ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Methylation of GLUT5 and Electromotile Responses During Chronic and Acute Sodium Salicylate Administration in the Cochlear Outer Hair Cells

Koklear Dış Tüy Hücrelerine Kronik ve Akut Sodyum Salisilat Uygulamasına GLUT5 Metilasyon ve Elektromotil Yanıtlar

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ABSTRACT

Aim: *Prestin molecule and transporter fructose GLUT5 in the lateral walls of outer hair cells are essential. This study aimed to investigate epigenetic alterations in the GLUT5 gene.*

Materials and methods: *The animals were divided into three groups randomly. No injection was received in the first group (n=3). Groups 2(n=3) and 3(n=6) were injected with intramuscular saline and sodium salicylate, respectively. Electrophysiological measurements were performed at 1st, 2nd, and 8th hours to evaluate the acute effect and in 2 weeks to evaluate the chronic effect. Methylation of CpG dinucleotides in the promoter can lead to dysregulated and repressed gene expression. Genomic DNA was isolated from bone tissues, treated with bisulfite, and analyzed using methylation specific PCR (polymerase chain reaction) (MSP). Epigenetic alterations in the GLUT5 gene were investigated by using the MSP method.*

Results: *There was a significant decrease in electrophysiological measurements at all frequencies in the acute effect ($p<0.01$), whereas there was no significant difference in the chronic effect for Group 3 ($p>0.05$). However, methylation of GLUT5 was observed to be increased during acute administration, followed by a decreasing trend to normal during the chronic period.*

Conclusion: *Our findings show that methylation of GLUT5 may decrease GLUT5 expression in lateral walls of outer hair cells, thereby changing prestin-bound fructose transport in cell membranes due to reduced GLUT5 expression.*

Keywords: *GLUT5; outer hair cells; cochlea; distortion product otoacoustic emissions; sodium salicylate*

ÖZET

Amaç: *Prestin molekülü ve taşıyıcı fruktoz GLUT5 dış tüy hücrelerinin lateral duvarlarında önemli bir parçasıdır. Bu çalışma GLUT5 genindeki epigenetik değişiklikleri araştırmayı amaçlamıştır.*

Materyal ve Metot: *Hayvanlar rastgele üç gruba ayrıldı. Birinci gruba enjeksiyon yapılmadı (n=3). Grup 2 (n=3) ve 3 (n=6)'e sırasıyla kas içi salin ve sodyum salisilat enjekte edildi. Elektrofizyolojik ölçümler akut etkiyi değerlendirmek için 1., 2. ve 8. saatlerde ve kronik etkiyi değerlendirmek için 2. haftada gerçekleştirilmiştir. GLUT5 genindeki epigenetik değişiklikler Metilasyon spesifik PCR yöntemi kullanılarak araştırıldı.*

Bulgular: *Akut etkide tüm frekanslarda elektrofizyolojik ölçümlerde anlamlı bir azalma görülürken ($p<0,01$), kronik etkide Grup 3 için anlamlı bir fark yoktu ($p>0,05$). Bununla birlikte, GLUT5 metilasyonunun akut uygulama sırasında arttığı ve ardından kronik dönemle birlikte normale dönme eğiliminde olduğu gözlenmiştir.*

Sonuç: *Bulgularımız GLUT5 metilasyonunun dış tüy hücrelerinin lateral duvarlarında GLUT5 ekspresyonunu azaltabileceğini ve böylece GLUT5 ekspresyonunun azalmasına bağlı olarak hücre membranında prestine bağlı fruktoz taşınımını değiştirebileceğini göstermektedir.*

Anahtar kelimeler: *GLUT5; dış tüy hücreleri; koklea; distorsiyon ürünü otoakustik emisyonlar; sodyum salisilat*

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Introduction

The mammalian cochlea is anatomically located within the organ of Corti and consists of two types of hair cells. The cochlea contains two types of hair cells: inner and outer hair cells[1]. These cells have different functions. While acoustic information is transferred to auditory nerve fibers via the electrical signals of inner hair cells (IHCs), outer hair cells (OHCs) enhance the stimulus mechanically by increasing vibrations of the cochlear division[2]. OHCs are known to amplify the vibration of the basilar membrane with a pattern often referred to as the traveling wave or cochlear amplification. One of their unique characteristics is their ability to alter their length in response to changes in membrane potential, known as electromotility[3]. Prestin is a transmembrane motor protein located on the lateral wall of OHCs[4]. Prestin is essential for the cochlear amplification process, electromotile responses and sharp frequency tuning[5,6]. GLUT5 (Glucose transporter type 5) is a fructose-specific transporter, which is reported to be abundantly expressed in the OHC lateral wall and was initially suggested as the OHC motor protein[7]. It has been shown that GLUT5 is not needed for OHC electromotility and cochlear amplification[8]. The discovery of prestin has become an academic interest concerning sugar transport in OHCs[1,9] as sugar transport is closely related to the response of OHC electromotility[10–12].

Cytoplasmic anions (predominantly Cl-) are utilized by prestin as an extrinsic voltage sensor and mediate changes responding to changes in OHC membrane potential[4]. By binding to prestin, these anions increase their affinity and move intracellularly with alteration in membrane voltage and simultaneous depolarization of OHCs, resulting in cell length shortening. In contrast, during hyperpolarization, they move towards the extracellular region, leading to increased length of the cells[4,5]. Suppose the cytoplasm does not have any monovalent anions. In that case, the shortened OHC length is maintained in maximum contraction because of the prestin molecules[5]. In addition, the blocking effect of salicylate on prestin is a well-known phenomenon in auditory physiology. Salicylate can also affect the physical properties of the stereocilia bundle by reducing bending stiffness[13,14]. When OHCs become damaged or dysfunctional due to excessive aspirin intake, this leads to decreased sensitivity in our ears and, eventually, hearing loss if left untreated[14–16].

Epigenetics is heritable gene expression modifications that occur without changes in the primary DNA sequence[17]. Recent studies investigating the effect of age, developmental variables and environmental variables (stress, etc.) on base sequences have demonstrated gene silencing by adding methyl groups through enzymatic mechanisms in DNA molecules in carcinogenesis and tumorigenesis. At the same time, demethylation was associated with gene activation[18–21]. This study aimed to investigate epigenetic alterations in the GLUT5 gene.

Materials and Methods

Animals

All animals were subjected to Bilateral otoscopic and audiological assessments, including 1-kHz high-frequency DPOAE and tympanometry tests. The tympanometry and DPOAE measurements were used to determine Otoacoustic Emissions (OAE) (Madsen Capella-Denmark/GN Otometrics A/S). The probe device was placed in the ear canal and stationary with measuring equipment. Animals have been placed in a soundproof room. All measurements were performed using sodium pentobarbital (40 mg/kg) when the guinea pigs were put under general anesthesia with a warming blanket, and the body temperature was maintained at 38°C.

Treatment Protocol

Twelve animals (n=24 ears) with normal electrophysiological results were randomly stratified into three groups: Group 1 was the control group, Group 2 was the placebo control, and Group 3 was the sodium salicylate group. The guinea pigs in Group 1 (negative control group, n=6 ears) received no drug. Group 2 (positive control group, n=6 ears) received physiological saline (NaCl 0.9%) at a dose of 200 mg/kg/day twice daily by subcutaneous injection for two weeks. Group 3 (experimental group, n=12 ears) received salicylate at 200 mg/kg/day twice daily by subcutaneous injection for two weeks[22,23]. Electrophysiological measurements to confirm the chronic effect[24] were performed immediately before sodium salicylate injection (Day 0), and at the end of 2 weeks, DPOAE measurements were performed 1 hour before and 2–8 hours after the injections to adjust the acute results. After electrophysiological recordings, all animals were sacrificed under 200 mg/kg sodium pentobarbital (high-dose anesthesia), followed by dissection of the temporal bones for DNA purification to assess the acute (0 to 8 hours) and chronic (8 hours

to 2 weeks) effects of salicylate administration[21,24]. The possible methylations in the promoter of the GLUT5 gene were evaluated by MSP. Freshly dissolved 200 mg/kg sodium salicylate from Sigma (St. Sigma-Aldrich; Germany) was administered intramuscularly twice-daily injections for two weeks[22,23,25–27].

Electrophysiological Recordings

The acoustic stimulation tones referred to as primary frequencies were f1 and f2. L1 and L2 as primary levels were adjusted individually, and the frequency ratio f2/f1 was fixed at 1.22. Stimulant degrees were fixed at 65- and 55-dB sound pressure levels (SPL) for L1 and L2, respectively. Several essential aspects were evaluated when generating DPOAE results between 0.75 and 8 kHz with 2f1-f2 mode. The duration of the test was 60 seconds. The amplitude at least 3 dB higher than the mean background noise level sampled in various frequencies surrounding the emission frequency is a widely accepted criterion for detecting and confirming DPOAE. S/N-R (frequency-specific signal/noise ratios) was evaluated in both ears of the experimental animals (guinea pigs)[24].

Genomic DNA Isolation

The temporal bones were frozen in liquid nitrogen and then shattered, and otic capsules were exposed and resected. These processes were performed in a separate porcelain bowl to prevent contamination of sample DNA and DNA from other tissues. The GeneMATRIX DNA (Cat No. 220342, Eurx, Poland) kit was used to isolate DNA from the resected otic capsules according to manufacturer specifications.

Methylation Specific Polymerase Chain Reaction

Methylation-specific PCR (MSP) is the most widely used tool for qualitative DNA methylation analysis, and it has many advantages, such as simplicity of design and implementation and sensitivity in detecting small amounts of methylated and aberrant DNA. Bisulfite conversion is a common approach for DNA methylation analysis in gene-specific analysis studies. By treating DNA with bisulfite, cytosine is converted to uracil. At the same time, 5-methylcytosine remains intact, providing information on methylated regions of DNA, even with a single nucleotide change. The MSP is a PCR-based method routinely used for gene-specific DNA methylation analysis after bisulfite treatment. DNA concentration and quantification were determined at 260/280 nm with Nano-200 nucleic acid analyzer spectrophotometry. Next, 10 µL of DNA was taken from each sample. Bisulfite treatment was done according to the EpiJET Bisulfite Conversion Kit (K1461, Thermo Fisher Scientific, USA) and followed the manufacturer's recommendations. This treatment transforms non-methylated cytosines to thymine but does not convert methylated cytosines. The CpG sequences in the promoter-exon1 connection of the GLUT5 gene were identified using MethPrimer 2.0 to change the region[27]. Methylation-specific PCR was carried out using the following protocols:

Polymerase chain reaction conditions for the methylated and unmethylated residues (Fig. 1):

Polymerase chain reaction primers for promoter-exon1 linkage zone of GLUT5 (SLC2A5),

Figure 1. *GLUT5 methylated and unmethylated primers in original sequence.*

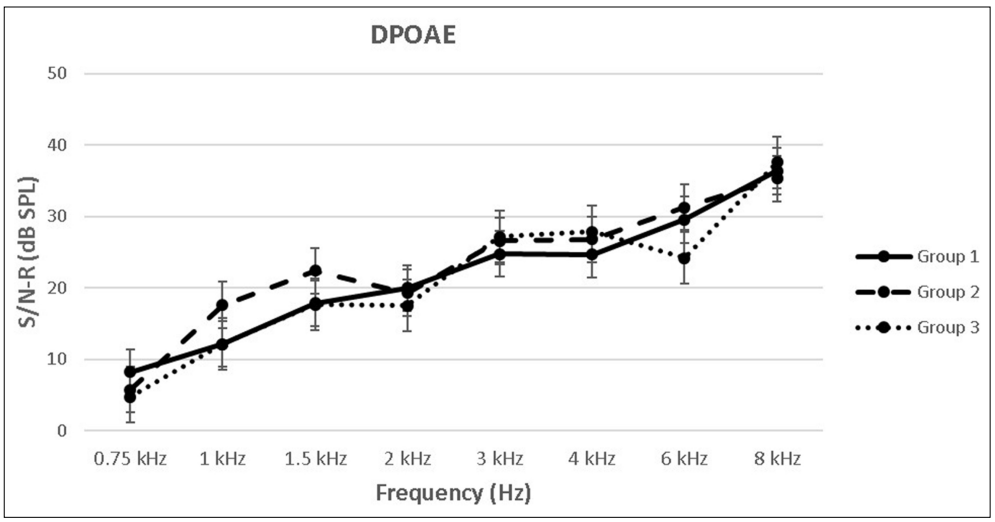

Figure 2. *Mean baseline S/N-R values in all groups.*

MetForward:
AAAGTAGAAGTGGATTTTAGGTAGCG-3'

MetReverse:
CAATACACTTTAACCACCGAAC, PCR product: 99 bp,

UnMetForward:
GAAGTGGATTTTAGGTAGTGAGG,

UnMetReverse:
CACAACAATACACTTTAACCACCA, PCR product: 98 bp.

Polymerase Chain Reaction Conditions and Agarose Gel Imaging

The buffer of PCR 1x, $MgCl_2$ (Thermo Fisher Scientific) and, two mM, Dimethylsulfoxide (DMSO): 5% (v/v) (Thermo Fisher Scientific) and, dNTP: 1.25 mM (Thermo Fisher Scientific) and, M/U Primer Forward: 10 pmol, M/U Primer Reverse: 10 pmol, Taq Polymerase: 1U (5U/μL) (Thermo Fisher Scientific) and sample DNA: 100 ng and dH_2O was used to reach a minimum of 20 μL and the conditions for PCR were as follows: 94°C 10'initiation, 94°C 40", 55°C 35", 72°C 35" 40 cycles, 72°C 7'ending.

All PCR products were spotted with EtBr (Thermo Fisher Scientific) on 2.5% agarose gel, and bands according to their pixel densities were evaluated using MiniBIS DNR Gel Analyzer[24,28].

Statistical Analysis

Statistical analyses were performed after checking the standard normal distribution of data (IBM Statistical Package for Social Sciences (SPSS) program version 19 for Windows; IBM Inc., Chicago, IL, USA). Since the data are unsuitable for parametric tests, intragroup comparisons were tested using the Wilcoxon test, and intergroup comparisons were made using the Mann-Whitney U test. The Kruskal-Wallis test was applied when more than two groups were involved in intergroup comparisons. Graphical results were plotted as mean ±1 standard error of the mean for each group.

Results

We evaluated the effect of sodium salicylate on the electromotive responses of OHCs using the S/N-R parameters in DPOAE measurements using the 2f1-f2 mode. During initial measurements ($p>0.05$), there was no statistical difference between the groups' DPOAE S / N-R responses. S / N-R values were above 3 dB at all frequency levels in the control group for DPOAE tests. An increase in S/N-R values was noted from low frequency to high frequency due to the mobility of OHCs in the cochlea (Fig. 2). Error bars represent one standard error of the mean and are plotted for Group 1, Group 2 and Group 3. They were comparable across all three groups.

There was no statistically significant difference in DPOAE S/N-R responses between 0.75 kHz and 8 kHz in Group 2 ($p>0.05$) (Fig. 3). Error bars represent one standard error of the mean and are plotted for the control and the other groups. They were comparable across all three groups. In Group 3, a statistically significant decrease was detected in DPOAE S/N-R responses starting at 1 hour ($p<0.01$), indicating the acute effect of sodium salicylate (Fig. 4) and a

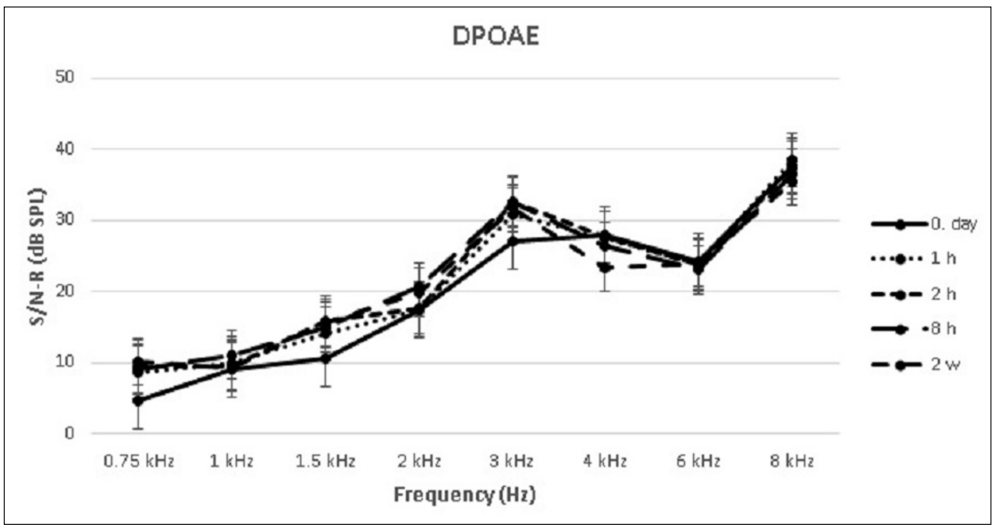

Figure 3. *S/N-R values during sodium salicylate medication administered to guinea pigs in Group 2. The figure depicts changes in DPOAEs during acute (1 hour and 8 hours) and chronic (8 hours to 2 weeks) periods.*

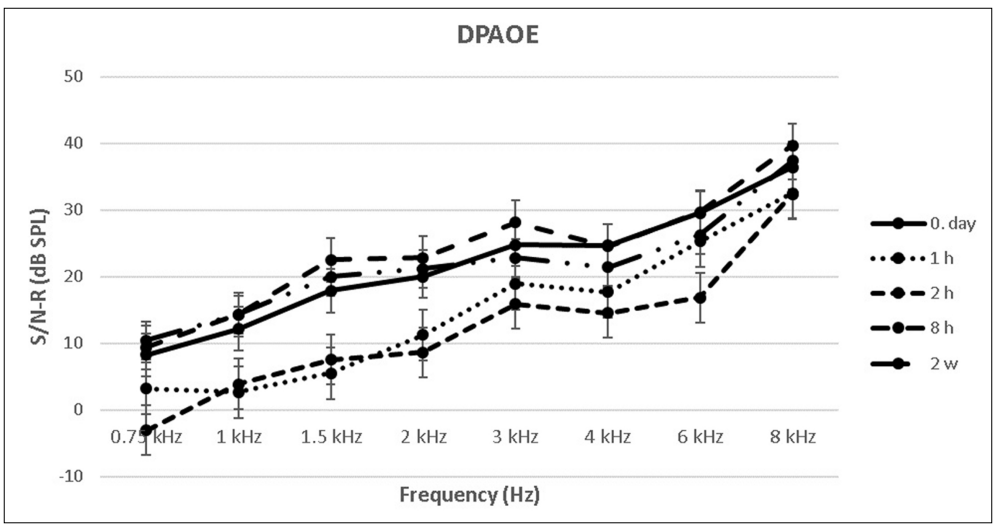

Figure 4. *S/N-R values during sodium salicylate medication administered to guinea pigs in Group 3. The figure depicts changes in DPOAEs during acute (1 hour and 8 hours) and chronic (8 hours to 2 weeks) periods.*

statistically significant decrease in DPOAE S / N-R responses ($p<0.01$) occurred at 2 hours after sodium salicylate injection; however, those changes were observed to return to baseline at 8 hours. Comparison of Group 1 and Group 3 during sodium salicylate administration showed a statistically significant decrease in DPOAE S/N-R responses in Group 1 ($p<0.01$). In contrast, this important difference was not observed at 8 hours ($p>0.05$).

There was a substantial increase in DPOAE S/N-R response ($p>0.05$). However, long-term salicylate administration's differences were insignificant (Fig. s 3 and 4). Starting from 8 hours, the increase and the DPOAE S/N-R responses declined to baseline values within two weeks. Figure 3 shows no statistically significant difference between the DPOAE S / N-R responses at Group 2 frequencies ($p>0.05$). There is no statistically significant difference between Group 2 and Group 1 ($p>0.05$) at any of the frequencies during long-term sodium salicylate administration.

The present research investigated GLUT5 gene promoter methylation in cochlear DNA from guinea pigs via a methylation-specific polymerase chain reaction. No methylation was observed in the GLUT5 gene promoter at 2 hours after acute salicylate administration in Group 1 and Group 2 (Fig. 5). However, significant

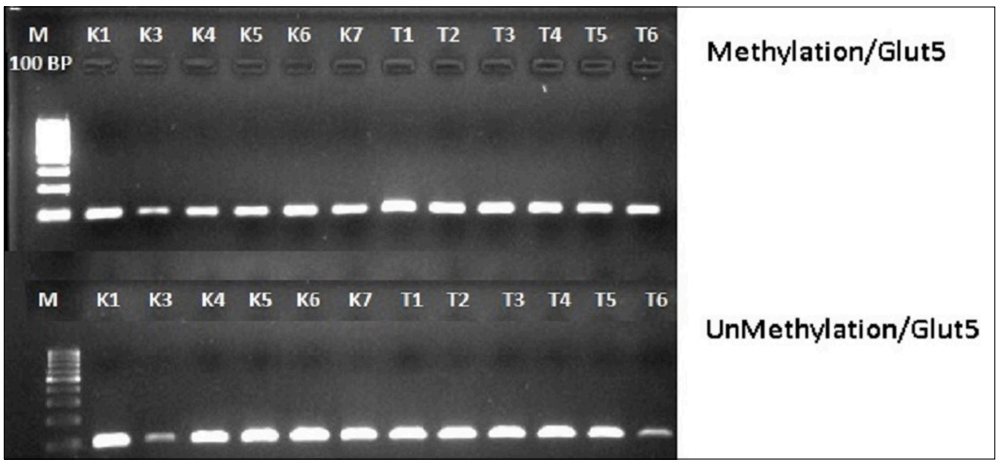

Figure 5. *Polymerase chain reaction shows increased methylation at 2 hours after sodium salicylate administration (T1–T6:Group 3). No methylation was observed in the GLUT5 gene sequence on DNA as the acute effect at 2 hours in Group 1 (K1–K4) and Group 2 (K4–K7).*

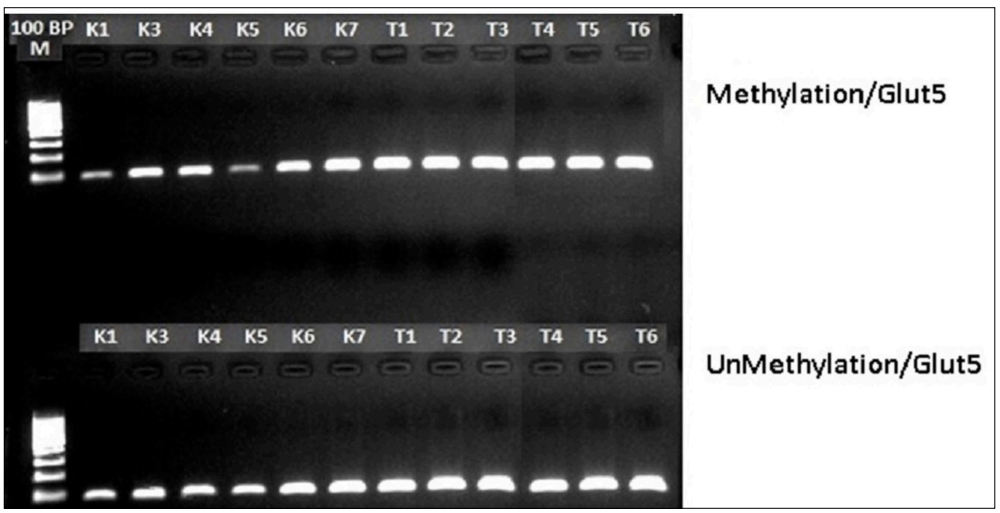

Figure 6. *Polymerase chain reaction results in all groups at two weeks (K1–K4:Group 1; K4–K7:Group 2; T1–T6:Group 3). No methylation was observed in the GLUT5 gene sequence on DNA, as the chronic effect was two weeks in any of the groups.*

methylation was observed with acute administration in Group 3 (Fig. 5). On the other hand, there was no methylation in the GLUT5 gene with chronic sodium salicylate administration (Fig. 6). In chronic salicylate administration, GLUT5 was shown to be unmethylated, showing the potential activity in the GLUT5 gene. Significant methylation was observed in the GLUT5 gene on DNA as the acute effect (Figure 5); however, a chronic effect on DNA was not observed in the same gene during sodium long-term salicylate administrations (Figure 6). Whereas DNA unmethylation of the region revealed the activated GLUT5 gene, DNA was not methylated in GLUT5 gene Group 1 and Group 2 (Fig. 6) under the effect of chronic salicylate administration at two weeks.

Discussion

Several studies have shown that age, developmental, and environmental factors (e.g., stress) may cause gene silencing by methylation of nucleotide sequences[29]. While this appears to cause only chemical changes in DNA by methylation/demethylation under the circumstances, it has also been correlated with gene activation/inhibition, for example, carcinogenesis. Salicylates are well-known ototoxic agents[24–27]. The acute effect of a single-dose salicylate injection reversibly reduces the mobility of outer hair cells. In contrast, chronic salicylate administration increases neuronal activity, often causing reversible hearing loss and tinnitus[25].

As a membrane protein, prestin can selectively transport the sugar molecule[4] on the lateral wall of OHCs. Salicylates can affect GLUT5[1], as well as affect sugar transport in guinea pig OHCs[19]. In recent studies, decreased DPOAE values were observed in the cochlea's outer hair cells under the acute effect of a single salicylate injection, while these values are back to baseline with chronic administration. In the present study, S/N-R levels in DPOAE measurements were significantly reduced when 200 mg/kg sodium salicylate was administered acutely by subcutaneous injection[30]. Studies on prestin have reported a potential hearing loss and decreased DPOAE responses in prestin-knockout experimental animals[5,24,26].

Sodium salicylate is known to block the OHC electromotility response[15]. Prestin acts as an extrinsic voltage sensor by binding Cl ions. Acting as an antagonist and competing with Cl ions, sodium salicylate competitively binds to the Cl-binding sites of prestin, by inhibiting OHC electromotility[13]. However, during long-term administration of sodium salicylate, S/N-R values significantly increase in DPOAE measurements. Salicylate binding to the Cl-binding sites of prestin has been found to have an acute effect on outer hair cells (OHCs), leading to their inactivation. This is because salicylates bind strongly with chloride ions, which are necessary for OHCs' normal functioning. Our findings show long-term salicylate administration may alter chloride ion concentrations and increase electromotile OHC responses. We think GLUT5 methylation during the acute effect of salicylate administration may block GLUT5 expression. As shown in our previous study[24], the decrease in DPOAE recordings during the acute effect suggests a possible association with the blockade of prestin.

Conclusion

Our current results have shown that salicylate administration may increase the methylation of GLUT5 during acute administration but return to baseline levels with chronic administration. This study suggests that epigenetic regulation of fructose transport may be prestin-dependent. Consequently, prestin-mediated transport of fructose may be associated with OHC electromotility.

The main result of this study is the methylation observed in the GLUT5 gene, which is a fructose transporter, under the acute effect of salicylate administration, and methylation of GLUT5 returned to baseline levels during chronic administration.

Ethics Approval

In this study, we used 12 healthy young or adult pigmented Cavia porcellus (Guinea pigs) with auropalpebral reflexes (weight range, 200–320 g) upon obtaining the approval of the Animal Care and Use Committee of the University on October 6, 2015.

References

1. Ashmore J. Cochlear outer hair cell motility. Physiol Rev. 2008;88(1):173–210.
2. Fettiplace R. Hair cell transduction, tuning, and synaptic transmission in the mammalian cochlea. Comprehensive Physiology. 2011;7(4):1197–227.
3. Kachar B, Brownell WE, Altschuler R, Fex J. Electrokinetic shape changes of cochlear outer hair cells. Nature. 1986;322(6077):365–8.
4. Zheng J, Shen W, He DZ, Long KB, Madison LD, Dallos P. Prestin is the motor protein of cochlear outer hair cells. Nature. 2000;405(6783):149–55.
5. Dallos P, Wu X, Cheatham MA, Gao J, Zheng J, Anderson CT, et al. Prestin-based outer hair cell motility is necessary for mammalian cochlear amplification. Neuron. 2008;58(3):333–9.
6. Bai JP, Navaratnam D, Santos-Sacchi J. Prestin kinetics and corresponding frequency dependence augment during early development of the outer hair cell within the mouse organ of Corti. Sci Rep. 2019;9(1):16460.
7. Nakazawa K, Spicer SS, Schulte BA. Postnatal expression of the facilitated glucose transporter, GLUT 5, in gerbil outer hair cells. Hear Res. 1995;82(1):93–9.
8. Wu X, Wang X, Gao J, Yu Y, Jia S, Zheng J, et al. Glucose transporter 5 is undetectable in outer hair cells and does not contribute to cochlear amplification. Brain Res. 2008;1210:20–8.
9. Parham K, Sohal M, Petremann M, Romanet C, Broussy A, Tran Van Ba C, et al. Noise-induced trauma produces a temporal pattern of change in blood levels of the outer hair cell biomarker prestin. Hear Res. 2019;371:98–104.
10. Wu X, Currall B, Yamashita T, Parker LL, Hallworth R, Zuo J. Prestin-prestin and prestin-GLUT5 interactions in HEK293T cells. Dev Neurobiol. 2007;67(4):483–97.
11. Matsuoka K, Wada K, Miyasaka Y, Yasuda SP, Seki Y, Nishito Y, et al. OHC-TRECK. A Novel System Using a Mouse Model for Investigation of the Molecular Mechanisms Associated with Outer Hair Cell Death in the Inner Ear. Sci Rep. 2019;9(1):5285.
12. Yang K, Huang ZW, Liu ZQ, Xiao BK, Peng JH. Long-term administration of salicylate enhances prestin expression in rat cochlea. Int J Audiol. 2009;48(1):18–23.

13. Oliver D, He DZ, Klocker N, Ludwig J, Schulte U, Waldegger S, et al. Intracellular anions as the voltage sensor of prestin, the outer hair cell motor protein. Science. 2001;292(5525):2340–3.
14. Hakizimana P, Fridberger A. Effects of salicylate on sound-evoked outer hair cell stereocilia deflections. Pflugers Arch. 2015;467(9):2021–9.
15. Tunstall MJ, Gale JE, Ashmore JF. Action of salicylate on membrane capacitance of outer hair cells from the guinea-pig cochlea. J Physiol. 1995;485(Pt 3)(3):739–52.
16. Chen B, Wang Y, Geng M, Lin X, Tang W. Localization of Glucose Transporter 10 to Hair Cells' Cuticular Plate in the Mouse Inner Ear. Biomed Res Int. 2018;2018:7817453.
17. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003;33 Suppl (3):245–54.
18. Brenet F, Moh M, Funk P, Feierstein E, Viale AJ, Socci ND, et al. DNA methylation of the first exon is tightly linked to transcriptional silencing. PLoS One. 2011;6(1):e14524.
19. Geleoc GS, Casalotti SO, Forge A, Ashmore JF. A sugar transporter as a candidate for the outer hair cell motor. Nat Neurosci. 1999;2(8):713–9.
20. Chang A, Chen P, Guo S, Xu N, Pan W, Zhang H, et al. Specific Influences of Early Acoustic Environments on Cochlear Hair Cells in Postnatal Mice. Neural Plast. 2018;2018:5616930.
21. Jiang M, Karasawa T, Steyger PS. Aminoglycoside-Induced Cochleotoxicity: A Review. Front Cell Neurosci. 2017;11:308.
22. Chen GD, Kermany MH, D'Elia A, Ralli M, Tanaka C, Bielefeld EC, et al. Too much of a good thing: long-term treatment with salicylate strengthens outer hair cell function but impairs auditory neural activity. Hear Res. 2010;265(1–2):63–9.
23. Radziwon KE, Stolzberg DJ, Urban ME, Bowler RA, Salvi RJ. Salicylate-induced hearing loss and gap detection deficits in rats. Front Neurol. 2015;6:31.
24. Bulut E, Budak M, Ozturk L, Turkmen MT, Uzun C, Sipahi T. DNA methylation of the prestin gene and outer hair cell electromotileresponse of the cochlea in salicylate administration. Turk J Med Sci. 2017;47(5):1626–33.
25. Huang ZW, Luo Y, Wu Z, Tao Z, Jones RO, Zhao HB. Paradoxical enhancement of active cochlear mechanics in long-term administration of salicylate. J Neurophysiol. 2005;93(4):2053–61.
26. Yu N, Zhu ML, Johnson B, Liu YP, Jones RO, Zhao HB. Prestin up-regulation in chronic salicylate (aspirin) administration: an implication of functional dependence of prestin expression. Cell Mol Life Sci. 2008;65(15):2407–18.
27. Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. Bioinformatics. 2002;18(11):1427–31.
28. Bharati J, Dangi SS, Mishra SR, Chouhan VS, Verma V, Shankar O, et al. Expression analysis of Toll like receptors and interleukins in Tharparkar cattle during acclimation to heat stress exposure. J Therm Biol. 2017;65:48–56.
29. Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD, Hanson MA. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. Pediatric research. 2007;61(7):5–10.
30. Ralli M, Troiani D, Podda MV, Paciello F, Eramo S, De Corso E, et al. The effect of the NMDA channel blocker memantine on salicylate-induced tinnitus in rats. Acta Otorhinolaryngologica Italica. 2014;34(3):198.