98059, 10 μM)로 먼저 전처치하면 Amiloride-sensitive current가 거의 정상 수준으로 되돌아왔다. 반면 p38과 JNKs inhibitors는 영향을 주지 못했다.

이상과 같은 결과에서 ENaC이 인체중이점막 상피세포의 수분흡수에 있어 중요한 역할을 하고 IL-1β가 그러한 ENaC의 역할을 억제하며 이는 PLC-PKC-ERK pathway가 연관되어 있음을 알 수 있었다.

핵심 되는 말: **Na^+** 이온통로, interleukin 1 beta, 신호전달