Investigation of Some Ion Channel Expressions in Cochlear Nucleus of Tinnitus Induced Rats*

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Abstract

Aim: The aim of this study is to gain a better understanding of how certain ion channels play a role in the molecular mechanisms of salicylate- and noise-induced tinnitus.

Method: The present study was conducted on thirty-two, 4-month-old, male Wistar Albino rats. Rats were equally divided into four groups; two experimental groups and two control groups. The assessment of tinnitus was based on a behavioral test which was modified from the conditional suppression method. Tinnitus was induced by sodium salicylate administration and noise exposure in rats in which the suppression ratios were zero (0). All animals in both experimental and control groups were decapitated in deep anaesthesia for 2 h after salicylate or saline administration and noise exposure, consecutively. Tissues from the left and right cochlear nucleus were dissected immediately in ice-cold RNA later (Invitrogen). Before reverse transcription, the RNA pools were arranged. Quantitative changes in HCN1, HCN2, HCN4, SCN1A, SCN2A1, SCN3A, TRPM2, TRPM7 and GAPDH mRNA expressions in the cochlear nucleus in both experimental and control groups were examined by quantitative real-time PCR method. Statistical data were analysed using the SPSS 21 program (Version 21.0, SPSS Inc., Chicago, IL, USA) with the Kruskal-Wallis and Mann-Whitney U tests.

Results: Fold changes in the expression levels of SCNA1, SCN2A1, SCN3A, TRPM2, TRPM7, CACNA1B, HCN1, HCN2 and HCN4 genes in both salicylate-induces tinnitus (SAT) and noise-induced tinnitus (NT) groups compared with the control group. According to these data, it is seen that the mRNA levels of all genes are lower in the cochlear nucleus area of the rats in both SAT and NT groups than in the control group. Considering each of these genes in NT group: SCNA1, SCN3A, TRPM7 genes slightly decreased; SCN2A1,

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ETHICAL STATEMENT: The ethical committee approval for the study was obtained from Fırat University, Animal Experiments Ethical Committee (Date: 06/12/2012, Number: 117) and the study was conducted in accordance with the principles of the Helsinki Declaration.

TRPM2, HCN1 and HCN4 genes slightly increased compared with the SAT group. For HCN2 gene fold changes were nearly the same in the NT and SAT groups.

Conclusion: The findings of this study suggest that tinnitus generation may be closely related to alterations in several key ion channel families activity including voltage-gated calcium channels, hyperpolarization-activated cyclic nucleotide–gated (HCN) channels, transient receptor potential (TRP) channels, voltage-gated sodium channels within the CN, specifically in response to salicylate-induced and noise-induced tinnitus models.

Keywords: Ion channels,qPCR, rat, tinnitus.

Kulak Çınlaması Oluşturulmuş Sıçanların Koklear Çekirdeklerinde Bazı İyon Kanalı Ekspresyonlarının İncelenmesi

Öz

Amaç: Bu çalışmanın amacı, belirli iyon kanallarının salisilat ve gürültü ile indüklenen tinnitusun moleküler mekanizmalarında nasıl bir rol oynadığını daha iyi anlamaktır.

Yöntem: Çalışma, 32 tane 4 aylık erkek Wistar Albino sıçanlar üzerinde gerçekleştirilmiştir. Sıçanlar, iki deney ve iki kontrol grubu olmak üzere dört gruba eşit olarak bölünmüştür. Tinnitus değerlendirmesi, koşullu baskılama yönteminden modifiye edilen bir davranış testine dayanmaktadır. Tinnitus, baskılama oranları sıfır (0) olan sıçanlarda sodyum salisilat uygulaması ve gürültü maruziyeti ile indüklenmiştir. Tüm deney ve kontrol gruplarındaki hayvanlar, salisilat veya salin uygulamasından ve ardışık gürültü maruziyetinden 2 saat sonra derin anestezi altında dekapite edilmiştir. Sol ve sağ koklear çekirdek dokuları hemen soğuk RNA later (Invitrogen) içersinde diseke edilmiştir. Ters transkripsiyondan önce, RNA havuzları düzenlenmiştir. Her iki deney ve kontrol grubundaki koklear çekirdekte HCN1, HCN2, HCN4, SCN1A, SCN2A1, SCN3A, TRPM2, TRPM7 ve GAPDH mRNA ekspresyonlarındaki niceliksel değişiklikler, nicel gerçek zamanlı PCR yöntemi ile incelenmiştir. İstatistiksel veriler, Kruskal-Wallis ve Mann-Whitney U testleri ile SPSS 21.0 programı (Version 21.0, SPSS Inc., Chicago, IL, USA) kullanılarak analiz edilmiştir.

Bulgular: SCNA1, SCN2A1, SCN3A, TRPM2, TRPM7, CACNA1B, HCN1, HCN2 ve HCN4 genlerinin ekspresyon düzeylerindeki katlamalı değişiklikler, hem salisilat ile indüklenen tinnitus (SAT) hem de gürültü ile indüklenen tinnitus (NT) grupları ile kontrol grubu arasında karşılaştırıldı. Bu verilere göre, tüm genlerin mRNA seviyelerinin, hem SAT hem de NT gruplarındaki sıçanların koklear çekirdek alanında kontrol grubundan daha düşük olduğu görülmektedir. NT grubundaki her bir bu genleri dikkate alındığında: SCNA1, SCN3A, TRPM7 genleri hafifçe azalmış, SCN2A1, TRPM2, HCN1 ve HCN4 genleri SAT grubu ile karşılaştırıldığında hafifçe artmıştır. HCN2 geni için katlanma değişiklikleri NT ve SAT gruplarında neredeyse aynıdır.

Sonuç: Bu çalışmanın bulguları, tinnitus oluşumunun salisilatla indüklenmiş ve gürültüyle indüklenmiş tinnitus modellerine yanıt olarak, özellikle CN içindeki voltajlı kalsiyum kanalları, hiperpolarizasyon-aktive siklik nükleotid-gated (HCN) kanalları, geçici reseptör potansiyeli (TRP) kanalları, voltajlı sodyum kanalları gibi birkaç önemli iyon kanalı ailesinin aktivitesindeki değişikliklerle yakından ilişkili olabileceğini önermektedir.

Anahtar Sözcükler: İyon kanalları, qPCR, sıçan, tinnitus.

Introduction

Subjective tinnitus is a universal disorder defined as a phantom sound that is pathologically interpreted by the brain as an acoustic signal that arises from abnormal neural activity in the auditory pathway without any acoustic source from outside or inside the body. It affects approximately 10-15% of the population, and about 20% of this rate needs medical attention because their quality of life is adversely affected. This condition develops especially in quiet environments and is literally qualified as "an annoying situation" by patients suffering from tinnitus[1-5].

Determining the presence of tinnitus in humans and its characteristic in the presence of tinnitus is not difficult. For this purpose, there are experimental studies created with noise or salicylate, and which are important for determining the general characteristics of tinnitus and investigating its relationship with the sounds in the body[6]. However, the use of animal models is imperative to investigate the physiological basis of tinnitus and to conduct experimental studies for potential treatment trials. However, determining the presence of tinnitus in animals is not so simple. For this purpose, Jastreboff, the poineer of the animal behavior test subjected animals to a paradigm based on a conditioned suppression method before inducing tinnitus with noise or salicylate and determination of the eligibility of this test[7-12].

In animals, as in humans, tinnitus can be induced by two methods: exposure to excessive noise and administration of sodium salicylate (or quinine). When the two methods are compared, it is seen that in the noise-induced tinnitus model, acoustic trauma is induced by exposing the subjects to excessive sound and various animal models have proven that noise exposure produces tinnitus[13-15] and in Jastreboff's model, tinnitus can be induced when 350 mg/kg dose of sodium salicylate is injected intraperitoneally for two consecutive days to experimental animals as well[8,16].

Although there is a lack of clarity on the mechanisms and anatomical initiation point underlying the development of tinnitus, the thought that decreased excitatory input in the auditory cortex is due to cochlear deafferentation through increased spontaneous firing rate in the cochlear nucleus, is strongly believed. The cochlear nucleus is the first center in the auditory pathway that processes acoustic signals from direct projections of the auditory nerve. In addition to cellular changes, changes at the molecular levels could be responsible for the pathophysiology of tinnitus[17-19].

In the last decade, animal research has clearly demonstrated that tinnitus is a pathology of synaptic plasticity[20]. Although many studies have been conducted to determine the basis of these mechanisms, they have not been completely clarified yet. The main purpose of this study is to gain a better understanding of how certain ion channels play a role in the molecular mechanisms of salicylate- and noise-induced tinnitus.

Material and Methods

Subjects

The present study was conducted on thirty-two, 4-month-old, male Wistar Albino rats weighing between 195 and 330 g. Rats were equally divided into four groups; two experimental groups and two control groups. In behaviour tests, twenty rats subjected to training. The experimental protocols were approved by the Local Animal Use Committees of Fırat University (Elazığ, Turkey), dated December 6, 2012, study number 117. The study was funded by Fırat University Scientific Research Projects Unit (FUBAP), study number VF.11.12 and was conducted at the Experimental Research Unit of Fırat University (FUDAM).

Assessment of Tinnitus

Behavioral tests were carried out by a conditional suppression method based on Jastreboff and colleagues' studies[8,21]. To evaluate the ability to combine silence and licking behavior of rats, a suppression ratio (SR) was calculated which is according to licking numbers (LN) of rats both during background sound is on and off. The formula is given below:

$$SR = (LN\ sound\ off/2min) / [(LN\ sound\ off/2min) + (LN\ sound\ on/28min)]$$

Suppression ratios between 0 and 0.4 were admitted to be successful at the end of this training session. According to behavioral test, rats with suppression ratios ranging from 0 to 0.2 were included in the experimental groups.

Tinnitus was induced by sodium salicylate administration in rats in which the suppression ratios were zero (0). 2 h after salicylate administration, the main 30-minute procedure was applied as in initial lick training. All licking numbers were counted and SRs were calculated once more to compare the difference between the two conditions. Suppression ratios equal to 0.4 or greater than 0.4 were admitted to perceive tinnitus.

Administration of Sodium Salicylate

According to Jastreboff's previous studies, sixteen rats were divided into two groups. Eight rats received a single dose of 350 mg/kg subcutaneous injection of sodium salicylate 2 h before decapitation as a 2-h post-salicylate injection group. The other eight rats received the same volume of saline as the control group[15,16].

Noise Exposure

Eight rats in the noise exposure group were anesthetized by xylazine/ketamine anesthesia supported by atropine administration. A pure tone of 10 kHz was delivered at 110 dB SPL for 3 h to induce tinnitus. Acoustic stimuli were calibrated at the head level of the rats. Eight rats in the control group were similarly anesthetized but unexposed to noise overexposure.

Quantitative Real-Time PCR Analysis

All animals in both experimental and control groups were decapitated in deep anaesthesia for 2 h after salicylate or saline administration and noise exposure, consecutively. Tissues from the left and right cochlear nucleus were dissected immediately in ice-cold RNA later (Invitrogen) under a stereomicroscope; placed in Rnase-free tubes and frozen in liquid nitrogen.

Total RNA was extracted from each frozen tissue; hence, frozen tissues were homogenised in Bullet Blender® 50 with Bullet Blender® Bead Lysis Kit according to the instructions of the kit. Supernatants were collected in Rnase-free tubes. Total RNA isolation was made with PureLink™ RNA Mini Kit. Total RNA levels in each tube were measured using Qubit 2.0 Fluorometer and calculated in mg/mL. Before reverse transcription, the RNA pools were arranged. Quantitative changes in HCN1, HCN2, HCN4, SCN1A, SCN2A1, SCN3A, TRPM2, TRPM7 and GAPDH mRNA expressions in cochlear nucleus in both experimental and control group were examined by quantitative real-time PCR method. Quantitative real-time PCR reactions were performed in Applied Biosystems 7500 Fast Real-Time PCR System and quantified by Applied Biosystem TaqMan primers designed specifically for rats. The data were collected and analysed using Biosystems 7500 Fast Real-Time PCR System Software. Rat GAPDH, an endogenous housekeeping gene was selected as an internal control. Each sample determination was performed in triplicate[21]. Statistical data were analysed using the IBM SPSS 21 program with the Kruskal-Wallis test.

Statistics: Data were presented as the mean ± standard deviation and the $p<0.05$ value was considered significant. HCN1, HCN2, HCN4, SCN1A, SCN2A1, SCN3A, TRPM2, TRPM7, expression levels in the cochlear nucleus tissue of the salicylate induced tinnitus (SAT), noise-induced tinnitus (NT) and control groups were analyzed by the Kruskal-Wallis test following the Mann-Whitney U test to define the diversity among the groups.

Results

SRs of the behavior test performed before tinnitus induction were calculated as zero (0) for ten rats in the SAT group and eight rats in the NT group. The rest of the rats in the NT group's SR were 0,1 and 0,2. Because these results provided the required "SR≤ 0.4" condition, tinnitus was induced in both groups according to their own procedures (Figure 1 and Table 1).

Figure 1. Supression rates of the rats in the salicylate-induced tinnitus group and noise-induced tinnitus group.

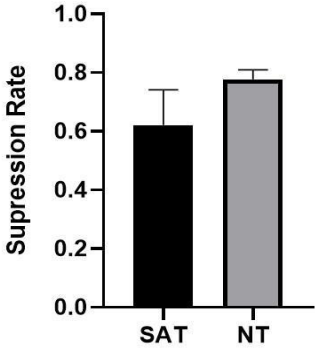


Table 1. Statistical analysis of suppression rates of the rats in salicylate-induced tinnitus group and noise-induced tinnitus group.

Group	Number	Minimum	Maximum	Mean	SD
SAT	10	0,44	0,78	0,6214	0,11986
NT	10	0,72	0,83	0,762	0,03170

There was no difference between the HCN1 and HCN2 channel groups but for the HCN4 channel groups there was a statistical difference between the control to HCN4 SAT group, control to HNC4 NT group and HCN4 SAT group to HCN4 NT group ($p<0.01$) (Table 2).

The statistical difference between every group of SCNA1, SCNA2A1 and SCN3A groups was significant. There was a statistical difference between the control to SCNA1 SAT group, control to SCNA1 NT group and SCNA1 SAT group to SCNA1 NT group; the control to SCN2A1 SAT group, control to SCN2A1 NT group and SCN2A1 SAT group to SCN2A1 NT group; the control to SCN3A SAT group, control to SCN3A NT group and SCN3A SAT group to SCN3A NT group ($p<0.05$) (Table 2).

The statistical difference between every group of TRPM2 and TRPM7 groups was significant. There was a statistical difference between the control to TRPM2 SAT group, control to TRPM2 NT group and TRPM2 SAT group to TRPM2 NT group; the control to TRPM7 SAT group, control to TRPM7 NT group and TRPM7 SAT group to TRPM7 NT group ($p<0.05$) (Table 2).

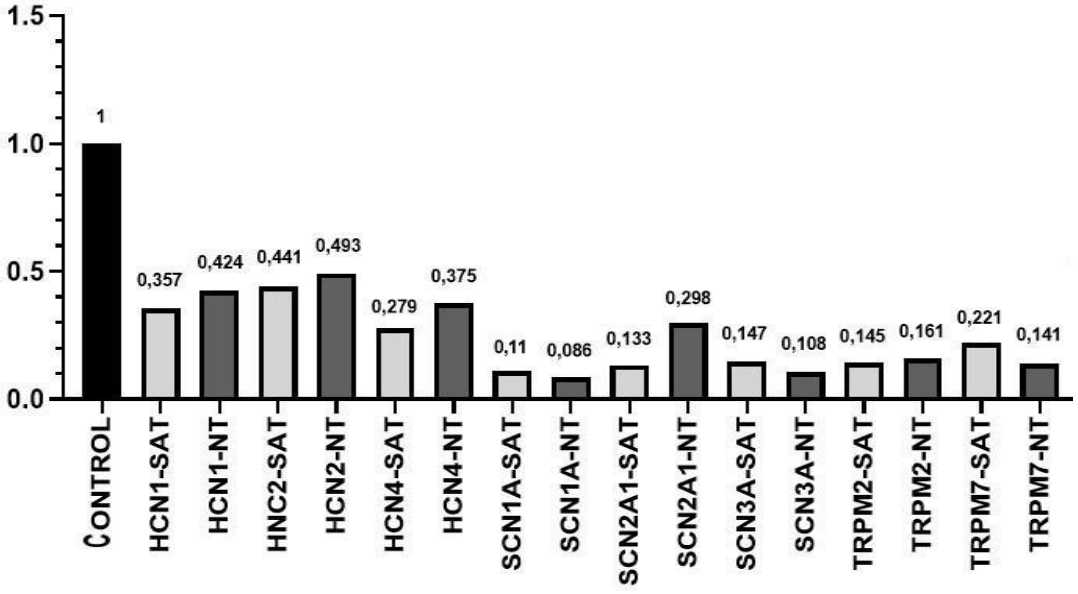
Figure 2 shows the fold changes in the expression levels of SCNA1, SCN2A1, SCN3A, TRPM2, TRPM7, HCN1, HCN2 and HCN4 genes in both salicylate-induces tinnitus (SAT) and noise-induced tinnitus (NT) groups compared with the control group. According to these data, it is seen that the mRNA levels of all genes are lower in the cochlear nucleus area of the rats in both SAT and NT groups than in the control group. Considering each of these genes in NT group: SCNA1, SCN3A, TRPM7 genes slightly decreased; SCN2A1, TRPM2, HCN1 and HCN4 genes slightly increased compared with the SAT group. For HCN2 gene fold changes were nearly the same in the NT and SAT groups.

Table 2. Statistical analysis of expression levels of ion channels in cochlear nuclei of rats in control group, salicylate-induced tinnitus group and noise-induced tinnitus group.

	Control Group				NT Group				SAT Group			
Ion Channel	**n**	**CT Mean**	**CT SD**	**ΔCT Mean**	**n**	**CT Mean**	**CT SD**	**ΔCT Mean**	**n**	**CT Mean**	**CT SD**	**ΔCT Mean**
HCN1*	3	28.03	0.066	-0.138	3	26.24	0.094	1.097	3	26.21	0.308	1.346
HCN2*	3	26.10	0.253	-2.068	3	24.09	0.029	-1.050	3	23.97	0.105	-0.888
HCN4*	3	29.08	0.092	0.91	3	27.46	0.043	2.321	3	27.61	0.042	2.746
SCN1A**	3	25.64	0.179	-4.986	3	24.32	0.078	-1.452	3	23.87	0.079	-1.807
SCN2A1**	3	28.93	0.062	-0.729	3	25.82	0.098	0.049	3	26.88	0.116	1.203
SCN3A**	3	29.90	0.043	-1.129	3	28.25	0.157	2.472	3	27.71	0.127	2.036
TRPM2M**	3	30.80	0.242	0.173	3	28.58	0.281	2.806	3	28.64	0.025	2.956
TRPM7**	3	29.45	0.045	-1.129	3	27.46	0.897	1.688	3	26.72	0.191	1.046

*p<0.01, **p<0.05

Figure 2. Fold changes of the ion channels in cochlear nuclei of rats in control group, salicylate-induced tinnitus group and noise-induced tinnitus group.



Discussion

Hyperpolarization-activated cyclic nucleotide–gated (HCN) channels, which are essential membrane proteins, are recognized as nonselective voltage-gated cation channels. Their function relies on the binding of cAMP to β-adrenergic receptors present in the plasma membranes of cardiomyocytes and neurons. The activation of HCN channels is triggered by membrane hyperpolarization, and they exhibit a high conductivity to Na+ and K+ ions, with a ratio ranging from 5:1 to 3:1. The HCN channels are the products of four genes, namely HCN1-4, and are extensively expressed in the heart as HCN1, HCN2, and HCN4, and in the central nervous system as HCN1-4[22-24]. In noise-induced tinnitus, it is suggested that downregulated expression of HCN channels has a protective effect against hyperactivity in fusiform cells of the dorsal cochlear nucleus (DCN) by generating hyperpolarization. However, the present study shows that decreased levels of HCN channels in the CN resulted in tinnitus, probably depending on the blockade of excitatory activity at this level causing an excitatory effect in the upper levels. In salicylate-induced tinnitus, blockade of calcium channels may result in loss of GABAergic transmission that leads to tinnitus by activating upper levels of the auditory pathway. The present study has supportive data that show decreased levels of HCN

chanels in the CN which may be responsible for enhanced evoked activity. It may be a consequence of an effort to normalize spontaneous spike rates in the auditory pathway[25].

In terms of the relationship between expression levels of HCN channels and N-type voltage-dependent calcium channels, downregulation of all of the given genes indicates suppressed HCN channel activity leading to prolong hyperpolarization which means limiting voltage-gated calcium channels and because of low intracellular calcium concentration reduction of NMDA and GABA mediated transmission, considering in both noise and salicylate induced tinnitus. Lack of stability in essential roles of HCN channels in neuronal excitability, rhythmic neuronal activity, dendritic integration, and synaptic transmission may cause a plastic chance to compensate for decreased excitatory activation of the ventral cochlear nucleus (VCN). There is a relief of inactivation of T- and N-type Ca^{2+} channels by resting membrane potential hyperpolarization that contributes to enhanced excitatory postsynaptic potential summation following pharmacological blockade of HCN channels, supporting the general principle that inducing tinnitus depends on decreased levels of excitatory stimuli while increased levels of inhibitory stimuli or vice versa[26-28].

TRPM7 gene belongs to the melastatin subfamily of the transient receptor potential family which encodes an ion channel and a serine/threonine protein kinase. TRPM7 channels are involved in increasing intracellular calcium levels and regulating magnesium ion homeostasis. As is known, magnesium is the second intracellular cation participating in synaptic transmission and preventing ion flow at resting membrane potentials by blocking NMDA receptors. It suppresses the overall excitability of neurons by decreasing the spontaneous firing rate. In addition, TRPM7 is also an important regulator of cellular Zn^{2+} which plays an antioxidant role under stressful conditions[29,30]. The present study is the first to observe expression levels of TRPM7 channels in the CN of rats in tinnitus models. According to the present study, downregulated TRPM7 gene expression was observed in the CN of rats, both in salicylate- and noise-induced tinnitus. Decreased levels of TRPM7 expression shows the same effect as the decreased levels of CaCN1b expression effect. Directly and indirectly, blockade of the channel by inhibition of GABAergic and NMDA-mediated transmission, intracellular calcium concentration is reduced, hyperpolarization time is prolonged and neuronal excitability is reduced. Decreased numbers of all ion channels related to the transportation of calcium ions may be one of the main reasons of the generation of tinnitus. On the other hand, reduced

neuronal excitability may be due to blockade of potassium conductances and GABA(A) receptors by magnesium ions or increased activity of the Na/Mg antiport mechanism.

TRPM2 is a member of the transient receptor potential melastatin family of cation channels that are permeable to Ca^{2+}, Na^{+}, and K^{+} cation channels. Their activation is induced by ADPR and NAD. Also, Ca^{2+} plays a critical role in this activation. As possible mechanism of intracellular Ca^{2+} is to enhance channel sensitivity to ADPR. TRPM2 is expressed in various tissues, widely including the brain, bone marrow, and cells of the immune system. In salicylate-induced tinnitus, according to Bal's study, inhibiting TRPM2 ion channels with flufenamic acid in the cochlear nuclei alters neural activity in the central auditory pathway by reducing neuronal excitability. This results in an amplification of inhibitory signals within the auditory nuclei, consequently leading to a reduction in signals that generate tinnitus. However, in the present study, our data shows that, contrary to the findings of Bal et al., the expression levels of TRPM2 in the cochlear nuclei of rats manifesting behavioral tinnitus are lower than those in the control group. Lower intracellular calcium levels may lead to prolonged hyperpolarization in neurons, which can alter the balance between inhibition and excitation[31-33].

In noise-induced tinnitus, to some extent, cochlear and cochlear nuclear damage was demonstrated in recent studies. It was reported that these damages caused increased spontaneous firing rates[18,34]. The present study is the first study for the expression changes of TRPM2 channels in rats exposed to noise exposure, and the results show the downregulation of these channels. In light of these data, unlike the general idea, decreased spontaneous firing rates related to low intracellular calcium levels may be expected through prolonged hyperpolarization.

Voltage-gated Na channels (VGSCs), a class of transmembrane proteins, are predominantly found on the membranes of excitable cells like neurons. Their primary function is to facilitate the transport of Na across the membrane and to convert electrical currents into transmitter release from the nerve terminal. The Voltage-Gated Sodium Channels (VGSCs) are crucial ion channels necessary for neuronal cells to generate excitability and perform normal physiological functions. They play a significant role in the rapid rising phase and the initial component of the falling phase of action potentials. In their resting state, they exist in a closed conformation. However, when the membrane potential is depolarized, the channels briefly open and then inactivate. Sodium channels have a refractory period during which they return to availability at rest. In mammals, ten VGSC subtypes have been identified, known as Nav1.1-Nav1.9 and NavX, based on

differences in the α-subunits. These are classified as “central” (NaV1.1, 1.2, 1.3, 1.6) and “peripheral” (NaV1.7, 1.8, 1.9) subunits, depending on their expression patterns. Recent studies have shown that Nav1.1, Nav1.2, and Nav1.3 are widely expressed in the rat central nervous system[35].

Given the significance of sodium currents in neurons, Liu et al. suggested that the blockade of sodium channels by salicylate could be due to the interaction of salicylate with the channel in its resting state. Even though salicylate might bind to the channel when it’s open, no alterations were observed in the voltage dependence of the activation state. It is hypothesized that when the Na current is inhibited by salicylate, salicylate interacts with the channel in its inactive state, without affecting the voltage dependence of activation. The shift of the inactivation curve of the Na current towards hyperpolarization, and the delayed recovery trajectory of the Na currents, are thought to be a result of the blockade by salicylate[35,36]. Considering these data, a supportive result was obtained from the present study. Compared with the control group there was a significant downregulation in the expression of SCNA1, SCN2A1 and SCN3A genes in the cochlear nucleus of rats showing behavioral evidence of salicylate-induced tinnitus. Downregulation may be a blocking effect of salicylate, which leads to shift the balance of the VCN neurons to inactivation. Considering both the downregulation and the blockage of sodium channels by salicylate, it seems that they play a synergistic role in the mechanism of tinnitus.

Regarding noise-induced tinnitus, Yin’s research indicated a notable reduction in the Nav1.6 channel within the anterior ventral cochlear nucleus (AVCN) of rats subjected to noise-induced tinnitus[36]. It was also observed that the downregulation of Nav1.6 in inhibitory neurons could result in diminished neural excitability of these neurons, potentially leading to increased activity in the DCN of tinnitus-affected rats. Furthermore, Nav1.6 might play a role in altering the balance between inhibition and excitation, thereby contributing to the heightened activity in the AVCN associated with tinnitus. The results of the present study suggested the same idea that downregulated expression of SCNA1, SCN2A1 and SCN3A genes in the VCN after noise-induced tinnitus play a critical role in the generation of tinnitus by increasing hyperactivity through decreasing excitability of the neurons[36]. Fryatt’s study revealed through qPCR data that there was a reduction in the expression of NaV1.1 and NaV1.6 mRNA in the spiral ganglion after noise exposure, when compared to the control group of rats. Considering results of the present study, it was assumed that decreased levels of sodium channels at

the beginning of the auditory pathway may have the same pattern in the lower parts of the pathway, which may be a compensation mechanism to fix the imbalance of excitability ability[37].

Conclusion

In conclusion, the findings of this study suggest that tinnitus generation may be closely tied to alterations in several key ion channel families activity including voltage-gated calcium channels, hyperpolarization-activated cyclic nucleotide–gated (HCN) channels, transient receptor potential (TRP) channels, voltage-gated sodium channels, and voltage-gated potassium channels, within the CN, specifically in response to salicylate exposure and noise-induced tinnitus models.

Notably, the study revealed that the downregulation of various ion channel genes within the CN may contribute to the development of tinnitus. This downregulation appears to result in several important effects, including; prolonged hyperpolarization of neurons, which may disrupt the balance between excitation and inhibition; reduced calcium influx, leading to decreased neurotransmitter release, particularly for inhibitory neurotransmitters like GABA; potential shifts in the balance between excitatory and inhibitory stimuli, playing a crucial role in tinnitus generation.

The results support the notion that ion channel dysregulation within the CN may play a crucial role in the development and maintenance of tinnitus, both in response to salicylate exposure and noise-induced tinnitus. Further research in this area is essential to deepen our understanding of tinnitus and to develop targeted interventions to manage and alleviate this challenging condition.

REFERENCES

1. Wilson JP, Sutton GJ. Acoustical correlates of tonal tinnitus. *CIBA Foundation Symposium*. 1981;85:82-10. doi: 10.1002/9780470720677.ch6.

2. Heller AJ. Classification and epidemiology of tinnitus. *The Otolaryngologic Clinics of North America*. 2003;36:239–248.

3. Bauer CA, Brozoski TJ. Tinnitus: Theories, Mechanisms, and Treatments. In: Schacht J, Popper AN, Fay RR, eds. Auditory Trauma, Protection and Repair. New York: Springer Science+Business Media. LLC; 2008:101-125.

4. Kizawa K, Kitahara T, Horii A, et al. Behavioral assessment and identification of a molecular marker in a salicylate-induced tinnitus in rats. *Neuroscience*. 2010;165:1323-1332.

5. Holmes S, Padgham ND. "Ringing in the ears": narrative review of tinnitus and ıts ımpact. *Biological Research for Nursing*. 2011;13(1):97-108.

6. Heffner HE, Hefner RS. *Behavioural Test For Tinnitus in Animals*. In: Eggermont JJ, Zeng FG, Popper AN, Fay RR, eds. Tinnitus. Springer Handbook of Auditory Reasearh. Newyork: Springer Science+Bussiness Media. 2012;21-58.

7. Estes WK, Skinner BF. Some quantitative properties of anxiety. *J Exp Psychol*. 1941;29:390-400.

8. Jastreboff PJ, Brennan JF, Sasaki CT. An animal model for tinnitus. *Laryngoscope*. 1988;98(3):280-286.

9. Jastreboff PJ, Brennan JF. Evaluating the loudness of phantom auditory perception (tinnitus) in rats. *Audiology*. 1994;33:202-217.

10. Jastreboff PJ, Sasaki CT. An animal model of tinnitus: a decade of development. *Am J Otol*. 1994;15:19–27.

11. Penner MJ, Jastreboff PJ. *Tinnitus: Psychophysical Observations in Humans and An Animal Model*. In: Clinical aspects of hearing. Newyork: Van De Springer; 1996:258-304.

12. Bauer CA, Brozoski TJ, Rojas R, et al. Behavioral model of chronic tinnitus in rats. *Otolaryngol Head Neck Surg*. 1999;121:457-462.

13. Kaltenbach JA, Afman CE. Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. *Hear Res*. 2000;140:165-172.

14. Bauer CA, Brozoski TJ. Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. *J Assoc Res Otolaryngol*. 2001;2:54–64.

15. Brozoski TJ, Bauer CA, Caspary DM. Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci*. 2002;22:2383–2390.

16. Jastreboff PJ, Brennan JF, Coleman JK, et al. Phantom auditory sensation in rats: an animal model for tinnitus. *Behav Neurosci*. 1988;102(6):811-822.

17. Pilati N, Large C, Forsythe ID, et al. Acoustic over-exposure triggers burst firing in dorsal cochlear nucleus fusiform cells. *Hearing Research*. 2012;283:98-106.

18. Shore S, Wu C. Mechanisms of noise-induced tinnitus: Insights from cellular studies. *Neuron*. 2019;103(1):8–20. doi: 10.1016/j.neuron.2019.05.008.

19. Han KH, Cho H, Han KR, et al. Role of microRNA-375-3p-mediated regulation in tinnitus. *International Journal of Molecular Medicine*. 2021;48:136.

20. Guitton MJ. Salicylate induces tinnitus through activation cochlear NMDA receptors. *Journal of Neuroscience*. 2003;23:3944-3952.

21. Li S, Kalappa BI, Tzounopoulos T. Noise-induced plasticity of KCNQ2/3 and HCN channels underlies vulnerability and resilience to tinnitus. *eLife*. 2015;4:e07242. doi: 10.7554/eLife.07242.

22. Puel JL, Guitton MJ. Salicylate-induced tinnitus: molecular mechanisms and modulation by anxiety. *Prog Brain Res*. 2007;166:41-146. doi: 10.1016/S0079-6123(07)66012-9.

23. Brozoski TJ, Bauer CA. The effect of dorsal cochlear nucleus ablation on tinnitus in rats. *Hearing Research*. 2005;206:227–236.

24. Shah MM. Cortical HCN channels: function, trafficking and plasticity. *J Physiol*. 2014;592(13):2711–2719.

25. Chang X, Wang J, Jiang H, et al. Hyperpolarization-activated cyclic nucleotide-gated channels: an emerging role in neurodegenerative diseases. *Front Mol Neurosci*. 2019;12:141. doi: 10.3389/fnmol.2019.00141.

26. Kaupp UB, Seifert R. Molecular diversity of pacemaker ion channels. *Annu Rev Physiol*. 2001;63:235-257.

27. Dribben WH, Eisenman LN, Mennerick S. Magnesium induces neuronal apoptosis by suppressing excitability. *Cell Death and Disease*. 2010;1:e63. doi: 10.1038/cddis.2010.39

28. Zou ZG, Rios FJ, Montezano AC, et al. TRPM7, Magnesium, and signaling. *Int J Mol Sci*. 2019;20:1877. doi: 10.3390/ijms20081877.

29. Bal R, Ustundag Y, Bulut F, et al. Flufenamic acid prevents behavioral manifestations of salicylate-induced tinnitus in the rat. *Arch Med Sci*. 2016;12(1):208–215.

30. Qaswal AB. Magnesium ions depolarize the neuronal membrane via quantum tunneling through the closed channels. *Quantum Rep*. 2020;2:57–63. doi: 10.3390/quantum2010005.

31. Olah ME, Jackson MF, Li H, et al. Ca2+-dependent induction of TRPM2 currents in hippocampal neurons. *J Physiol*. 2009;587:965-979.

32. Jiang LH, Yang W, Zou J, Beech DJ. TRPM2 channel properties, functions and therapeutic potentials. *Expert Opinion on Therapeutic Targets*. 2010;9:973-988.

33. Dehmel S, Pradhan S, Koehler S, et al. Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus possible basis for tinnitus-related hyperactivity? *Journal of Neuroscience*. 2012;32(5):1660-1671. doi: 10.1523/JNEUROSCI.4608-11.2012.

34. Wang J, Ou SW, Wang YJ. Distribution and function of voltage-gated sodium channels in the nervous system. *CHANNELS*. 2017;11(6):534–554.

35. Liu Y, Li X. Effects of salicylate on voltage-gated sodium channels in rat inferior colliculus neurons. *Hear Res*. 2004;193(1-2):68-74. doi: 10.1016/j.heares.2004.03.006.

36. Yin M, Xia C, Cong W, et al. Aberrant expression of Nav1.6 in the cochlear nucleus correlates with salicylate-induced tinnitus in rats. *Biochemical and Biophysical Research Communications*. 2020;526:786-792.

37. Fryatt AG, Mulheran M, Egerton J, et al. Ototrauma induces sodium channel plasticity in auditory afferent neurons. *Molecular and Cellular Neuroscience*. 2011;48:51-61. doi: 10.1016/j.mcn.2011.06.005.