Dexamethasone increases fluid absorption through Na^+/H^+ exchanger 3 activation in normal human middle ear epithelial cells

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Abstract

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Background: The proper homeostasis of the liquid lining the surface of the middle ear cavity is vitally important for maintaining a fluid-free middle ear cavity. Disruption of this homeostasis leads to fluid collection in the middle ear cavity and results in otitis media with effusion (OME).

Purpose: The purpose of this study was to investigate the molecular and functional expression of the Na^+/H^+ exchanger (NHE)s in normal human middle ear epithelial (NHMEE) cells and to evaluate the role of NHEs in fluid absorption and the effect of

dexamethasone (DXM) on NHE function and NHE-dependent fluid absorption in NHMEE cells.

Materials and Methods: Western blot analysis was performed for NHE1, -2, and -3 in NHMEE cells. The fluid absorption rate was measured after liquid application on the luminal surface of the cells. Intracellular pH (pH_i) was measured using the pH-sensitive fluorescent probe bis-(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM. NHE activity was determined as Na^+-induced *pHi* recovery from an acid load achieved by luminal exposure to 40 mmol/l NH_4Cl.

Results: NHE1, -2 and -3 were all expressed in the NHMEE cells. The *pHi* recovery rate was suppressed by inhibition of NHE 2 and -3 with HOE694 at concentrations greater than 50 μM. Inhibition of NHE3 with 650 μM of HOE694 or S3226 significantly decreased the fluid absorption rate. Dexamethasone increased the Na^+-induced *pHi* recovery rate which was reversed by the inhibition of NHE-3 with 650 μM of HOE694. Dexamethasone treatment up-regulated NHE3 expression in a dose-dependant manner. The fluid absorption rate was increased by treatment with Dexamethasone (10^{-7} M) and reversed by the inhibition of NHE3.

Conclussion: This study have shown that NHE3 are involved in the regulation of both pH*i* and fluid absorption on the luminal surface of NHMEE cells. Dexamethasone stimulates NHE3 expression and NHE3-dependent fluid absorption in NHMEE cells. These findings provide a new insight into mechanisms that regulate periciliary fluid and the therapeutic mechanisms behind steroid treatment of otitis media with effusion.

Key words: dexamethasone, otitis media with effusion, fluid absorption, middle ear, Na^+/H^+ exchanger

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I. Introduction

One of the major functions of the middle ear epithelium is to provide a fluid free middle ear cavity. Middle ear epithelial cells control the periciliary fluid volume by regulating both fluid and electrolyte absorption and secretion[1]. Disruption of these processes can lead to fluid collection in the middle ear cavity, and result in otitis media with effusion (OME). However, little is known about the mechanisms of

secretion and absorption of fluid and electrolyte in the middle ear epithelium.

Various mechanisms that regulate Na^+ transport across luminal cell membranes have been identified. These include the electrogenic process of Na^+ absorption via an epithelial Na^+ channel (ENaC) [2]and the electroneutral process of Na^+/H^+ exchange (NHE)s[3]. The NHE gene family is comprised of nine members that are categorized by cellular localization. There are resident plasma membrane isoforms including NHE1 (basolateral) and NHE2 (luminal), recycling isoforms (NHE3-5), as well as intracellular isoforms (NHE6, 7, 9)[4]. Among them, the NHE 1, 2 and 3 isoforms are the most characterized members of this gene family. The NHEs are the major determinants of intracellular pH (*pHi*), and also facilitate NaCl absorption in epithelial cells[5,6]. Previous studies also reported that the NHEs play an important role in fluid absorption in various tissues including the intestine and the bile duct[7,8]. The NHEs are also expressed in proximal and distal human airways, and they are involved in the regulation of *pHi* in human airway epithelial cells[9,10]. Because the vectorial Na^+ transport by epithelial cells regulates the volume of airway surface liquid (ASL) in airway epithelial cells[11], the NHEs may have an important function in regulating the ASL volume. However, it is unclear whether the NHEs have a regulatory function in fluid absorption in airway epithelial cells.

Na^+ absorption is also a major driving force of fluid absorption in the middle ear epithelial cells[12,13]. Amiloride-sensitive ENaC is a major determinant in transepithelial current and Na^+ absorption in the middle ear epithelial cells of Mongolian gerbil[13]. Interestingly, it was found that NHE-dependent transepithelial current also exist in normal human middle ear epithelial (NHMEE) cells in a preliminary study using an Ussing chamber. However, there has been no report on the expression or function of

NHEs in middle ear epithelial cells.

Glucocorticoids are one of the useful treatment modalities in otitis media with effusion[14]. Although modulation of ion transport processes is considered the underlying action of this hormone[15,16], its mechanism has not been clearly studied. Because glucocorticoids are known to activate Na^+ channels including NHEs[17,18,19], glucocorticoids might regulate fluid absorption by modulating NHEs in the airway epithelia, including the middle ear epithelial cells.

The purpose of this study was to investigate the molecular and functional expression of the NHEs in NHMEE cells and to evaluate the role of NHEs in fluid absorption in NHMEE cells. In addition, I investigated the effect of dexamethasone (DXM) on NHE functions and NHE-dependent fluid absorption in NHMEE cells.

II. Materials and Method

2.1. Cell culture

Primary cultures of NHMEE cells were prepared as previously described[20,21]. The NHMEE cells were acquired from normal human middle ear mucosa. All procedures were approved by the Institutional Review Board of Yonsei Medical Center. Passage-2 NHMEE cells were plated on a collagen-coated semipermeable membrane with a 0.45-μm pore size (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[20] and incubated for 7–8 days until the cells formed a functionally polarized monolayer.

2.2. Western blotting

The NHMEE cells were lysed and boiled for 5 min in sample buffer, separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis on 8% acrylamide minigels and blotted onto nitrocellulose membranes. After incubating in a blocking buffer, the membrane was treated with diluted anti-NHE1, -2 antibody (1:1000). The monoclonal anti-NHE1 antibodies (91 kDa), and polyclonal anti-NHE2,antibodies (90 kDa) were obtained from Chemicon International (Temecula, CA) and polyclonal antibody specific for NHE 3 (93 kDa) was a gift from Dr. Orson W Moe, University of Texas Southwestern Medical C enter, Dallas, Texas, USA[22]. The membranes were treated with appropriate secondary antibodies and the signal was detected by means of enhanced chemiluminescence (ECL plus system; Amersham, Aylesbury, UK).

2.3. Measurement of fluid absorption capacity

The role of NHEs in fluid absorption was evaluated by measuring the capacity of epithelial cell to modulate the absorption of luminal liquid[23]. Briefly, the surface liquid was aspirated, and 100 µl of Krebs Ringer bicarbonate (KRB) solution containing 2% blue dextran (BD), a cell-impermeant fluid volume marker dye, was applied luminally. Dexamethasone (10^{-7} M) and/or HOE 694 (1, 50 and 650 µM) were also mixed into the luminal solution in some cultures. Microaliquots (2–5 µl) of luminal liquid were sampled at indicated times, and analyzed. BD concentration was measured optically to calculated remaining volume[23].

2.4. Measurements of Intracellular pH (pH_i) and NHE Activity

pH_i was measured in the monolayers using the pH-sensitive fluorescent probe bis (2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM. Cells were loaded with BCECF-AM for 10 min. at room temperature in solution A containing 2.5 µM BCECF-AM and mounted in the miniature Ussing chamber and BCECF fluorescence was recorded and calibrated using a previously described protocol[24]. Briefly, the fluorescence at excitation wave-lengths of 490 and 440 nm was recorded using a recording setup (Delta Ram; PTI Inc.), and the 490/440 ratios were calibrated intracellularly by perfusing the cells with solutions containing 145 mM KCl, 10 mM HEPES, and 5 µM nigericin with the pH adjusted to 6.2–7.6. NHE activity was determined as Na^+-induced *pHi* recovery from an acid load achieved by exposure to 40 mmol/l NH_4Cl using the methods of Roos and Boron (1981)[25] with modification.

Figure 1 shows a typical pH-recovery experiment for determination of NHE activity. The addition of NH_4^+ to the luminal membrane produced rapid intracellular alkalinization (7.92±0.12 *(n = 11)*) due to the influx of NH_3 into the cells. Subsequent removal of extracellular NH_4^+ caused a rapid fall in *pHi* due to dissociation of NH_4^+ into H^+, which remains in the cells, and NH_3, which can rapidly leave the cytoplasm. This caused a large undershoot of the *pHi* to 6.65 ± 0.09, significantly below the starting value. When Na^+ containing solution was applied on the luminal cell surface, the *pHi* subsequently recovered (at an average rate of 0.39±0.08 ΔpH unit/min), as a result of NHE activity present in the apical membrane of the NHMEE cells. Typically, the first 10–40 s of the initial linear portion of the pH_i recovery was fitted to a linear equation using the Felix software (version 1.4; PTI Inc.).

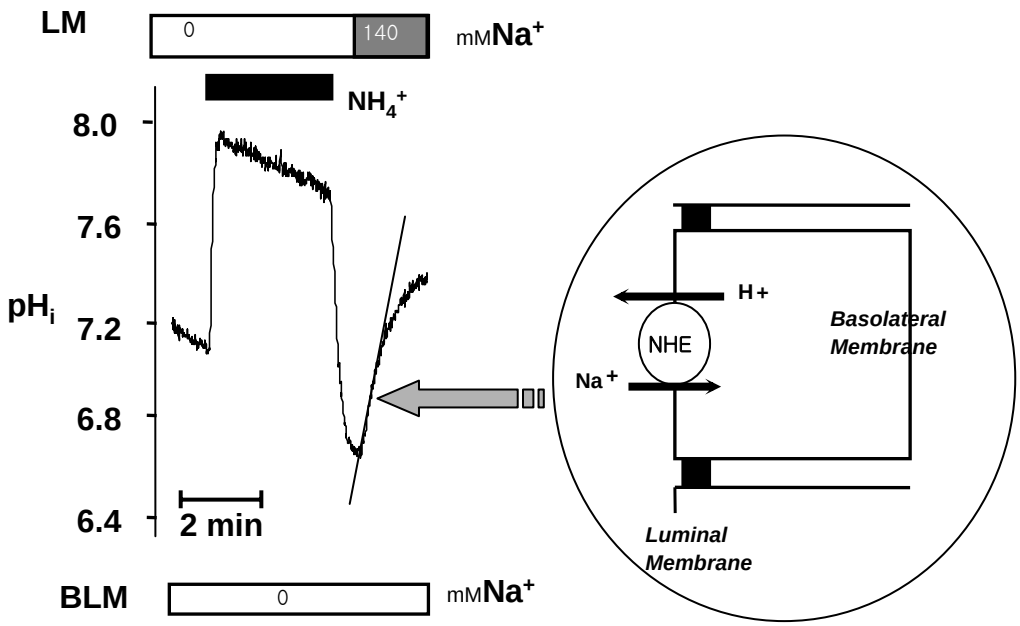


Fig. 1. Na^+/H^+ Exchanger (NHE) activity in the luminal membrane of normal middle ear epithelial cells. The pH_i of NHMEE cells was measured using BCECF as described in the Materials and Methods section. The membrane-specific NHE activities of polarized NHMEE cells were analyzed using a double perfusion chamber with separate applications of 140mM Na^+. The application of Na^+ to the luminal side evoked an increase in *pHi*. *LM*, luminal membrane; *BLM*, basolateral membrane.

2.5. Solutions and chemicals

The HEPES-buffered solution contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10mM glucose and 10 mM HEPES (pH 7.4 with NaOH). The Na-free solution contained 140 mM N-methyl-D-glucamine chloride (NMDG-Cl), 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES (pH 7.4 with Trisbase). The osmolarity of all solutions was adjusted to 310 mOsm with the major salt prior to use. The BCECF-AM was purchased from Molecular Probes (Eugene, OR). All other chemicals, including 3-methylsulphonyl-4-piperidinobenzoyl guanidine methanesulfonate (HOE694)[26], were purchased from Sigma. The specific NHE3 inhibitor, S3226(3-[2-(3-guanidino-2-methyl-3-oxopropenyl)-5-methyl-phenyl]-N-isopropylidene-2-methyl-acrylamide dihydrochloride), was kindly provided by Aventis Pharma (Frankfurt, Germany)[27].

2.6. 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. A value of $p < 0.05$ was considered statistically significant.

III. Results

3.1. Molecular expression and functional activity of NHE isoforms on the luminal surface of NHMEE cells

Western blot analysis was performed to detect the presence of human NHE1, -2, and -3 in NHMEE cells. As shown in Fig. 2, NHE1 (91 kDa), NHE2 (90 kDa) and NHE3 (87 kDa) were all expressed in the NHMEE cells. To distinguish the role of NHE isoforms in Na^+-induced *pHi* recovery, an NHE-specific inhibitor (HOE694) was used. Prior studies have established the following inhibitor constant (*K*i) values for the NHE1, -2, and -3 isoforms: 0.16, 5, and 650 μM, respectively[28]. As previously mentioned, the initial rate of apical NHE activity was 0.39±0.08 ΔpH unit/min. The recovery rate in the presence of 1 μM HOE694 (0.38±0.09 ΔpH unit/min) was not significantly different from the rate of the control. However, Na^+-induced *pHi* recovery was inhibited to HOE694 at concentrations greater than 50 μM. The *pHi* recovery rate was decreased to 0.21± 0.02 ΔpH unit/min by 50 μM HOE694 and almost abolished by 650 μM HOE694(0.05±0.01 ΔpH unit/min) (Fig. 3A & B). These results suggest that NHE2 and -3 regulate *pHi* in the luminal surface of NHMEE cells.

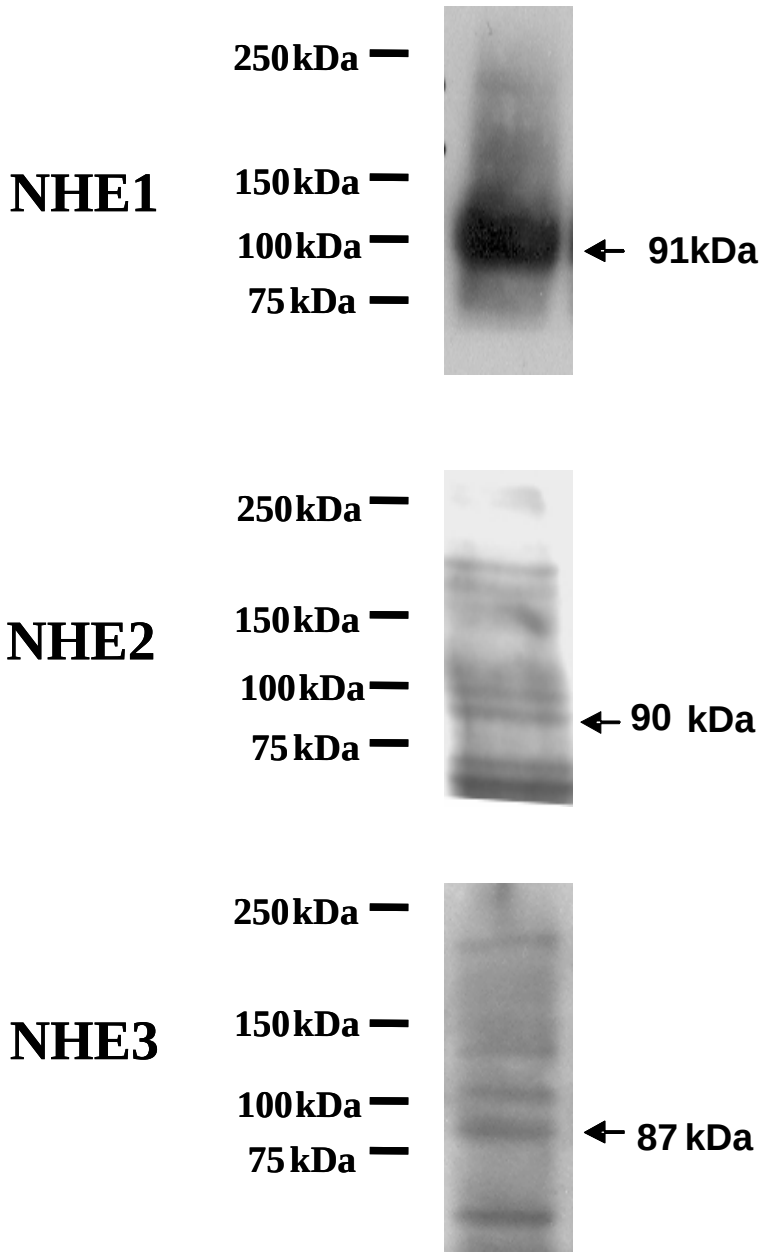
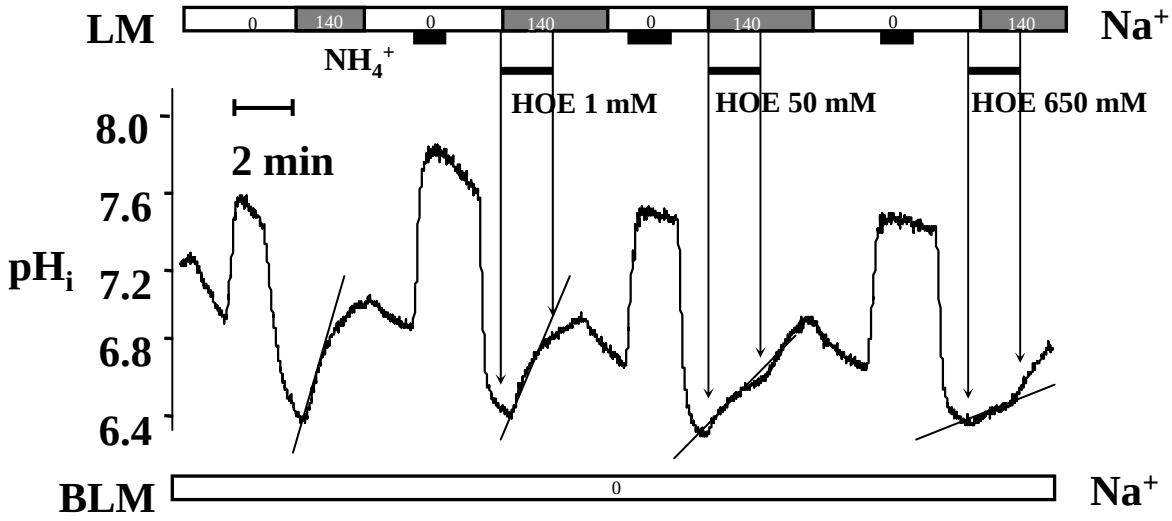
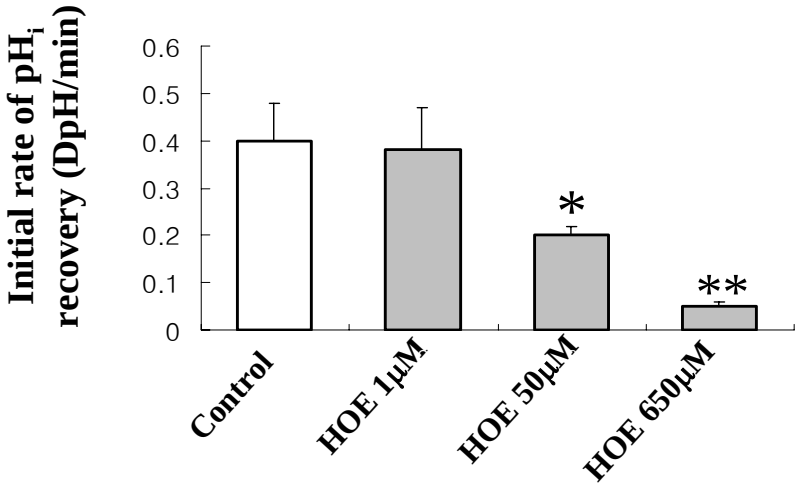


Fig. 2. Western blotting for Na^+/H^+ Exchanger (NHE) isoforms in normal human middle ear epithelial cells. NHE1 (91 kDa), NHE2 (90 kDa) and NHE3 (87 kDa) were all expressed in the NHMEE cells.

A


LM
0
140
0
140
0
140
0
140
Na+
NH4+
HOE 1 mM
HOE 50 mM
HOE 650 mM
8.0
2 min
7.6
pHi
7.2
6.8
6.4
BLM
0
Na+

B


Initial rate of pHi recovery (DpH/min)
0.6
0.5
0.4
0.3
0.2
0.1
0
*
**
Control
HOE 1μM
HOE 50μM
HOE 650μM

C

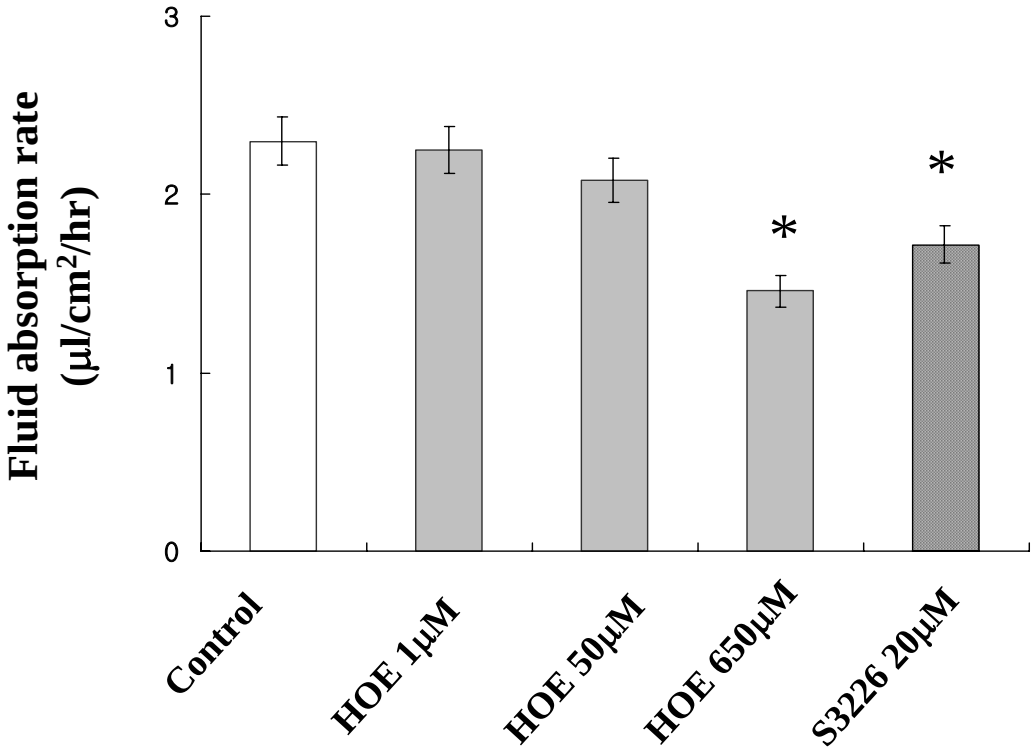


Fig. 3. The role of Na^+/H^+ Exchanger (NHE) isoforms in *pHi* regulation and fluid absorption in the luminal membrane of normal human middle ear epithelial cells. The Na^+-induced *pHi* recovery rate was measured in the presence of different concentrations of HOE694. ***A***, A representative trace showing the effects of HOE 694. *LM*, luminal membrane; *BLM*, basolateral membrane. ***B***, Summarized results of six experiments. ***C***. 100 μl of KRB solution (0.1% DMSO) with/without various concentrations of HOE694 or 20 μM of S3226 were applied on the luminal surface and the fluid absorption rate was measured as described in the Mterials and methods section. Six hundred fifty μM of HOE694 and 20 μM of S3226 inhibited fluid absorption. * indicates $p < 0.05$, **, $p < 0.01$, difference from control.

3.2. The role of NHEs in fluid absorption

In order to evaluate the role of NHEs in fluid absorption, the absorption rate of fluid of NHMEE cells was measured after application of 100 μl of fluid on the luminal surface of cells. The cells absorbed fluid at the rate of 2.3 μl/cm^2/hr while in a resting state. Treatment with 1 μM or 50 μM of HOE694 did not change the absorption rate significantly (2.25±0.31 μl/cm^2/hr and 2.08±0.22 μl/cm^2/hr, respectively). However, 650 μM HOE964 significantly decreased the absorption rate by 1.46±0.19 μl/cm^2/hr and 20 μM S3226, a specific inhibitor for NHE3, also decreased the rate by 1.72±0.24 μl/cm^2/hr (Fig. 3C). These data suggest that NHE3 plays a critical role in fluid absorption in the luminal surface of the NHMEE cells.

3.3. The effect of dexamethasone on NHEs activity

The effect of dexamethasone on the Na^+-induced *pHi* recovery was then evaluated. The recovery rate was greatly increased after exposure to dexamethasone (control: 0.39±0.08 ΔpH unit/min; dexamethasone treated: 1.42±0.08 ΔpH unit/min). Interestingly, treatment with dexamethasone stimulated Na^+-induced *pHi* recovery (Fig. 4 A & B). I interpreted the HOE 694 (650 μM)-sensitive, Na^+-induced *pHi* recovery rate as NHE3 activity, which was greatly increased after dexamethasone treatment (control: 0.36±0.08 ΔpH unit/min, dexamethasone treated: 0.99±0.08 ΔpH unit/min, $p < 0.05$) (Fig. 4C). These results suggest that dexamethasone increases NHE3 activity in the luminal surface of NHMEE cells.

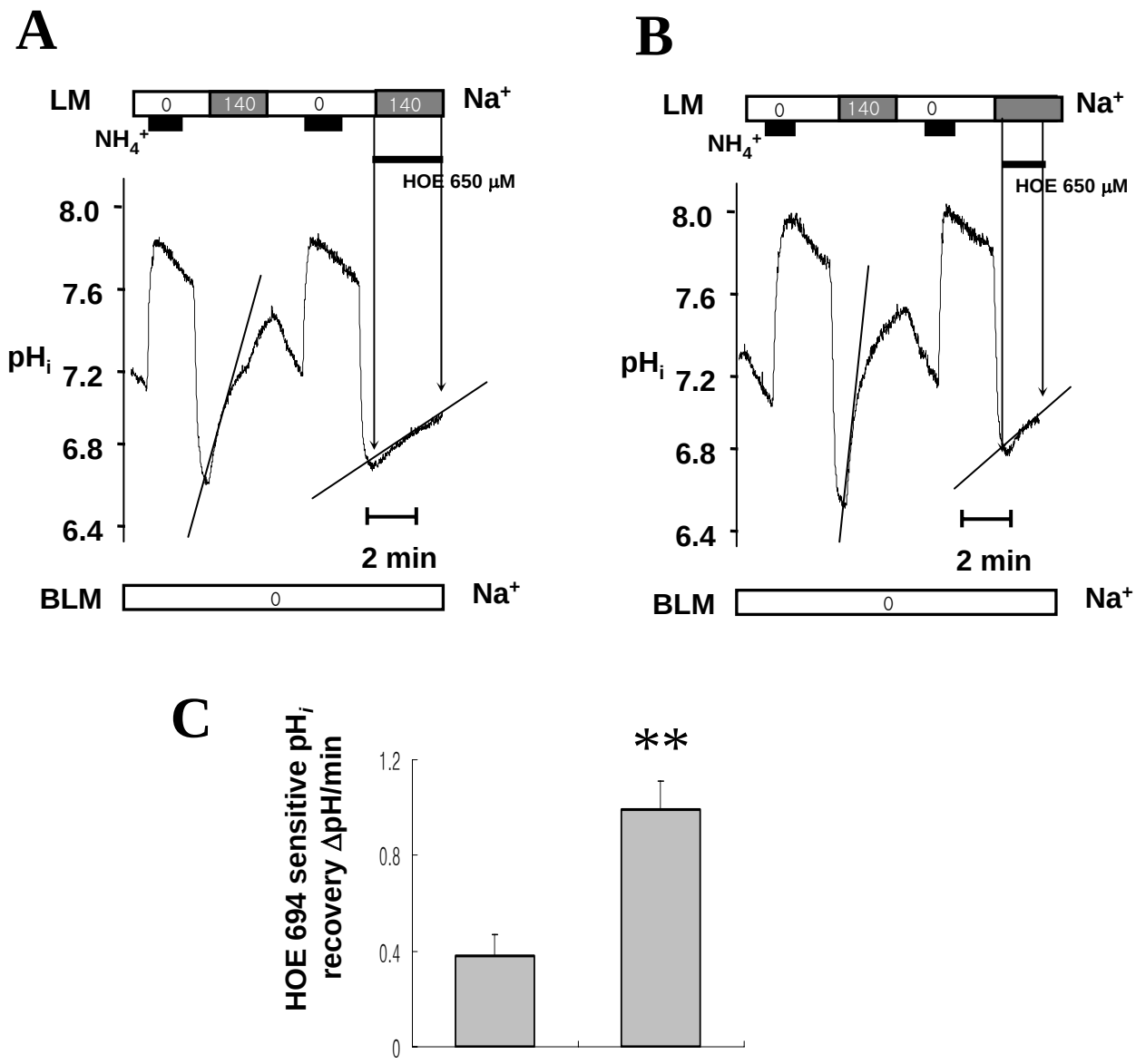


Fig. 4. Activation of Na^+-induced pHi recovery by dexamethasone.The Na^+-induced *pHi* recovery rate was measured after treatment with dexamethasone (DXM) (10^{-7} M for 12 hrs). Representative traces of control and DXM-treated samples are presented in ***A*** and ***B***.. *LM*, luminal membrane; *BLM*, basolateral membrane. ***C.*** Effects of DXM stimulation on NHE3 was determined by comparing the HOE694 (650 μM)-sensitive ΔpH unit/min. ** indicates $p < 0.01$, difference from control. *LM*, luminal membrane; *BLM*, basolateral membrane.

3.4. The effect of dexamethasone on NHEs expression and NHE3-dependent fluid absorption.

The effect of dexamethasone on the expression of NHE isoforms was determined by western blotting. Dexamethasone up-regulated NHE3 expression in a dose-dependant manner (with a maximum effect at 10^{-7} M). However, NHE1 and -2 expressions were not changed after dexamethasone treatment (Fig. 5A). Lastly, I determined whether dexamethasone increased NHE3-dependent-fluid absorption capacity in NHMEE cells. The fluid absorption rate was increased 220% by the treatment with 10^{-7} M dexamethasone (control: 2.19 ± 0.11 μl/cm^2/hr: dexamethasone treated: 4.82 ± 0.33 μl/cm^2/hr). Treatment with 1 μM and 50 μM of HOE694 did not affect dexamethasone-induced fluid absorption. However, when the cells were incubated with 650 μM HOE694, the effect of dexamethasone almost disappeared (3.32 ± 0.23 μl/cm^2/hr) (Fig. 5B). Addition of 20 μM S3226 inhibited dexamethasone-induced fluid absorption to 3.56 ± 0.33 μl/cm^2/hr. These data indicate that dexamethasone stimulated both NHE3 expression and NHE3-dependent fluid absorption in NHMEE cells

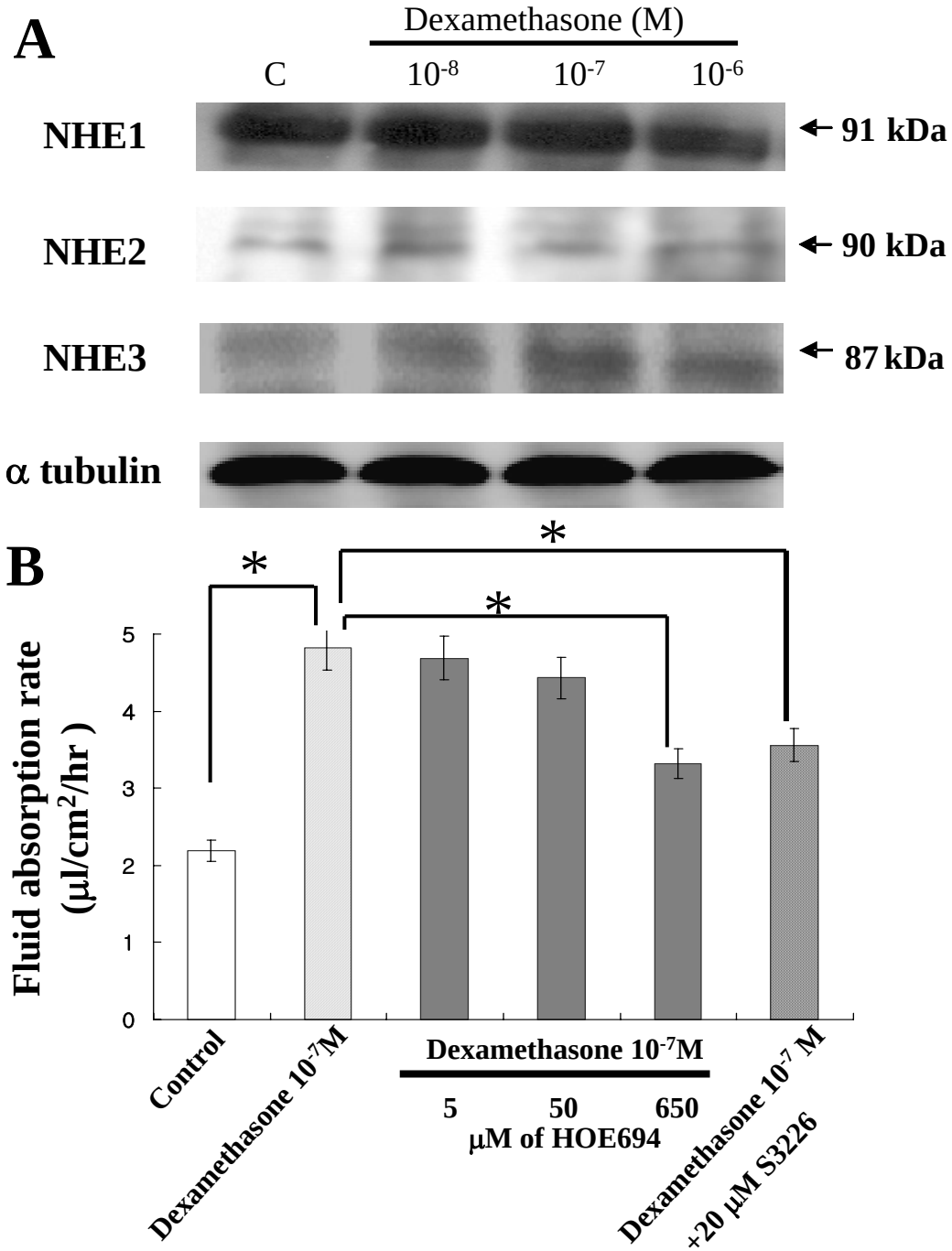


Fig. 5. Activation of NHE 3 expression and NHE 3- dependent fluid absorption by dexamethasone. .***A***. Western blotting was performed to determine expression levels of the NHE isoforms. Dexamethasone (DXM) increases NHE3 expression (with a maximum effect at 10^{-7}M). NHE1 and -2 expression were not changed after dexamethasone treatment. ***B.*** The fluid absorption rate was measured after application of 100 μl of KRB solution on the luminal surface of NHMEE cells. Dexamethasone stimulates the fluid absorption rate and this stimulatory effect is inhibited by 650 μM of HOE694 or 20 μM of S3226. * indicates $p < 0.05$.

IV. Discussion

The NHE gene family plays an integral role in electroneutral Na^+ absorption in various cell types. However, the role of NHEs in the airway epithelium remains controversial. Several researchers reported that only NHE1 mRNA was expressed in isolated human airway epithelial cells, while NHE2 and -3 were not detected[9,29]. In contrast, NHEs have been shown to be involved in *pHi* regulation in the luminal membrane of Calu-3 cells, a serous cell model of airway epithelium[10]. I also have shown that NHE 1, -2 and –3 are expressed in human nasal epithelium[30]. In the present study, NHE1, -2,and -3 were found to be expressed in NHMEE cells. This discrepancy might originate from the differential expression of ion channels between the lower and upper respiratory epithelium. Although NHE1, -2 and –3 were all expressed in NHMEE cells, only NHE2 and NHE3 are functionally active in *pHi* regulation on the luminal surface of NHMEE cells.

Several lines of evidence suggest that NHE plays an important role in fluid absorption in various epithelia including those of the kidney and intestine[31,32,33,34]. Because the vectorial Na^+ absorption has important implications in ASL volume and composition, I investigated whether the NHEs play a role in the fluid absorption in NHMEE cells. In order to do so, I measured the absorption capacity of NHMEE cell after fluid application on the luminal surface. Using this approach, I have demonstrated that the NHE3 isoform is responsible for fluid absorption in the NHMEE cells, because fluid absorption was significantly decreased by inhibition of NHE3 with 650 μM of HOE694 or 20 μM of S3226. Among the NHE isoforms, NHE3 has been known to be the most important isoform in fluid absorption in various

cell types. NHE3 knockout mice fail to absorb the fluid secreted from the cholangiocytes[33], and the inhibition of NHE3 results in diarrhea by disruption of normal fluid absorption in the intestine[34]. The results of this study suggest that NHE3 also contributes to fluid absorption in respiratory-type epithelia such as the middle ear epithelium and might play an important role in maintaining the middle ear cavity in a fluid-free state. However the effect of NHE3 inhibition on fluid absorption is much smaller than the effect on pH regulation in this experiments. These results may be explained by contribution of other sodium channels such as epithelial Na^+ channels and paracellular sodium transport to fluid absorption in airway epithelium.

Previous studies established that glucocorticoids activate NHE function by genomic or non-genomic pathways in various cell types[17,18,19]. Because this studies revealed that NHE contributes both to *pHi* regulation and fluid absorption in NHMEE cells, I evaluated the effects of dexamethasone on the function of NHEs in NHMEE cells. DXM up-regulated NHE-3 expression and stimulated NHE3-dependent *pHi* recovery in this experiments. NHE 1, -2 were not affected by dexamethasone treatment. More importantly, I found that dexamethasone stimulated NHE3-dependent fluid absorption in NHMEE cells. Exceptional stimulation of NHE 3 by dexamethasone in NHMEE cells is consistent with previous reports where glucocorticoids stimulated fluid absorption by increasing NHE3 activity in intestinal epithelium, proximal renal tubular epithelium and cholangiocytes[31,32,33]. These results are the first evidence that NHE3 is involved in fluid absorption and that glucocorticoids such as dexamethasone stimulate the absorption process in respiratory-type epithelial cells.

V. Conclusion

The purpose of this study was to investigate the molecular and functional expression of the NHEs in NHMEE cells and to evaluate the role of NHEs in fluid absorption in NHMEE cells. In addition, I investigated the effect of dexamethasone (DXM) on NHE functions and NHE-dependent fluid absorption in NHMEE cells.

The results are summarized as followings.

1. NHE1 (91 kDa), NHE2 (90 kDa) and NHE3 (87 kDa) were all expressed in the NHMEE cells and NHE2, NHE3 regulate *pHi* in the luminal surface of NHMEE cells.
2. NHE3 plays a critical role in fluid absorption in the luminal surface of the NHMEE cells.
3. Dexamethasone increases NHE3 activity in the luminal surface of NHMEE cells.
4. Dexamethasone stimulated both NHE3 expression and NHE3-dependent fluid absorption in NHMEE cells.

These findings provide new insight into the regulation periciliary fluid and the mechanisms utilized by steroid in the treatment of otitis media with effusion.

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국문요약

사람 정상 중이점막 상피세포에서 dexamethasone의 Na^+/H^+ exchanger 3 활성화를 통한 삼출액 흡수증가

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배경: 중이 점막을 덮고있는 점액의 적절한 조절 및 유지는 중이 점막 상피세포의 중요한 기능의 하나이며 중이강을 삼출액이 없도록 유지해 주는데 매우 중요하다. 이러한 항상성의 장애는 중이강 내의 삼출액 저류로 이어지고 결과적으로 중이염을 일으킨다.

목적: 본 논문의 목적은 먼저 Na^+/H^+ exchanger (NHE)가 사람 정상 중이점막 상피세포에서 발현되는지 여부와 이 이온 채널이 사람 정상 중이

점막 상피세포에서 발현된다면 기능적으로 활성화되어 있는지 증명하는 것이며 또한 중요한 이비인후과 질환의 하나인 삼출성 중이염에서 발병과 치료에 중요한 역할을 할 것으로 생각 되는 세포의 삼출액 흡수에 있어서 Na^+/H^+ exchanger가 어떤 역할을 하는지 증명 하고 마지막으로 사람 정상 중이점막 세포에서 Na^+/H^+ exchanger에 의존하는 삼출액 흡수와 Na^+/H^+ exchanger의 기능에 대한 dexamethasone의 효과를 증명하는 것 이다.

재료 및 방법: NHE1,2,3가 사람 정상 중이점막 상피세포에서 발현되는 것을 증명하기 위해 Western blot analysis를 사용하였다. 사람 정상 중이점막 세포의 용액 흡수 속도는 세포의 표면에 용액을 도포한 후에 세포 표면에 남아있는 용액의 BD의 농도를 측정 하여 흡수된 용액의 양을 측정함으로서 속도를 측정하였다. 세포 내 pH는 형광 물질인 bis-(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM를 이용해 측정하였다. Na^+/H^+ exchanger의 활성도 는 세포에 40 mmol/l NH_4Cl을 주었을 때 가해진 세포 내 산성화로부터 Na^+ 을 주었을 때 발생하는 pH 회복속도를 측정함으로써 간접적으로 결정하였다.

결과: NHE1,2,3 모두 사람 정상 중이점막 상피세포에서 발현되었다. PH의 회복속도는 HOE694 50 μM 이상의 농도에서 NHE2와 NHE3의 기능저하로 인해 억제되었다. 650 μM의 HOE694와 S3226으로 NHE3를 억압하였을 경우 삼출액 흡수속도가 의미 있게 감소되었다. Dexamethasone은

NHE3의 발현을 농도에 비례하여 증가시켰다. 삼출액 흡수속도는 Dexamethasone처리에 의해서 증가되었고 NHE3의 억제에 의해서 감소되었다.

결론: 본 논문에서는 NHE3가 사람 정상 중이점막 상피세포에서 pH와 삼출액 흡수에 모두 작용한다는 것을 증명하였다. Dexamethasone은 사람 정상 중이점막 상피세포에서 NHE3의 발현을 증가시키고 NHE3에 의한 삼출액흡수를 증가시킨다. 이러한 결과는 삼출성 중이염에서 임상적으로 흔히 사용해 왔던 스테로이드 치료에 관한 치료 기전에 이론적 바탕이 될 수 있으며 기도 상피세포의 점액 조절에 관한 여러 기전 중에서 새로운 발견이라 할 수 있다.

핵심되는 말 : 삼출액흡수; Na^+/H^+ exchanger; 중이염;