Expression and localization of α_{2A}-adrenergic receptor in the rat post-natal developing cochlea

Chaoyong Tian, Yang Yang, Yao Li, Fei Sun, Juan Qu, Dingjun Zha

Department of Otolaryngology Head and Neck Surgery, Xijing Hospital, Air Force Medical University, Xi'an, Shannxi Province, China

ABSTRACT

Lots of adrenergic receptors (ARs) are widely present across the auditory pathways and are positioned to affect auditory and vestibular functions. However, noradrenergic regulation in the cochlea has not been well characterized. In this study, a rat model of noise-induced hearing loss was developed to investigate the expression of α_{2A}-adrenergic receptor (AR) after acoustic trauma, then, we investigated the expression of α_{2A}-AR in the developing rat cochlea using immunofluorescence, qRT-PCR, and Western blotting. We found that the expression of α_{2A}-AR significantly increased in rats exposed to noise compared with controls. Immunofluorescence analysis demonstrated that α_{2A}-AR is localized on hair cells (HCs), spiral ganglion neurons (SGNs), and the stria vascularis (SV) in the postnatal developing cochlea from the post-natal day (P) 0 to P28. Furthermore, we observed α_{2A}-AR mRNA reached a maximum level at P14 and P28 when compared with P0, while no significant differences in α_{2A}-AR protein levels at the various stages when compared with P0. This study provides direct evidence for the expression of α_{2A}-AR in HCs, SGNs, and the SV of the cochlea, indicating that norepinephrine might play a vital role in hearing function within the cochlea through α_{2A}-AR.

Key words: adrenergic receptor; hair cells; spiral ganglion neurons; stria vascularis; rat.

Correspondence: Prof. Dingjun Zha, Department of Otolaryngology Head and Neck Surgery, Xijing Hospital, Air Force Medical University, 127 Changle Western Road, 710032, Xi'an, Shannxi Province, China. Tel. +86.29.84775381. E-mail: zhadjun@fmmu.edu.cn

Contributions: DZ, FS, CT, experiment design; CT, YY, YL, experiments performing, data collection; CT, YY, data analysis; CT, JQ, DZ, contribution to the interpretation of the results and manuscript writing. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval: all animal procedures were conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the Air Force Medical University (Ethic Code: IACUC-20220192), Xi'an, Shannxi Province, China.
Availability of data and materials: All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Funding: supported by the National Natural Science Foundation of China (Grant No.82171161 and No.81870732), National Key Research and Development Plan (2019YFB1311605), the Natural Science Foundation of Shaanxi (2018PT-01, 2020JZ-32), Shaanxi Provincial Clinical Medical Research Center (2021LCZX-15), and the Research Promotion project from Xijing Hospital (XJZT18X23).

Introduction

The mammalian cochlea consists of hair cells (HCs), spiral ganglion neurons (SGNs) and stria vascularis (SV), which play an important role in auditory function. Morphological studies have confirmed that the sympathetic nerve fibers are distributed in the cochlea,[1-5] but no adrenergic nerve terminals have been found in the organ of Corti or SV.[6] Therefore, the way by which the sympathetic nervous system (SNS) regulates peripheral auditory function is still not clear. The neurotransmitters in postganglionic sympathetic nerve fibers mainly include norepinephrine (NE), epinephrine, acetylcholine (ACh), purine, and polypeptides. Noradrenergic regulation of the central auditory system occurs at several levels, including the cochlear nucleus, [7] the lateral superior olive,[8] medial geniculate nucleus,[9,10] and auditory cortex,[11-14] where NE alters intrinsic and synaptic physiology. NE exerts a modulatory effect on neuronal activities through the activation of α_2-adrenergic receptors (ARs).[15-20] However, noradrenergic regulation in the cochlea has not been well characterized. Sympathetic neurons influence activity in target organs by releasing NE through ARs to play a role in target organs.[21] There are three types of ARs exist: α_1 (α_{1A}, α_{1B}, α_{1D}), α_2 (α_{2A}, α_{2B}, α_{2C}) and β (β_1, β_2, β_3) receptors. These ARs belong to the superfamily of G protein-coupled receptors (GPCRs),[22] which have many key findings in the cochlea, such as stem cell fate, planar cell polarity (PCP), hearing loss, and hearing protection.[23,24] The α_1,[25] α_{1A},[26] α_2,[27-31] β,[32,33] β_1,[25,34,35] and β_2[25,35-37] ARs are widely expressed across the peripheral auditory system, with varying expression patterns, and are well positioned to influence inner ear function. However, little is known about developmental changes in cochlear ARs expression.

Experiments using stimulation or transection have documented a protective effect in the SNS regarding noise-induced hearing loss (NIHL).[38-42] However, $Dbh^{-/-}$ mice lacking measurable NE or epinephrine were as susceptible to noise exposure as controls.[43] Cellular localization studies have provided some insights regarding the possible role of α_2-ARs in inner ear function.[27] We previously reported that NE suppression of the GABA response is regulated by α_2-ARs, which reduce intracellular cAMP formation through the inhibition of adenylyl cyclase.[30] Although there are multiple pathways by which the SNS modulates hearing function, the mechanisms of ARs in the development of the rat auditory system are unclear. To address these in the present study, firstly, we observed the α_{2A}-AR expression in a rat model of NIHL to demonstrate that the receptor may play a role in auditory process; after that, we investigated the expression of α_{2A}-AR in the developing rat cochlea *via* immunohistochemistry, qRT-PCR analysis, and Western blotting.

Materials and Methods

Animals

Sprague-Dawley rats were purchased from the Laboratory Animal Center of the Air Force Medical University. All the experiments were approved by the Institutional Animal Care and Use Committee of the Air Force Medical University (Ethic Code: IACUC-20220192), Xi'an, Shannxi Province, China. Post-natal rats were decapitated without anesthesia while adult animals were decapitated under deep anesthesia *via* an intraperitoneal injection of 10% chloral hydrate (250 mg/kg).[44] Rats were housed under standard conditions (temperature 23±2°C, humidity 60±5%, 12 h light/dark cycle).

Noise exposure

In this study, rats of both sexes (8-10 weeks old, weighing 180-250 g) were exposed to white noise at 115 dB sound pressure level (SPL) for 6 h per day for two consecutive days. The control group was placed in the noise booth but not exposed to noise. Exposure to noise was performed in a double-walled soundproof room where rats had free access to food and water. A Radio Shack Super Tweeter (Tandy Corp, Fort Worth, TX, USA), which was located above the cages, generated a noise (white noise, 115 dB SPL) that was then amplified by a power amplifier (Yamaha, Japan) and delivered to a loudspeaker. Sound levels of the noise exposure were confirmed by a sound level meter (Bruel and Kjaer, Shanghai, China) at multiple locations within the cabinet to ensure uniformity of the sound field and were measured before and after exposure to ensure stability.

Auditory brainstem responses

After rats were anesthetized, the active electrodes were inserted into subcutaneous tissue at the vertex, the reference electrode was inserted in the mastoid area of the test ear, and the ground electrode was placed at the base of the tail. Tucker Davis Technology (TDT) System-III hardware and SigGenRZ/BioSigRZ software (Tucker-Davis Technologies, Alachua, FL, US) were used to record the response. Alternating click stimuli were used for response detection and delivered using a headphone MF1 1172, which filtered the acoustic range to 100-3000 Hz. The speaker was in this experiment 1 cm away from the outer ear canal of the rat. Stimulus began with 90 dB intensity (21/s) of tone-burst (frequencies of 4, 8, 16, 24 and 32 kHz), with 10 dB decreased once, and then in 5 dB intervals near threshold until no responses were detected. The first wave was used to determine thresholds for each frequency.

Distortion product otoacoustic emissions (DPOAEs)

The emissions were measured using a TDT WS-8 computer workstation (Tucker-Davis Technologies). DP-grams of DPOAE amplitudes as a function of f1 and f2 frequencies were represented. Frequencies were acquired with an f2/f1 ratio of 1.2. At one day after noise exposure, frequency was incremented from 4 kHz to 32 kHz through 2 kHz and 4 kHz steps. The detection threshold was determined as a DPOAE of 3 dB above the noise floor.

Immunofluorescence

For immunofluorescence analysis, the cochleae from post-natal day (P)0, P7, P14, P21 and P28 rats were fixed overnight at 4°C in 4% paraformaldehyde in phosphate-buffered saline (PBS). The tissues were decalcified in 10% EDTA in PBS (P0, P7, and P14 for 2 h; P21 and P28 for 3 to 7 days), followed by dehydration in 30% sucrose solution overnight at 4°C. The samples were embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, USA) at -20°C, and mid-modiolar 10-µm-thick cross-sections of the cochleae were obtained using a cryostat microtome (CM1850; Leica, Wetzlar, Germany). The sections were collected on glass slides.

The cochlear sections were incubated for 40 min at 37°C with a blocking solution made of PBS (0.1% Triton X-100 in PBS) with 5% normal donkey serum (NDS; Jackson ImmunoResearch, West Grove, PA, USA). The primary antibody was applied to the cochlea sections overnight at 4°C. For primary antibodies, we used anti-α_{2A}-AR (ab85570, 1:200; Abcam, Cambridge, UK), and anti-β_3 (TUJ-1; MAB1637, 1:1000; Millipore, Burlington, MA, USA). After rinsing three times in PBS, secondary antibody incubation was performed at room temperature for 2 h. For secondary antibodies, we used two types of donkey anti-rabbit: A-21206 (Alexa 488 conjugated, 1:200; Thermo Fisher Scientific, Waltham, MA, USA) or AP182C (Alexa 594 conjugated, 1:200; Millipore). After

washing in PBS, the sections were labeled with rhodamine-conjugated phalloidin[45] (PHDR1, 1:200; Cytoskeleton, Inc., Denver, CO, USA) for 10 min at room temperature in the dark. Sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1:1000; Solarbio Corporation, Beijing, China) for 10 min at room temperature in the dark and mounted on glass slides with Mowiol 4-88 (81381-50G; Sigma Aldrich, Steinheim am Albuch, Germany) mounting medium. Every experiment also included a negative control where the primary antibody was omitted.

The sections were observed using a fluorescence microscope (Olympus Corp., Tokyo, Japan). The images were captured using Olympus confocal software FV10-ASW 1.7a and adjusted for brightness and contrast.

mRNA expression levels of α_{2A}-AR

Cochlea were obtained from Sprague- Dawley rat pups of both sexes (P0, P7, P14, P21, and P28). Total RNA was extracted at 4°C using the E.Z.N.A.® Total RNA Kit I (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The obtained total RNA was reverse transcribed into complementary DNA (cDNA) using LifeECO TC-96/G/H(b)C according to the manufacturer protocols (Bioer Technology, Hangzhou, China) *via* the RevertAid First Strand cDNA Synthesis Kit (K1622; Thermo Fisher Scientific). Gene-specific mRNA analyses were performed using the standard protocol for TB Green® Premix Ex Taq™ II (TaKaRa Biotechnology, Kusatsu, Japan). The $2^{-\Delta\Delta Ct}$ method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous reference was used to determine the relative levels of gene expression. The primer sequences for α_{2A}- AR, [46, 47] and GAPDH [47] are listed in Table 1.

Western blotting

At least six cochleae tissues (P0, P7, P14, P21, P28, and P56-P70) were collected for each Western blot. Total proteins were extracted from the cochleae *via* the Protein Extraction Kit (Solarbio, Beijing, China). Sample protein concentrations were measured *via* the BCA Protein Assay Kit (Beyotime, Haimen, China). The proteins in each group were separated using CFAS Any KD PAGE gels (PE008; Shanxi ZHHC Biotechnology Co., Ltd., China). After electrophoresis, proteins were blotted onto polyvinylidene fluoride membrane (PVDF; Millipore) (200 mA, 1 h). They were then blocked with 5% milk dissolved in TBS-Tween (0.1%) for 1 h at 37°C and then incubated with a primary antibody against α_{2A}-AR (1 µg/mL dilution, ab85570; Abcam) and GAPDH (1:4000 dilution, 10494-1-AP; Proteintech, Rosemont, IL, USA) in blocking buffer overnight at 4°C. The membranes were washed with TBS-Tween (3×10 min) and incubated with a secondary antibody (1:5000 dilution, CoWin Biotech, Beijing, China) at room temperature for 2 h. The E-BLOT Touch Imager (e-BLOT, Shanghai, China) was used to capture the chemiluminescence. All bands of interest were quantified and normalized to their respective GAPDH bands.

Statistical analysis

All data are presented as the mean ± SD. Analyses of ABR threshold and DOPAE amplitudes were completed using a two-way analysis of variance (two-way ANOVA) followed by the Tukey-Cramer test. Analyses of α_{2A}-AR expression at various stages were completed using a one-way analysis of variance (one-way ANOVA) followed by Dunnett's test. Analyses of α_{2A}-AR expression in noise and control group were completed using a Student's *t*-test. All statistical analyses were performed using GraphPad Prism version 8.3.0 for Windows (GraphPad Software, San Diego, CA, USA). Significance was set as $p<0.05$.

Results

A rat model of NIHL was developed to investigate the expression of α_{2A}-AR (Figure 1A), the ABR threshold was slightly higher in noise group at all frequencies tested than in the control group (n=10, two-way ANOVA, $p<0.0001$), meanwhile, the mean DOPAE amplitudes of the noise group were lower than that of the control group at all tested frequencies, with statistical significance (n=10, two-way ANOVA, $p<0.0001$) (Figure 1 B,C). Western blotting was used to investigate the expression of α_{2A}-AR between two groups (Figure 1D). We found that the expression of α_{2A}-AR significantly increased in rats exposed to noise compared with controls (n=10, Student's *t*-test, $p<0.05$). It suggested that the receptor plays an important role in NIHL. To investigate the distribution patterns of α_{2A}-AR in the rat cochlea, we carried out immunofluorescence staining during postnatal development (P0, P7, P14, P21, and P28). We found that α_{2A}-AR were predominantly immunoreactive in the HCs, SGNs, and cells of the SV from P0 to P28 in the rat cochlea (Figure 2, Supplementary Figure 1). The distributions of α_{2A}-AR in the cochlea were predominantly immunoreactive in the plasma membrane and cytoplasm of HCs, SGNs, and cells of the SV at different developmental stages. In detail, we conducted triple-labeling using a marker of HCs and cells in the SV [48], phalloidine, to observe whether α_{2A}-AR was expressed in HCs and cells in the SV (Figures 3 and 4). Furthermore, we performed triple-labeling using an antibody against TUJ-1, which is a marker of SGNs, to determine whether α_{2A}-AR were expressed in SGNs (Figure 5). No immunoreactivity was observed in the negative controls (Supplementary Figure 2). We quantified α_{2A}-AR mRNA expression in the developing rat cochleae *via* qRT-PCR. As shown in Figure 6, α_{2A}-AR mRNA reached a maximum level at P14 and P28 when compared to the P0 (n=6, one-way ANOVA, $p<0.05$). Western blotting revealed that α_{2A}-AR proteins were present in the developing rat cochlea (Figure 7). We detected immunoreactive bands of 51 kDa corresponding to α_{2A}-AR in cochlear tissue. GAPDH (35 kDa) was used as an internal control. Furthermore, we quantified α_{2A}-AR protein expression levels at various stages relative to GAPDH as a control. Inconsistent with the qRT-PCR data, we found no significant differences in α_{2A}-AR protein levels at the various stages when compared to the P0 (n=6, one-way ANOVA, $p>0.05$).

Table 1. Primers for real-time polymerase chain reaction

Gene	Forward (5′-3′)	Reverse (5′-3′)
GAPDH	GTCTTCACCACCACGGAGAAGGC	ATGCCAGTGAGCTTCCCGTTCAGC
α_{2A}-AR	GGTAAGGTGTGGTGCGAGAT	CAGCGCCCTTCTTCTCTATG

GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Discussion

Organ of Corti, SGNs, and SV are regarded as three major cochlear structures that can independently degenerate and influence the degree of hearing loss.[49,50] The SNS projections into the inner ear have two main components:[3,51,52] a vessel-associated component and a vessel-independent component.[4-6] In pathological states, the SNS is associated with NIHL *via* cochlear blood flow (CBF) regulation, and the function of NE in CBF has been well established [53-60] ARs also play a role in hearing disorders such as vertigo by regulating inner ear ion exchange.[61] Although both α_1- and α_2-ARs have been localized in the cochlea, α_2-ARs is the predominant receptor in this region.[29] Furthermore, while a selective α_1-antagonist increased CBF, a selective α_2-antagonist, β-antagonist, and non-selective α-antagonist had no effect on CBF.[62] α_1-ARs have been localized in the chinchilla cochlea *via* the α_1-AR antagonist [H]-prazosin.[63] These receptors may be related to the cochlear microvasculature, although the anatomical locations have not yet been determined. The spiral modiolar artery contains α_{1A}-AR, which mediate vasoconstriction at branch points.[26] α_2-AR have been implicated in physiological responses and innervation in the organ of Corti.[64] NE may regulate auditory transmission, *via* α_2-ARs, through suppression of the GABA response.[30] The HCs in the rat neonatal cochlea express all three α_2-ARs subtypes.[28] The expression of α_{2A}-, α_{2B}-, and α_{2C}-ARs has been confirmed in rat SGNs, and brimonidine acts directly on these receptors to protect the SGN.[27,65] α_1-, β_1-, and β_2-ARs also have been immunohistochemically identified within the organ of Corti and SGNs.[25] β_1-AR play a role in regulating ion exchange in the SV.[66] Meanwhile, β_2-AR mRNA is present in the SV, and is implicated in the generation of gerbil nonstrial lateral wall tissues.[37] Isoproterenol, *via* β-ARs, increases endolymphatic hydrostatic pressure in variable ways in the vestibule and cochlea.[67] The signaling pathway of SNS regulation of peripheral auditory function is not yet clear. In addition, the location and role of these receptors are still unclear.[36] Here, we investigated the expression of α_{2A}-ARs in the developing mammalian cochlea with the goal of supporting a theoretical explanation of the function of the SNS in auditory function. Our immuno-

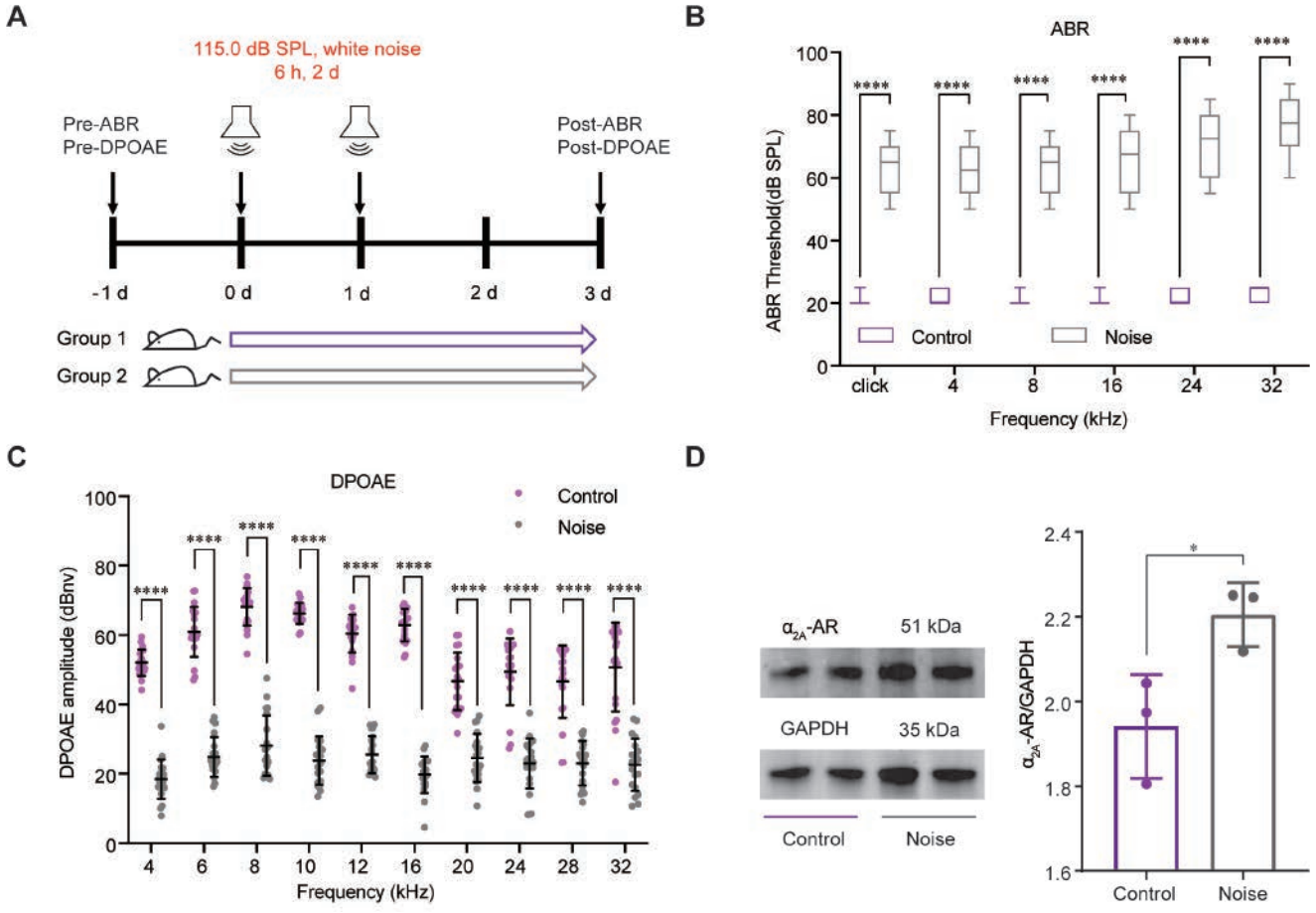

Figure 1. Traumatic noise exposure activates α_{2A}-AR expression in rat cochlea. **A**) Schematic of the within-animal experimental design; animals were exposed to white noise at 115 dB SPL for 6 h per day during two consecutive days; ABR thresholds and DPOAE amplitudes were measured one-day pre-exposure and one-day post-exposure. **B**) Noise-induced ABR thresholds were significantly higher than control group at one-day post-noise; data were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test (n=10 per group); ****p<0.0001. **C**) Noise-induced DPOAE amplitudes confirmed a significant decrease compared to control at one-day post-noise; data were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test (n=10 per group); ****p<0.0001. **D**) Western blots using whole cochlear tissue homogenates reveal specificity for α_{2A}-AR and show an increase in band densities for α_{2A}-AR examined one day after the noise exposure compared with unexposed controls; data were analyzed using a Student's *t*-test (n=6 per group); *p<0.05.

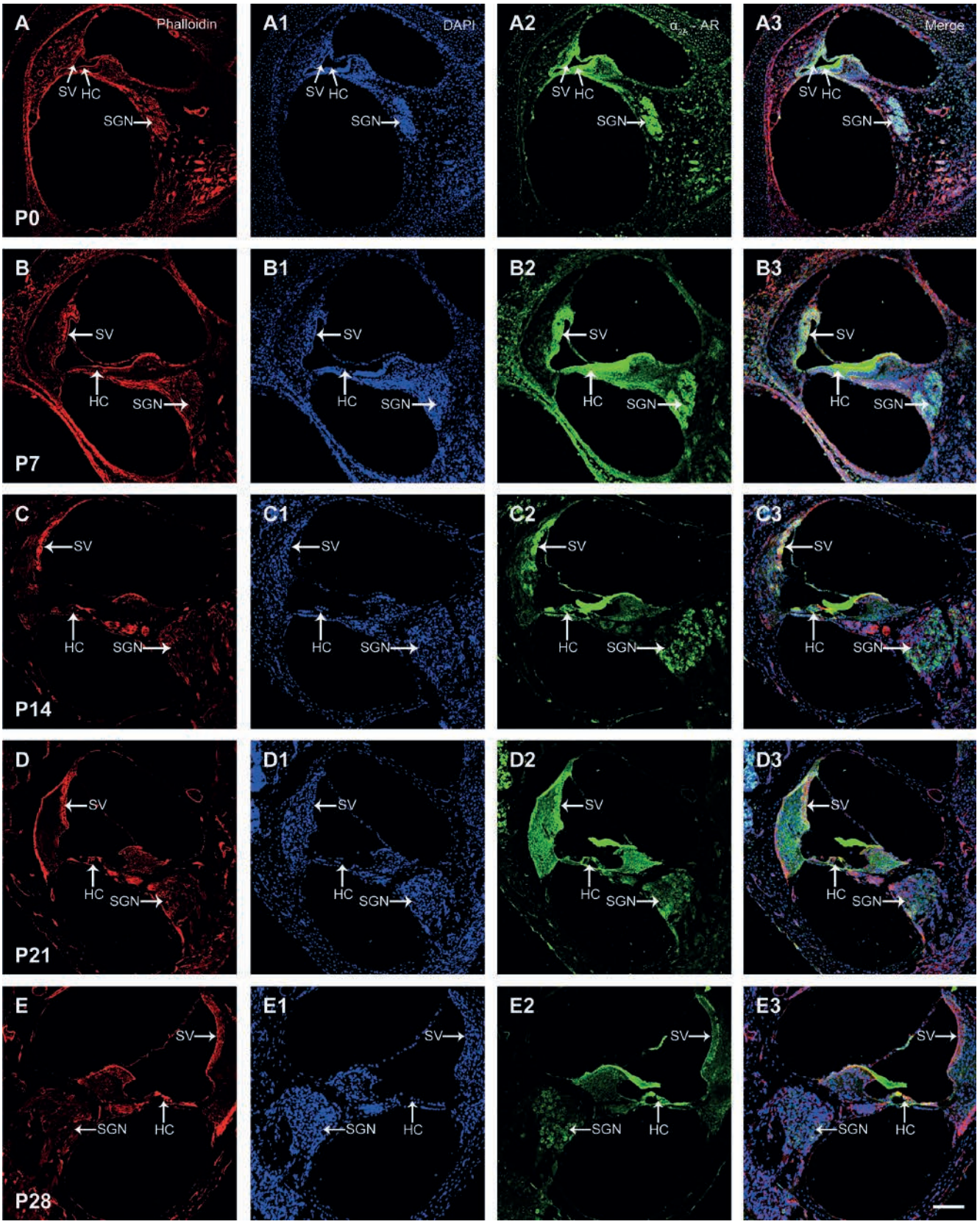

Figure 2. Immunofluorescence of α_{2A}-AR within the developing cochleae tissues at various stages. **A-A3**) P0 group. **B-B3**) P7 group. **C-C3**) P14 group. **D-D3**) P21 group. **E-E3**) P28 group. Immunolabelling of a transverse section of developing cochleae tissues at various stages using phalloidine (red), DAPI (blue), and an antibody against α_{2A}-AR (green). α_{2A}-AR was mostly expressed in HCs, SGNs, and the SV. HCs, hair cells; SGNs, spiral ganglion neurons; SV, stria vascularis. Scale bars: 100 μm.

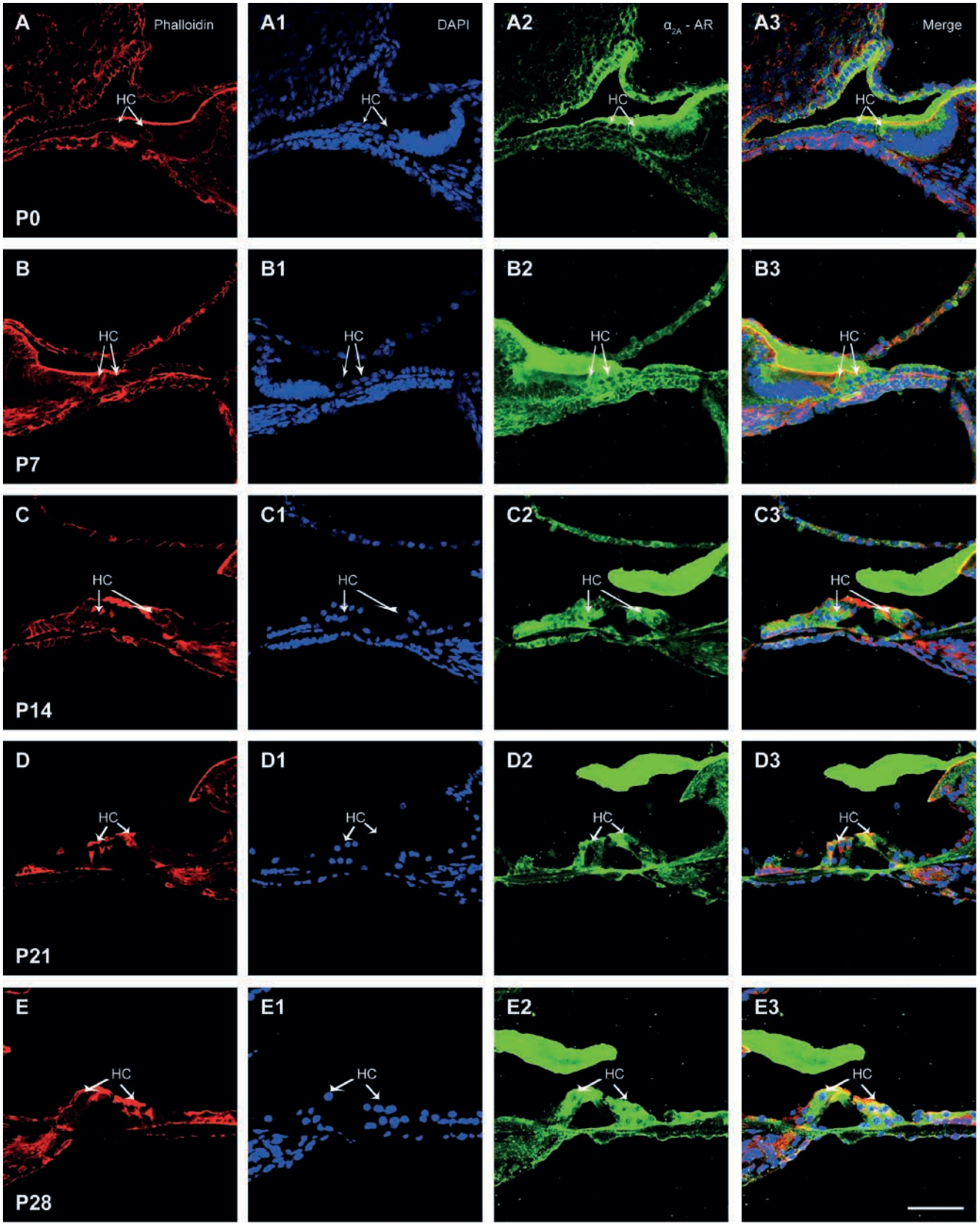

Figure 3. Immunofluorescence of α_{2A}-AR in HCs at various stages. **A-A3**) P0 group. **B-B3**) P7 group. **C-C3**) P14 group. **D-D3**) P21 group. **E-E3**) P28 group. Co-immunolabelling *via* phalloidine (red), DAPI (blue), and an antibody against α_{2A}-AR (green) focused on HCs in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. HCs, hair cells. Scale bars: 50 μm.

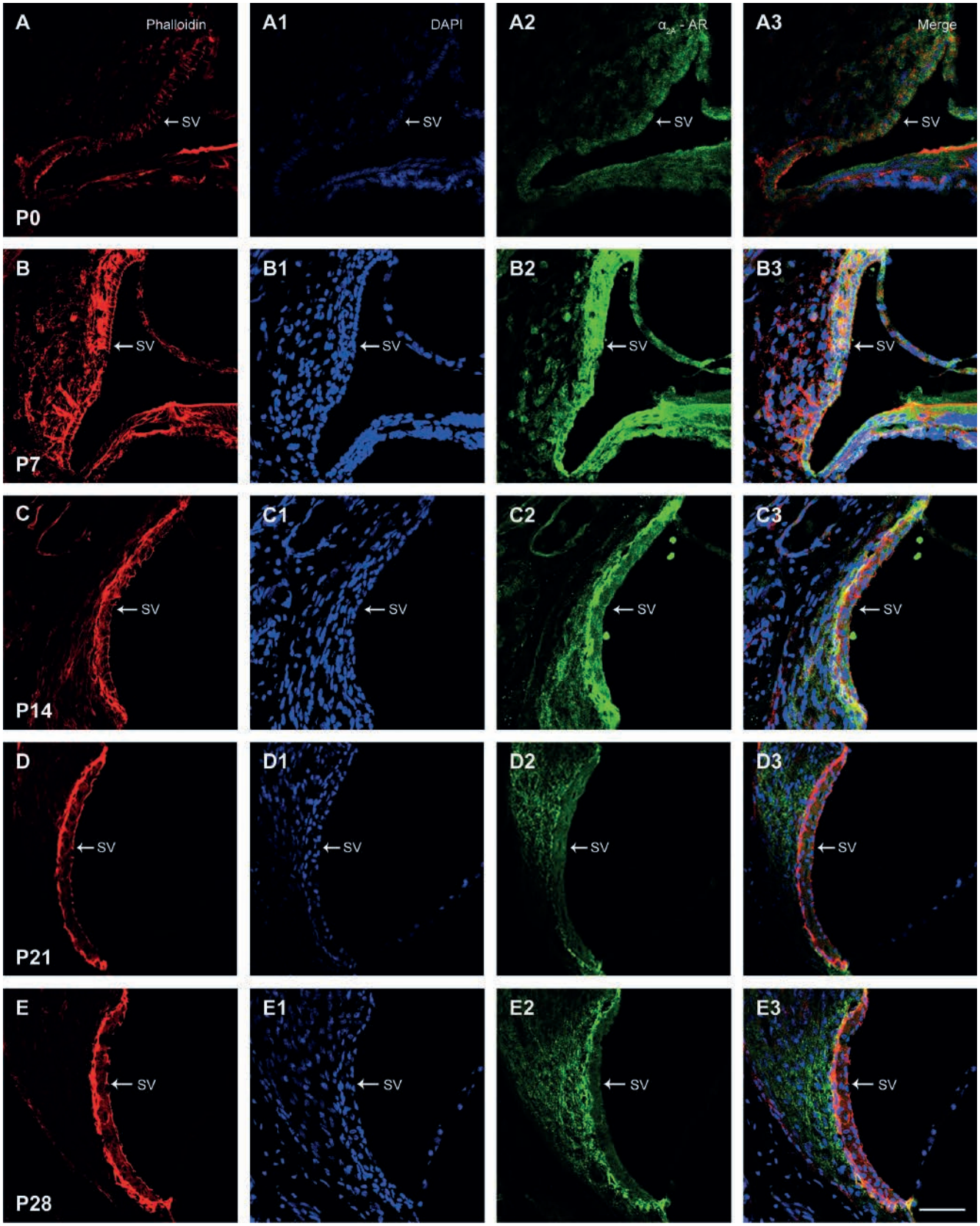

Figure 4. Immunofluorescence of α_{2A}-AR in SV at various stages. **A-A3**) P0 group. **B-B3**) P7 group. **C-C3**) P14 group. **D-D3**) P21 group. **E-E3**) P28 group. Co-immunolabelling *via* phalloidine (red), DAPI (blue), and an antibody against α_{2A}-AR (green) focused on SV in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. SV, stria vascularis. Scale bars: 50 μm.

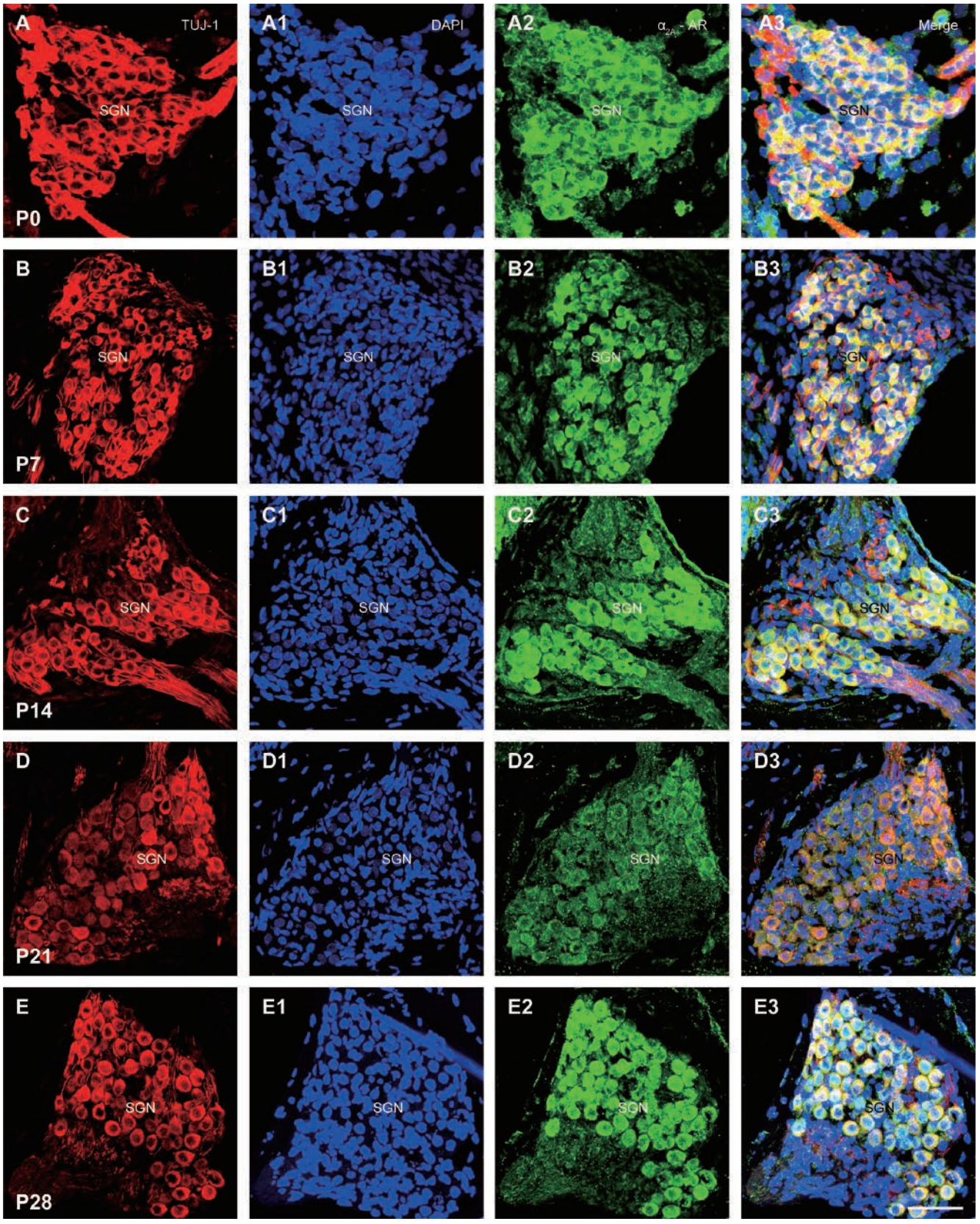

Figure 5. Immunofluorescence of α_{2A}-AR in SGNs at various stages. **A-A3**) P0 group. **B-B3**) P7 group. **C-C3**) P14 group. **D-D3**) P21 group. E-E3) P28 group. Co-immunolabelling *via* TUJ-1 (red), DAPI (blue), and an antibody against α_{2A}-AR (green) focused on SGNs in transverse sections of P0, P7, P14, P21, and P28 rat cochlea. SGNs, spiral ganglion neurons. Scale bars: 50 μm.

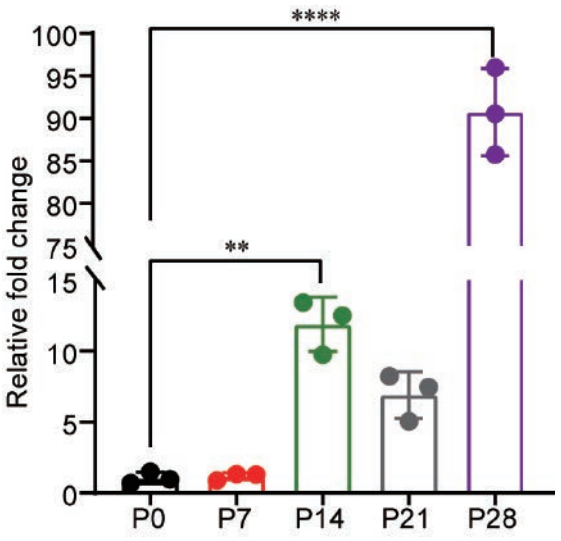

Figure 6. Expression of α_{2A}-AR mRNA in the developing rat cochlea. qRT-PCR data showing significantly higher levels of α_{2A}-AR in P14 and P28 rat cochlea compared with P0. **p<0.005; ****p<0.0001.

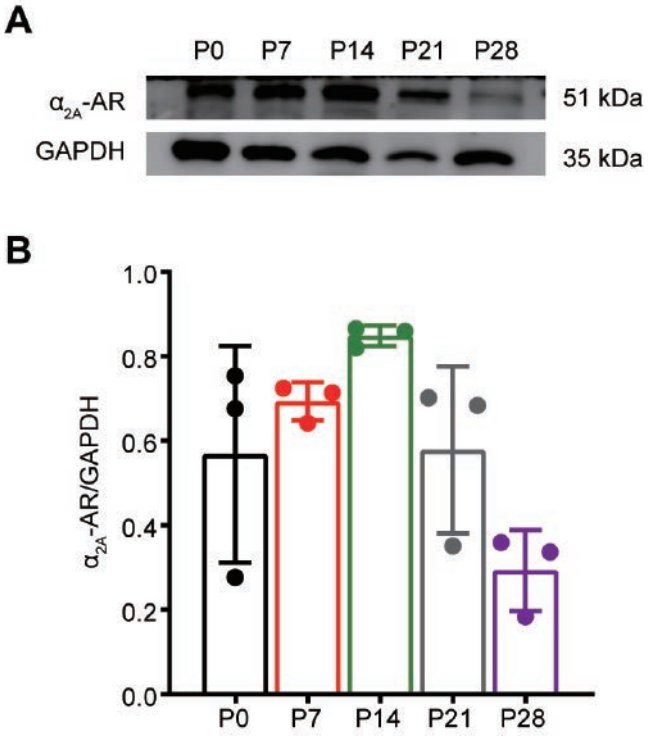

Figure 7. Developmental expression of α_{2A}-AR in the postnatal rat cochlea. **A**) Representative immunoblots for α_{2A}-AR and GAPDH in the rat cochlea during postnatal development. **B**) α_{2A}-AR protein levels; no significant differences in expression levels were detected in the rat cochlea compared with P0; the band intensities for GAPDH demonstrate equal protein loading among the five stages. p>0.05.

fluorescence data confirmed that α_{2A}-AR are expressed in cochlear HCs, SGNs, and SV of the cochlea. Furthermore, we observed α_{2A}-AR expression in the developing rat cochlea in terms of both mRNA and protein strengthens the credibility of our immunofluorescence results. Together, the findings of this study, therefore, allow us to speculate that NE might play a key role in the auditory functions of the cochlea *via* the receptor.

Cai *et al*. showed that the α_2-ARs expressed in the rat cochlea and the vestibular labyrinth, however, they only selected the early postnatal period (P3 and P8) by immunohistochemistry.[28] In this study, we provide direct evidence for the expression patterns of α_{2A}-AR in the rat cochlea. ARs were localized in different regions in the cochlea, indicating that these receptors may play a role in the processing of peripheral auditory information through multiple signaling pathways. The diverse distribution of this receptor in the mammalian cochlea also corresponds to the diversity of functions. Therefore, our data supported the results of previous studies.

In the current study, we selected α_{2A}-AR to initially explore their expression in the developing rat cochlea, with the goal of providing a theoretical basis for subsequent studies on the distribution and function of other receptors. Data regarding the role of NE in auditory processing remain limited. Additional studies are needed with various experimental techniques to explore the role of NE and its receptors in peripheral auditory modulation in multiple species. Investigations of the expression and function of other ARs (α_{1A}, α_{1B}, α_{1D}, α_{2B}, α_{2C}, β_1, β_2, β_3), in addition to explorations of the precise molecular mechanisms underlying ARs modulation in the mammalian cochlea, it will increase our understanding of the role of the SNS in auditory function. In terms of clinical applications, the roles of catecholamines in cochlear functions such as responses to noise injury are topics worthy of future investigation. In summary, α_{2A}-AR is predominantly expressed in the HCs, SGNs, and SV cells in the rat cochlea. Our findings indicate that NE might play a vital role in hearing function within the cochlea through its receptors.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant No.82171161 and No.81870732), National Key Research and Development Plan (2019YFB1311605), the Natural Science Foundation of Shaanxi (2018PT-01, 2020JZ-32), Shaanxi Provincial Clinical Medical Research Center (2021LCZX-15), and the Research Promotion project from Xijing Hospital (XJZT18X23).

References

1. Nagaraj BS, Linthicum FH Jr. Autonomic innervation of the human middle ear: an immunohistochemical study. Am J Otolaryngol 1998;19:75-82.
2. Uddman R, Kitajiri M, Sundler F. Autonomic innervation of the middle ear. Ann Otol Rhinol Laryngol 1983;92:151-4.
3. Hozawa K, Kimura RS. Cholinergic and noradrenergic nervous systems in the cynomolgus monkey cochlea. Acta Otolaryngol 1990;110:46-55.
4. Borg E, Densert O, Flock A. Synaptic vesicles in the cochlea. Acta Otolaryngol 1974;78:321-32.
5. Spoendlin H, Lichtensteiger W. The adrenergic innervation of the labyrinth. Acta Otolaryngol 1966;61:423-34.
6. Densert O, Flock A. An electron-microscopic study of adrenergic innervation in the cochlea. Acta Otolaryngol 1974;77:185-97.
7. Kuo SP, Trussell LO. Spontaneous spiking and synaptic depression underlie noradrenergic control of feed-forward inhibition. Neuron 2011;71:306-18.

8. Hirao K, Eto K, Nakahata Y, Ishibashi H, Nagai T, Nabekura J. Noradrenergic refinement of glutamatergic neuronal circuits in the lateral superior olivary nucleus before hearing onset. J Neurophysiol 2015;114:1974-86.
9. Tebecis AK. Effects of monoamines and amino acids on medial geniculate neurones of the cat. Neuropharmacology 1970;9:381-90.
10. Torda C. Effects of noradrenaline and serotonin on activity of single lateral and medial geniculate neurons. Gen Pharmacol 1978;9:455-62.
11. Edeline JM, Manunta Y, Hennevin E. Induction of selective plasticity in the frequency tuning of auditory cortex and auditory thalamus neurons by locus coeruleus stimulation. Hear Res 2011;274:75-84.
12. Manunta Y, Edeline JM. Effects of noradrenaline on frequency tuning of rat auditory cortex neurons. Eur J Neurosci 1997;9:833-47.
13. Manunta Y, Edeline JM. Effects of noradrenaline on frequency tuning of auditory cortex neurons during wakefulness and slow-wave sleep. Eur J Neurosci 1999;11:2134-50.
14. Martins AR, Froemke RC. Coordinated forms of noradrenergic plasticity in the locus coeruleus and primary auditory cortex. Nat Neurosci 2015;18:1483-92.
15. Arima J, Kubo C, Ishibashi H, Akaike N. alpha2-Adrenoceptor-mediated potassium currents in acutely dissociated rat locus coeruleus neurones. J Physiol 1998;508:57-66.
16. Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF. Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. J Neurosci 1999;19:1599-609.
17. Qu Y, Eysel UT, Vandesande F, Arckens L. Effect of partial sensory deprivation on monoaminergic neuromodulators in striate cortex of adult cat. Neuroscience 2000;101:863-8.
18. Ishibashi H, Umezu M, Jang IS, Ito Y, Akaike N. Alpha 1-adrenoceptor-activated cation currents in neurones acutely isolated from rat cardiac parasympathetic ganglia. J Physiol 2003;548:111-20.
19. Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol 2006;80:53-83.
20. Devilbiss DM, Page ME, Waterhouse BD. Locus ceruleus regulates sensory encoding by neurons and networks in waking animals. J Neurosci 2006;26:9860-72.
21. Sternini C. Organization of the peripheral nervous system: autonomic and sensory ganglia. J Investig Dermatol Symp Proc 1997;2:1-7.
22. Hein L. Adrenoceptors and signal transduction in neurons. Cell Tissue Res 2006;326:541-51.
23. Zhang Z, Chai R. Hear the sounds: the role of G protein-coupled receptors in the cochlea. Am J Physiol Cell Physiol 2022;323:C1088-C99.
24. Ma X, Guo J, Fu Y, Shen C, Jiang P, Zhang Y, et al. G protein-coupled receptors in cochlea: Potential therapeutic targets for hearing loss. Front Mol Neurosci 2022;15:1028125.
25. Khan KM, Drescher MJ, Hatfield JS, Ramakrishnan NA, Drescher DG. Immunohistochemical localization of adrenergic receptors in the rat organ of corti and spiral ganglion. J Neurosci Res 2007;85:3000-12.
26. Gruber DD, Dang H, Shimozono M, Scofield MA, Wangemann P. Alpha1A-adrenergic receptors mediate vasoconstriction of the isolated spiral modiolar artery in vitro. Hear Res 1998;119:113-24.
27. Cai J, Li J, Liu W, Han Y, Wang H. alpha2-adrenergic receptors in spiral ganglion neurons may mediate protective effects of brimonidine and yohimbine against glutamate and hydrogen peroxide toxicity. Neuroscience 2013;228:23-35.
28. Cai J, Li J, Mao Y, Bai X, Xu L, Wang H. Immunohistochemical localization of alpha2-adrenergic receptors in the neonatal rat cochlea and the vestibular labyrinth. J Mol Neurosci 2013;51:1010-20.
29. Carrasco VN, Prazma J, Faber JE, Triana RJ, Pillsbury HC. Cochlear microcirculation. Effect of adrenergic agonists on arteriole diameter. Arch Otolaryngol Head Neck Surg 1990;116:411-7.
30. Zha DJ, Wang ZM, Lin Y, Liu T, Qiao L, Lu LJ, et al. Effects of noradrenaline on the GABA response in rat isolated spiral ganglion neurons in culture. J Neurochem 2007;103:57-66.
31. Martin F, Marianowski R, Tu TY, Herman P, Tran Ba Huy P. Modulation of cyclic AMP production by strial marginal cells from gerbil in culture. Hear Res 1994;81:33-41.
32. Ishii K, Zhai WG, Akita M. Effect of a beta-stimulant on the inner ear stria vascularis. Ann Otol Rhinol Laryngol 2000;109:628-33.
33. Schicknick H, Henschke JU, Budinger E, Ohl FW, Gundelfinger ED, Tischmeyer W. beta-adrenergic modulation of discrimination learning and memory in the auditory cortex. Eur J Neurosci 2019;50:3141-63.
34. Fauser C, Schimanski S, Wangemann P. Localization of beta1-adrenergic receptors in the cochlea and the vestibular labyrinth. J Membr Biol 2004;201:25-32.
35. Kim BG, Kim JY, Jung J, Moon IS, Yoon JH, Choi JY, et al. beta1- and beta2-adrenergic stimulation-induced electrogenic transport by human endolymphatic sac epithelium and its clinical implications. Sci Rep 2017;7:42217.
36. Schimanski S, Scofield MA, Wangemann P. Functional beta2-adrenergic receptors are present in nonstrial tissues of the lateral wall in the gerbil cochlea. Audiol Neurootol 2001;6:124-31.
37. de Minteguiaga C, Clerget MS, Tran Ba Huy P, Elalouf JM. beta2-adrenergic but not vasopressin V2 receptor mRNAs are expressed in the stria vascularis of the rat inner ear. Pflugers Arch 1998;436:940-7.
38. Wada T, Takahashi K, Ito Z, Hara A, Takahashi H, Kasakari J. The protective effect of the sympathetic nervous system against acoustic trauma. Auris Nasus Larynx 1999;26:375-82.
39. Hildesheimer M, Sharon R, Muchnik C, Sahartov E, Rubinstein M. The effect of bilateral sympathectomy on noise induced temporary threshold shift. Hear Res 1991;51:49-53.
40. Horner KC, Giraudet F, Lucciano M, Cazals Y. Sympathectomy improves the ear's resistance to acoustic trauma—could stress render the ear more sensitive? Eur J Neurosci 2001;13:405-8.
41. Giraudet F, Horner KC, Cazals Y. Similar half-octave TTS protection of the cochlea by xylazine/ketamine or sympathectomy. Hear Res 2002;174:239-48.
42. Hildesheimer M, Henkin Y, Pye A, Heled S, Sahartov E, Shabtai EL, et al. Bilateral superior cervical sympathectomy and noise-induced, permanent threshold shift in guinea pigs. Hear Res 2002;163:46-52.
43. Maison SF, Le M, Larsen E, Lee SK, Rosowski JJ, Thomas SA, et al. Mice lacking adrenergic signaling have normal cochlear responses and normal resistance to acoustic injury but enhanced susceptibility to middle-ear infection. J Assoc Res Otolaryngol 2010;11:449-61.
44. Yu B, Zhang G, An Y, Wang W. Morroniside on anti-inflammation activities in rats following acute myocardial infarction. Korean J Physiol Pharmacol 2018;22:17-21.
45. Wang P, Zhang P, Huang J, Li M, Chen X. Trichostatin A protects against cisplatin-induced ototoxicity by regulating expression of genes related to apoptosis and synaptic function. Neurotoxicology 2013;37:51-62.

46. Matsubara A, Miyashita T, Inamoto R, Mori N. Presence of adrenergic receptors in rat endolymphatic sac epithelial cells. J Membr Biol 2013;246:109-14.
47. Lee G, Park H, Park HS, Lee JG. Modulation of alpha 1 adrenergic receptors on urinary bladder in rat spinal cord injury model. Int Neurourol J 2012;16:62-8.
48. Lamm K, Zajic G, Schacht J. Living isolated cells from inner ear vessels: a new approach for studying the regulation of cochlear microcirculation and vascular permeability. Hear Res 1994;81:83-90.
49. Schuknecht HF, Gacek MR. Cochlear pathology in presbycusis. Ann Otol Rhinol Laryngol 1993;102:1-16.
50. Ohlemiller KK. Age-related hearing loss: the status of Schuknecht's typology. Curr Opin Otolaryngol Head Neck Surg 2004;12:439-43.
51. Spoendlin H, Lichtensteiger W. The sympathetic nerve supply to the inner ear. Arch Klin Exp Ohren Nasen Kehlkopfheilkd 1967;189:346-59.
52. Spoendlin H. Autonomic innervation of the inner ear. Adv Otorhinolaryngol 1981;27:1-13.
53. Perlman HB, Kimura R, Fernandez C. Experiments on temporary obstruction of the internal auditory artery. Laryngoscope 1959;69:591-613.
54. Thalmann R, Miyoshi T, Thalmann I. The influence of ischemia upon the energy reserves of inner ear tissues. Laryngoscope 1972;82:2249-72.
55. Santi PA, Duvall AJ, 3rd. Stria vascularis pathology and recovery following noise exposure. Otolaryngology 1978;86:ORL354-61.
56. Ren T, Brown NJ, Zhang M, Nuttall AL, Miller JM. A reversible ischemia model in gerbil cochlea. Hear Res 1995;92:30-7.
57. Lamm K, Arnold W. Noise-induced cochlear hypoxia is intensity dependent, correlates with hearing loss and precedes reduction of cochlear blood flow. Audiol Neurootol 1996;1:148-60.
58. Nuttall AL. Sound-induced cochlear ischemia/hypoxia as a mechanism of hearing loss. Noise Health 1999;2:17-32.
59. Mom T, Bonfils P, Gilain L, Avan P. Origin of cubic difference tones generated by high-intensity stimuli: effect of ischemia and auditory fatigue on the gerbil cochlea. J Acoust Soc Am 2001;110:1477-88.
60. Shi X. Physiopathology of the cochlear microcirculation. Hear Res 2011;282:10-24.
61. Kim BG, Kim JY, Jung J, Moon IS, Yoon JH, Choi JY, et al. beta(1)- and beta(2)-adrenergic stimulation-induced electrogenic transport by human endolymphatic sac epithelium and its clinical implications. Sci Rep 2017;7:42217.
62. Ohlsen KA, Baldwin DL, Nuttall AL, Miller JM. Influence of topically applied adrenergic agents on cochlear blood flow. Circ Res 1991;69:509-18.
63. Liu SY, Pitovski DZ, Shivapuja BG. Alpha 1-adrenergic receptors in the mammalian cochlea. Acta Otolaryngol 1996;116:710-3.
64. Drescher MJ, Drescher DG, Khan KM, Hatfield JS, Ramakrishnan NA, Abu-Hamdan MD, et al. Pituitary adenylyl cyclase-activating polypeptide (PACAP) and its receptor (PAC1-R) are positioned to modulate afferent signaling in the cochlea. Neuroscience 2006;142:139-64.
65. Cortada M, Levano S, Bodmer D. Brimonidine protects auditory hair cells from in vitro-induced toxicity of gentamicin. Audiol Neurootol 2017;22:125-34.
66. Wangemann P, Liu J, Shimozono M, Schimanski S, Scofield MA. K+ secretion in strial marginal cells is stimulated via beta 1-adrenergic receptors but not via beta 2-adrenergic or vasopressin receptors. J Membr Biol 2000;175:191-202.
67. Inamoto R, Miyashita T, Matsubara A, Hoshikawa H, Mori N. The difference in endolymphatic hydrostatic pressure elevation induced by isoproterenol between the ampulla and the cochlea. Auris Nasus Larynx 2017;44:282-7.

Received: 11 April 2023. Accepted: 22 July 2023.

European Journal of Histochemistry 2023; 67:3748
doi:10.4081/ejh.2023.3748