Contribution of beta1- and beta2-adrenergic receptors to cochlear function

Authors:

Diana Mendus

Piera Smeriglio

Mirna Mustapha

Corresponding author:

Dr. Mirna Mustapha, Stanford University, Edwards Building, 300 Pasteur Drive, Room R111A, Stanford, CA 94305-5453.

E-mail: mirnam@stanford.edu

Address: Department of Otolaryngology – Head and Neck Surgery, Stanford University, Stanford, CA, USA, 94305

Abbreviations

ABR, Auditory Brainstem response; BC, body condition; AR, adrenergic receptor; beta-AR, beta-adrenergic receptor; DPOAE, Distortion product otoacoustic emission; KO, knock out; OHC, outer hair cells; dB, decibel; SPL, sound pressure level; WT, wild type.

Abstract

Sympathetic innervation is heavily present in the cochlea. However, its role in the development and maintenance of normal hearing remains debatable. Beta1-adrenergic receptors 1 (beta1-ARs) and beta2-adrenergic receptors (beta2-ARs) are two types of receptors that are stimulated by the sympathetic nervous system and are expressed by many cochlear cell types. In this study, we have analyzed the functional consequences of the lack of beta-ARs in the cochlea. We have first evaluated hearing thresholds using auditory brainstem response and distortion product otoacoustic emissions in young and aged knockout mice lacking beta1-ARs, beta2-ARs, or both beta1-ARs and beta2-ARs. Secondly, we tested the sensitivity of these mice to acoustic overexposure.

Hearing tests revealed similar normal thresholds in all beta- adrenergic receptors mice when compared to age matched wild type controls. When exposed to noise mice lacking beta1- but not beta2- AR exhibited a subtle protection of hearing thresholds at the low frequencies only. These results suggest that despite being expressed by many cochlear cell types these beta-ARs are not playing a crucial role in hearing development and maintenance.

Highlights

- Sound-evoked brainstem responses were compared between mice lacking beta1- and beta2 -adrenergic receptors and wild type controls.
- Outer hair cell function of these mice was evaluated by distortion product otoacoustic emissions.
- There was no clear evidence of reduction in cochlear function in beta1- and beta2-adrenergic receptor knockouts at any age.
- Lack of beta1-adrenergic receptor confers subtle protection from noise trauma in the low frequency region.

Keywords
Beta-Adrenergic receptors, Mouse models, Noise trauma, hearing thresholds

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1. Introduction

There are three main types of nerves that innervate the cochlea: afferent, efferent, and the sympathetic fibers (Spoendlin, 1984; Shibamori et al., 1984; Warr, 1992). The afferent fibers, or spiral ganglion neurons are bipolar neurons that form peripheral synapses with the inner and outer hair cells. Spiral ganglion neurons carry all the auditory information from the sensory inner hair cells to the auditory brainstem. The efferent, or olivocochlear neurons, originate in the brainstem and project to the organ of Corti. These fibers are involved in the regulation of auditory nerve activity (Warr et al. 1986; Warr, 1992; Liberman,1990).

Cochlear sympathetic innervation originates from the thoracic or cervical system, and predominately acts on beta-ARs. Immunohistochemical and mRNA expression analysis reported distribution of the beta-ARs in the organ of Corti, despite the lack of direct sympathetic innervation (Fauser et al., 2004; Khan et al., 2007).

The role of sympathetic innervation in the cochlea was previously investigated and protective effects of sympathectomy on noise trauma were reported (Borg, 1982; Hildesheimer et al., 1991; Horner et al., 2001, Bielefeld and Hendelson, 2007). A Genetic mouse model of impaired adrenergic signaling, with no measurable adrenaline or noradrenaline, however, did not confirm the previously suggested protective role during noise exposure (Maison et al., 2010). Both sympathectomy model and mouse model lacking sympathetic neurotransmitters did not clarify the role of adrenergic receptors in adrenergic signaling. Hence, we investigated the functional role of the beta1- and beta2-AR in the cochlea. We performed physiological recordings of cochlear function to address these questions: (1) what are the physiological consequences of the deletion of beta1-AR and/or beta2-AR, and, (2) does the deletion of either beta1-AR or beta2-AR results in a protective effect against acoustic overexposure?

2. Materials and Methods

1. Animals

Beta-adrenergic receptors null mice were generated and generously provided by Dr. Kobilka, recipient of the 2012 Nobel Prize in Chemistry. Mice of either sex lacking beta1-AR (Rohrer et al., 1996), beta2-AR, (Chruscinski et al., 1999) or both beta1- and beta2-ARs (Rohrer et al., 1999), and their littermate WT controls were used for these experiments. These mice are on a mix background C57BL/6J, 129Sv, DBA and FVB/N. Homozygotes beta2-AR mice were crossed with the homozygotes beta1-AR (BL/6J, 129Sv, DBA) to generate the double heterozygotes that they were used subsequently as breeders to generate the double mutants. Therefore, these mice were on a mix background. All procedures were completed with national animal care guidelines and were approved by the Stanford University Administrative Panel on Laboratory Animal Care.

2. Acoustic overexposures

The acoustic overexposure stimulus was broadband noise, 4-16 kHz at 100 dB SPL for 2 hours. During noise exposure, animals were awake and unrestrained within individual boxes without a lid. Five boxes were placed directly under the horn of the sound delivery loud speaker within a small reverberant chamber. Noise calibration to the target SPL was performed immediately before

each exposure session using a sound pressure meter with 60-120 dB range (Radioshack). SPL variations between experiments and across the cages were less than 1 dB.

3. Physiological tests

Auditory brainstem response (ABR) measurements were conducted as described in Mendus et al., 2014. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Acoustic stimuli at 8, 16 and, 32 kHz were delivered to the mouse's ear canal via plastic tubes connected to a speaker. ABRs were recorded via subdermal needle electrodes inserted at the vertex, behind the left pinna, and in the left leg. The sound intensity level decreased in 5 dB steps from 80 dB SPL (for 8 and 16 kHz) or from 100 dB SPL (for 32 kHz) to 0 dB SPL. The ABR threshold was defined as the lowest stimulus level at which replicable waves I and V could be identified in the response waveform.

Distortion Product Otoacoustic Emissions or DPOAEs, were measured using a probe tip microphone placed in the external auditory canal. Responses were recorded for primary tones with a frequency ratio of 1.2, and with the level of f2 equal to the f1 intensity level. The range of f2 varied from 4 to 90 kHz and intensity stepped from 20 to 80 dB, in 10 dB increments. To avoid distortion of non-physiological origin, stimulus over 80 dB SPL were not used. Ear canal sound pressure was amplified and digitally sampled, and the 2f1-f2 DPOAE amplitude and surrounding noise floor readings were extracted. The threshold at each frequency was calculated when the DPOAE was more the 5 dB SPL and two standard deviations above the noise floor. If a DPOAE response was not detected at 80 dB SPL, the threshold was arbitrarily set to 80 dB SPL for averaging purposes. All statistical analyses were performed using t-tests.

3. Results

1. Lack of beta-adrenergic receptors does not cause hearing impairment

The cochlea has abundant adrenergic innervation from the sympathetic nervous system (Spoendlin and Lichtensteiger, 1966; Lichtensteiger and Spoendlin, 1967; Spoendlin, 1981). It appears that the distribution of beta1- and beta2- ARs in the cochlea is different between inner and outer hair cells, and different between cochlear turns (Fauser et al., 2004; Khan et al., 2007). In order to assess cochlear function in the absence of either or both beta-ARs, we measured auditory thresholds as early as 1 month of age in beta1-AR, beta2-AR, beta1/2-AR double knockouts (KOs) and their littermate's wild type (WT) controls (Figure1).

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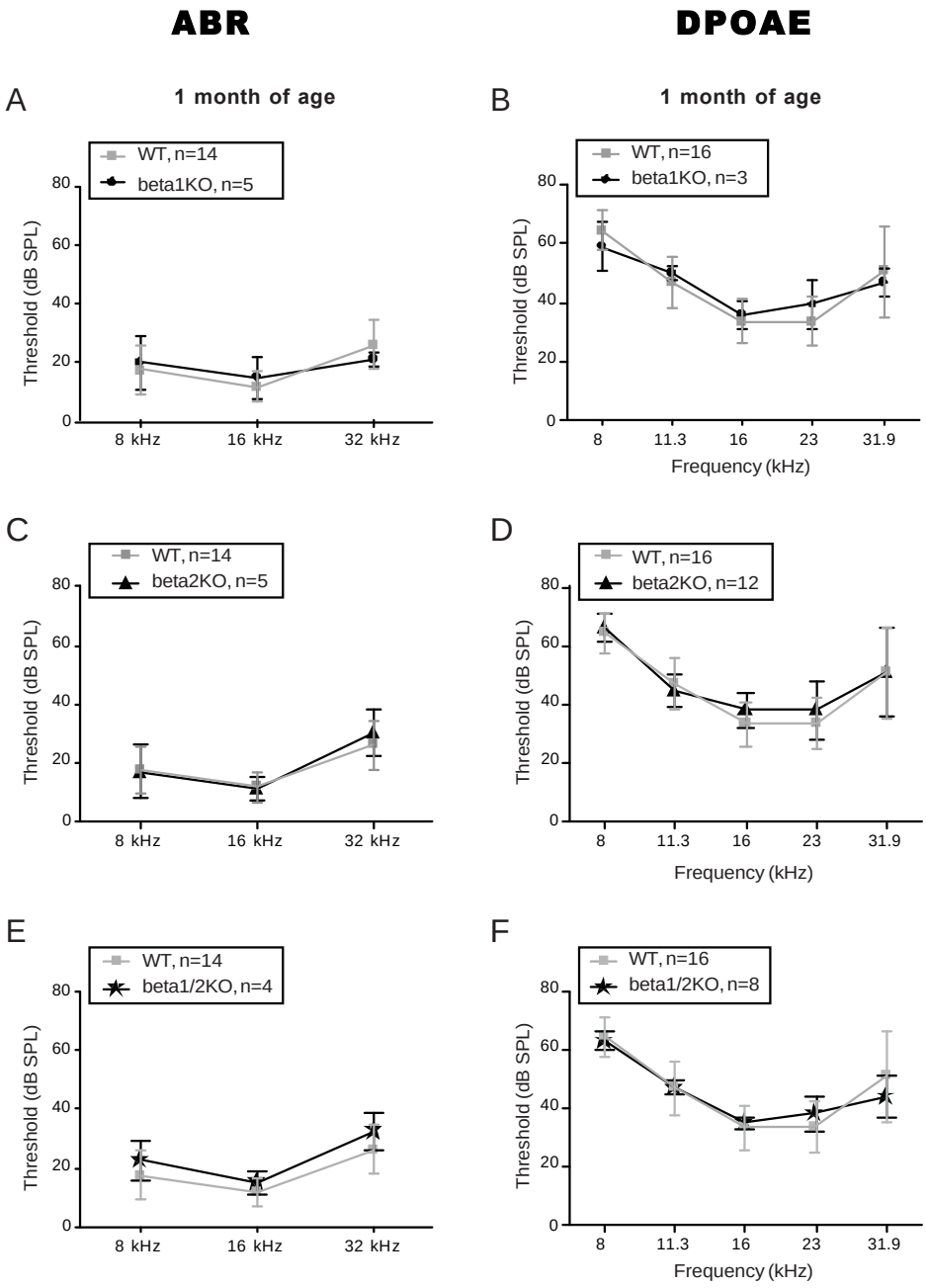

Figure 1: Assessment of hearing thresholds in beta-adrenergic receptor KOs at 1 month of age. ***A***, ***C***, and ***E,*** Auditory brainstem response (ABR) recording for three frequencies did not reveal significant difference in auditory thresholds between WT control and beta1-AR KO (***A***), beta2-AR KO (***C***) or beta1/2-AR double KO (***E***) mice. ***B***, ***D*** and ***F***, Functional measurements of OHCs by distortion product otoacoustic emission (DPOAE) tests showed no difference in thresholds at all tested frequencies between WT and beta1- (***B***), beta2- (***D***) or beta1/2- (***F***) AR KOs. Values depicted as means ± sd. T-test: * $p<0.05$.

No significant differences were observed in measurements of outer hair cells (OHCs) function by DPOAE, or in neuronal response as tested by ABR, in any of the groups tested. This observation is in accordance with previous work by Maison and colleagues, where they showed that deletion of beta-AR ligands adrenalin and noradrenalin did not altered the auditory function (Maison et al., 2010). However, they found that mice lacking adrenergic signaling in the ear were prone to middle ear infections, resulting in elevated DPOAE thresholds (Maison et al., 2010). Our beta-AR KO mice, however, did not reveal elevation in the DPOAE thresholds.

Cochlear function in older mice that lack adrenergic signaling has not been reported so far. Therefore, we performed additional testing on two groups of beta2-AR KO mice, one group at 6 months of age and another group at 12 months of age (Figure 2). Both groups were compared with WT littermates on the same background. We saw a similar degradation in hearing sensitivity between WT and beta2-AR KO mice (as assessed by t-test). At 6 months of age, an increase of about 20dB in ABR thresholds were seen at the high frequency region in both beta2-AR KO and WT animals. DPOAE responses were slightly elevated in both groups as well. When tested at 12 months of age, both beta2-AR KO and WT mice showed ABR and DPOAE thresholds shift at all frequencies tested (Figure 2).

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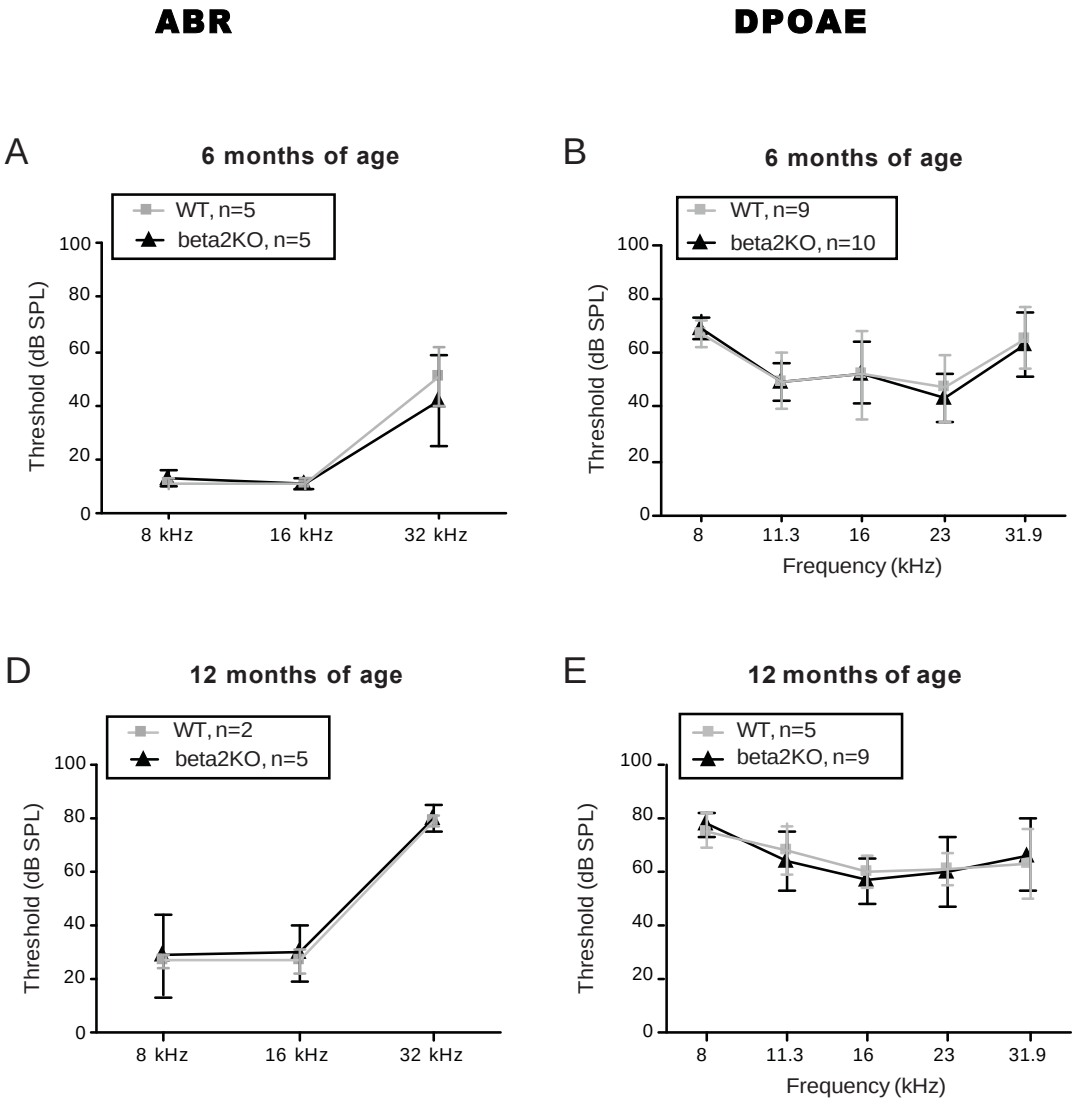

Figure 2. Assessment of hearing thresholds in aged beta2- adrenergic receptor KOs mice. ***A***, ***C***, ABR threshold tests revealed no significant difference between WT and beta2-AR KO mice at 6 months (***A***) and 12 months (***C***) of ages. ***B***, ***D***, Functional measurements of OHCs using DPOAEs showed also similar thresholds in WT and KOs at all frequencies tested. Values depicted as means ± sd. T-test: * $p<0.05$.

2. Subtle hearing threshold protection in beta1- but not beta2-adrenergic receptor mice was observed following acoustic overexposure

Early studies suggested a protective role played by the sympathetic system on noise damaged ears function (Borg, 1982; Hildesheimer et al., 1991; Horner et al., 2001, Bielefeld and Hendelson, 2007). In addition, the potential implication of beta-ARs in the regulation of potassium secretion raises the question about the role of these receptors in the noise-damaged ears (Wangeman et al., 2000). Therefore, we measured auditory responses to noise trauma in beta1- and beta2-AR KO mice and compared them to WT littermates. Age matched mice were divided into three groups based on their genotypes: beta1-AR KOs, beta2-AR KOs, and WT littermates. The baseline ABR and DPOAE responses for all mice at 5 months of age were recorded (Figure 3A, B and Figure 4A, B). One week later, all three groups of mice underwent 2 hours of broadband noise exposure (4-16 kHz) at 100 dB for 2 hours. We collected ABR and DPOAE measurements at 24 hours, 2 weeks, and 8 weeks following noise exposure (Figure 3 and 4). Hearing tests in noise exposed beta1- AR KO mice revealed a subtle hearing protection in these mutants at the low frequencies (8kHz) but not at the mid and high frequencies (16kHz and 32kHz) (Figures 3) when compared to WT controls. This subtle protection was observed right after noise exposure but was only significant 2 weeks (as assessed by t-test; $p < 0.05$) post-exposure. However, no significant shift in the hearing thresholds at any of the frequencies tested was observed in beta2-AR KO mice (figure 4).

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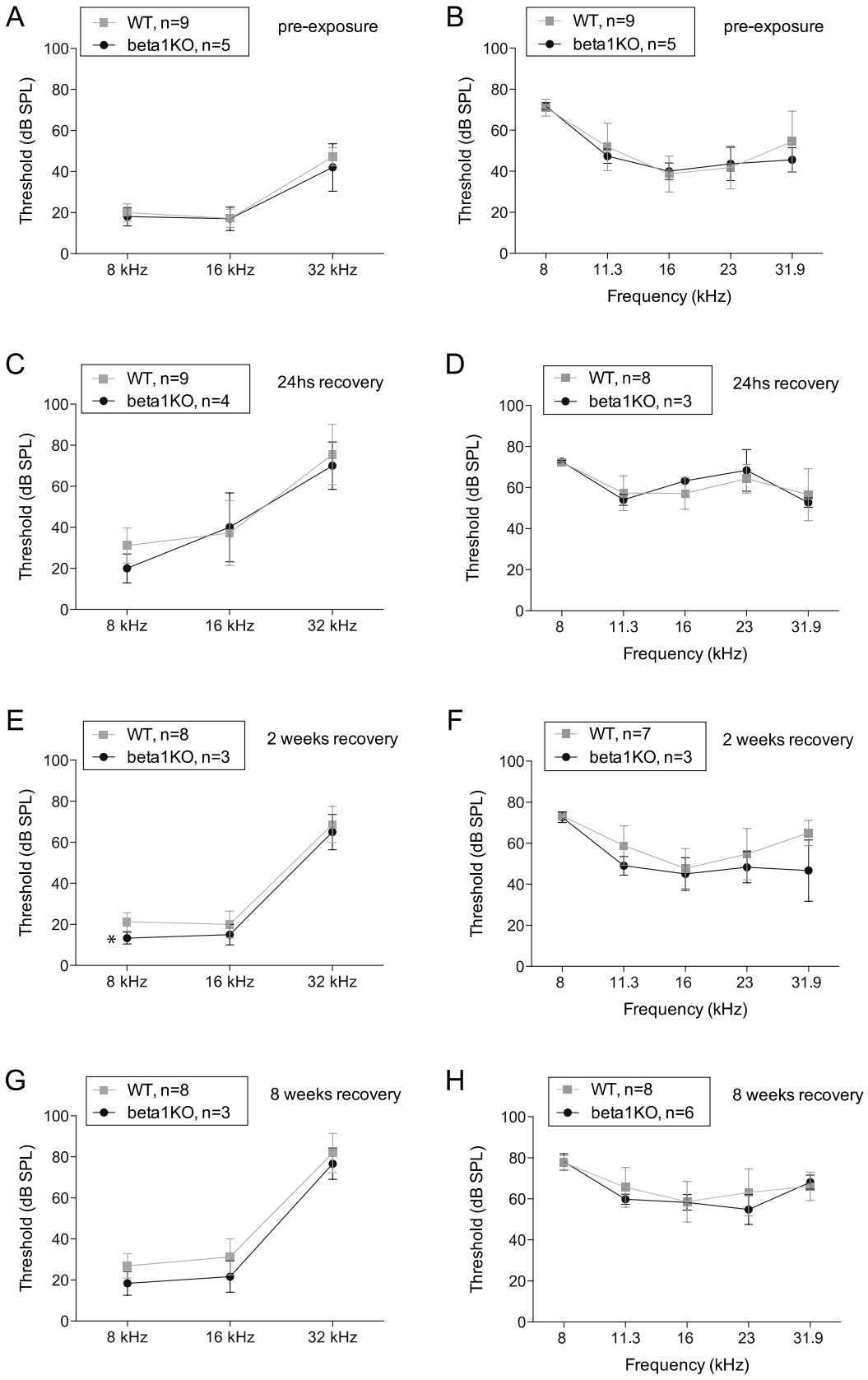

Figure 3. Assessment of hearing sensitivity in noise exposed beta1- adrenergic receptor KOs ears. ***A*** and ***B***, ABR (***A***) and DPOAE (***B***) tests in 5 months old beta1-AR KO and WT littermate mice one week prior to acoustic overexposure. ***C***, ***D***, 24 hours after noise exposure (100 dB SPL broadband 4 -16 kHz), mice were tested for ABR (***C***) and DPOAE (***D***) thresholds. DPOAE responses were similar between the two groups (***D***), however, ABR responses at lower frequencies were more sensitive in beta1-AR KO compare to WT controls. ***E***, ***F***, Tests for ABR (***E***) and DPOAE (***F***) 2 weeks after noise overexposure revealed similar thresholds recovery in WT and beta1-AR KOs. ***G*** and ***H***, The difference in ABR tests at 8 kHz observed 2 weeks after exposure between WT and beta1-AR KO mice persisted at 8 weeks (***G***) post noise exposure. DPOAE thresholds were similar between genotypes, although higher than pre-noise exposure levels. Values depicted as means ± sd. T-test: * $p<0.05$.

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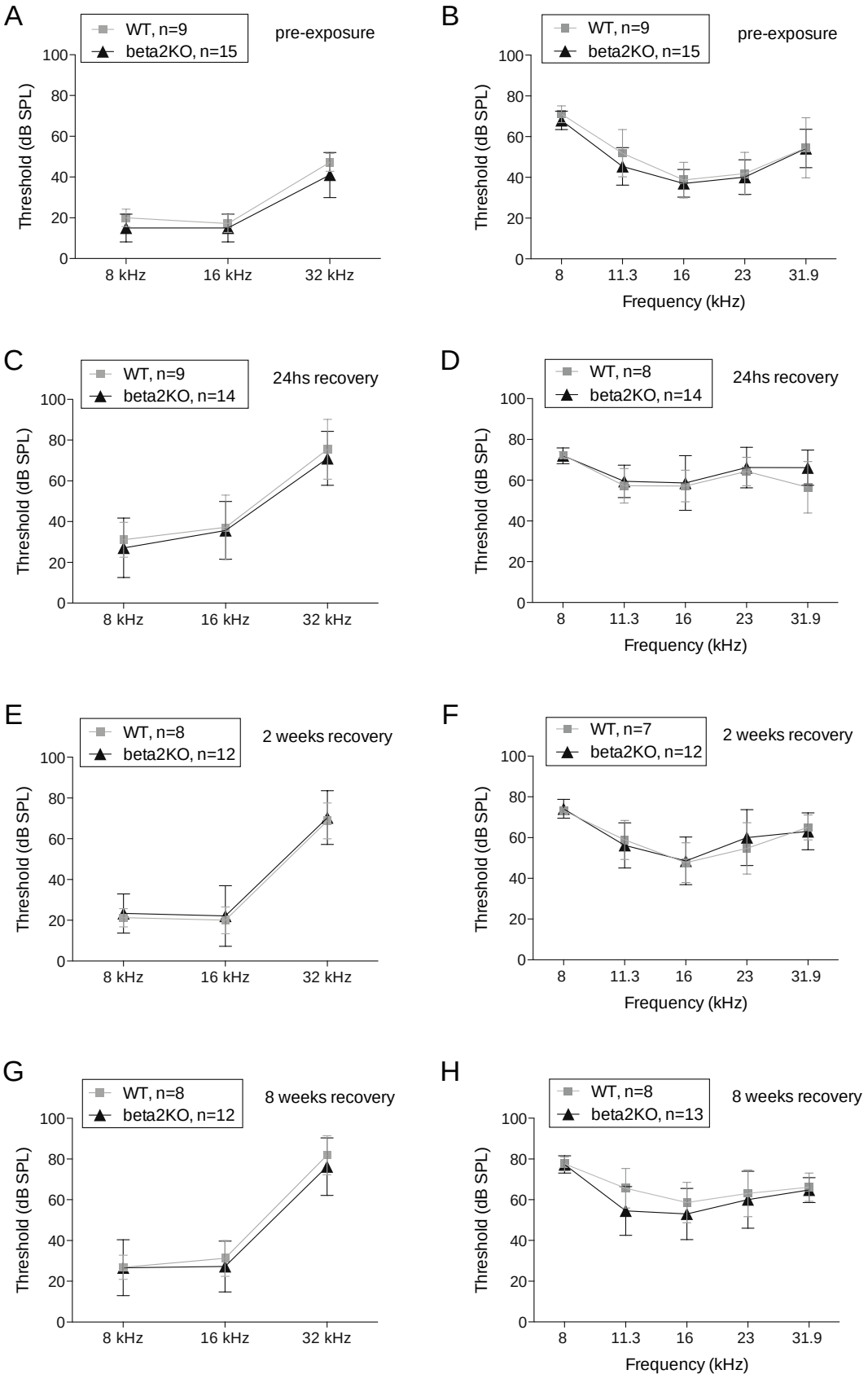

Figure 4. Assessment of hearing sensitivity in noise exposed beta2- adrenergic receptor KOs ears. ***A***, ***C***, ***E***, ***G***, ABR responses were similar between WT and beta2-AR KO mice 1 week before noise exposure (***A***), 24 hours after noise exposure (***C***), after 2 weeks of recovery from the delivered noise trauma (***E***) and after 8 weeks of recovery from noise trauma (***G***). No recovery at high frequency region was seen in both WT and beta2-AR KO animals (***E***, ***G***). ***B***, DPOAE responses (performed 1 week before noise overexposure) were similar between WT and beta2-AR KO mice at 5 month of age. ***D***, Noise trauma led to elevated thresholds at all frequencies. No difference in damage response was observed between the two genotypes. ***F*** and ***H***, Recovery of DPOAE thresholds: 2 weeks (***F***) and 8 weeks (***H***) after noise trauma. No significant differences were found between beta2-AR KOs and their WT littermates. Values depicted as means ± sd. T-test: * $p<0.05$.

Discussion

Recent reports on beta-adrenergic have detected expression of these receptors in several cochlear cell types including the stria vascularis (Schimanski et al., 2001; Fauser et al., 2004; Khan et al., 2007). Despite the expression of these receptors throughout the cochlea, their contributions to the proper cochlear function have not been investigated yet. Here we took advantage of the available knockout mice generated by Dr. Kobilka (Rohrer et al., 1993; Chruscinski et al., 1999) to address the role of these receptors in hearing.

1. Beta-adrenergic receptors in cochlear function of young and aged mice

One assumption we made, for looking into physiological cochlear responses, was that the sympathetic system profusely innervates many structures of the cochlea and controls many important processes for hearing function. For example, the stellate ganglion (or sympathetic ganglion) has been shown to be responsible for the sympathetic regulation of cochlear blood flow (Laurikainen et al., 1993). Stimulation or removal of the stellate ganglion can decrease or increase, respectively, cochlear blood flow (LaRouere et al., 1989; Laurikainen et al., 1993; Ren et al., 1993). It has also been reported that changes in cochlear blood flow are reflected in ABR and DPOAE responses. Decreased amplitudes of both measurements were observed with compression of the internal auditory artery (Telischi et al., 1998; Telischi et al., 1999). Interestingly, defect in vasodilation was observed in beta2-AR KO mice (Chruscinski et al., 1999) suggesting a potential important role for this receptor in cochlear blood flow. Additionally, adrenergic receptors were implicated in the regulation of potassium secretion in marginal cells of the stria vascularis, and consequently, in the maintenance of normal endocochlear potential (Wangemann et al., 2000). Together these observations raise the question regarding the role of adrenergic receptors in proper hearing development and maintenance.

We have measured hearing using ABR and DPOAE tests in young and aged single and double beta1- and beta2- AR KO mice and showed normal hearing threshold levels in these mutants at all tested ages. It has been shown that both ABR and DPOAE thresholds rise 1 dB for every mV decrease in the

endocochlear potential (EP) levels (Sewell, 1984). For these reasons we have tested both ABR and DPOAEs as a reflection of EP levels since the EP test requires scarifying the mouse and therefore not being able to follow this same mouse with age. The findings presented in this study do not suggest a significant defect in the hearing level in both single and double beta-adrenergic receptors mutants. This finding may be due to the subtle role that these receptors are playing in hearing or a compensatory effect from other receptors such as the alpha- adrenergic receptors.

2. Beta-adrenergic receptors and noise trauma

In the second set of experiments, we probed the role of beta-ARs in the cochlea following noise exposure. We assumed that deletion of beta-ARs might play a protective role in the noise-exposed ears. Our assumption was based on previous reports indicating that the loss of adrenergic innervation protects the ear from acoustic injury (Borg, 1982; Horner et al., 2001; Giraudet et al., 2002; Hildesheimer et al, 2002; Bielefeld and Henderson, 2007). Interestingly, Maison and colleagues disproved these studies by showing no difference in response to noise exposure in mice that were lacking the ligands adrenaline and noradrenaline (Maison et al., 2010). In their study, they used a genetic approach instead of the surgical or pharmacological approaches used in earlier studies. In our study, we used a different genetic approach, deleting the receptor as opposed to the ligand. Our observations fall right in the middle between earlier reports on protective effects of sympathectomy and recent the study on adrenalin/noradrenalin-deficient mice. In beta1-AR KOs, we have observed protection of roughly 10 dB SPL in the basal turn of the cochlea, which is in agreement with previous studies (Borg et al., 1982, Hildesheimer et al., 1991; Horner et al., 2001). However, middle and basal turns of the cochlea in the beta1-AR KOs and all turns in the beta2-AR KOs were similar to their control WT littermates. The observed similarities are in agreement with a previously described genetic approach of adrenergic pathway disruption (Maison et al., 2010). The difference in response to noise exposure between beta1-AR and beta2-AR KO mice may be attributed to potassium secretion in the stria vascularis. It was previously shown that potassium secretion in the stria vascularis is dependent on beta1-AR stimulation and not beta2-AR stimulation (Wangemann et al., 1995; Wangemann et al., 2000). The normal auditory thresholds observed in beta1-AR KO mice may be to the subtle effect of this receptor on potassium secretion and/or that the lack of beta1-AR could be compensated by another receptor or physiological mechanism (Wangemann et al., 2001; Wangemann, 2002). It is possible that this subtle effect to be more significant in conditions like acoustic trauma to the cochlea, and subsequently, high potassium recycling in the cochlear duct.

In conclusion, this study shows that mice lacking beta1-AR and beta2-AR, have normal auditory thresholds as measured by ABR and DPOAE. These mice responded to acoustic trauma similar to their control littermates and exhibited similar rates of hearing loss. However, our results revealed a subtle hearing protection at the low frequencies in absence of beta1-AR following noise exposure.

5. Acknowledgement

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The authors declare no competing financial interests.

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