Overexpression of SK2 Channels Enhances Efferent Suppression of Cochlear Responses without Enhancing Noise Resistance

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Abstract

Cochlear hair cells express SK2, a small-conductance Ca\(^{2+}\)-activated K\(^+\) channel thought to act in concert with Ca\(^{2+}\)-permeable nicotinic acetylcholine receptors (nAChRs) \(\alpha9\) and \(\alpha10\) in mediating suppressive effects of the olivocochlear efferent innervation. To probe the \textit{in vivo} role of SK2 channels in hearing, we examined gene expression, cochlear function, efferent suppression and noise vulnerability in mice overexpressing SK2 channels. Cochlear thresholds, as measured by auditory brainstem responses and otoacoustic emissions were normal in overexpressers, as was overall cochlear morphology and the size, number and distribution of efferent terminals on outer hair cells. Cochlear expression levels of SK2 channels were elevated 8-fold, without striking changes in other SK channels or in the \(\alpha9/\alpha10\) nAChRs. Shock-evoked efferent suppression of cochlear responses was significantly enhanced in overexpresser mice, as seen previously in \(\alpha9\) overexpresser mice (Maison et al. 2002); however, in contrast to \(\alpha9\) overexpressers, SK2 overexpressers were not protected from acoustic injury. Results suggest that efferent-mediated cochlear protection is mediated by other downstream effects of ACh-mediated Ca\(^{2+}\) entry, different from those involving SK2-mediated hyperpolarization and the associated reduction in outer hair cell electromotility.
Introduction

Outer hair cells (OHCs) in the mammalian cochlea operate as forward and reverse transducers, both transforming sound-evoked cochlear vibrations into electrical potentials and converting their own transmembrane potentials back into mechanical motion (Brownell et al. 1985). The latter process, known as somatic electromotility, plays a key role in the amplification of cochlear motion and thus in the generation of responses at threshold sound levels (Dallos and Fakler 2002; Liberman et al. 2002). OHC function is regulated by the medial olivocochlear (MOC) efferent system, a sound-evoked negative feedback loop terminating on OHCs (Guinan 1996a). Activation of this feedback system elevates cochlear thresholds and reduces the ear's vulnerability to acoustic injury (Kujawa and Liberman 1997).

The MOC/OHC synapse is cholinergic (Eybalin, 1993). Studies of isolated hair cells suggest that ACh-gated Ca\(^{2+}\) entry through \(\alpha9/\alpha10\) nicotinic acetylcholine receptors (nAChRs) activate a hyperpolarizing current mediated by co-localized small-conductance Ca\(^{2+}\)-activated K\(^+\) channels (SK2) (Elgoyhen et al. 1994a; Elgoyhen et al. 2001; Fuchs and Murrow 1992; Oliver et al. 2000). In addition, ACh application decreases OHC stiffness via Ca\(^{2+}\)-activated modification of OHC motor and/or cytoskeletal proteins (Dallos et al. 1997). Correspondingly, in vivo studies show that MOC activation elicits two types of suppression: a "fast" (\(\tau=100\) msec) effect thought to arise from OHC hyperpolarization, and a "slow" effect (\(\tau=10\) sec) thought to arise from a wave of Ca\(^{2+}\)-induced Ca\(^{2+}\) release propagating along the OHC basolateral membrane (Sridhar et al. 1997; Sridhar et al. 1995). Circumstantial evidence has suggested that the protective effects of shock-evoked MOC effects vis-à-vis acoustic vulnerability are mediated by slow rather than fast effects of ACh release (Reiter and Liberman 1995).

Gene targeting studies have begun to probe the roles of nAChRs and SK channels in the in vivo responses to MOC stimulation. Loss of \(\alpha9\) nAChR results in loss of fast and slow MOC-mediated suppression as well as subtle changes in the morphology of MOC terminals on OHCs (Vetter et al. 1999). Overexpression of \(\alpha9\)AChR receptors results in enhanced MOC suppressive effects and renders the ear more resistant to acoustic injury (Maison et al. 2002a). Deletion of SK2 channels also eliminates MOC-mediated suppression of cochlear responses, but also causes dramatic post-natal degeneration of MOC terminals which complicates interpretation of the loss of MOC function (Vetter et al. 2005).

Here, we study the effects of SK2 overexpression on inner ear function. SK2 overexpresser mice show enhanced MOC-evoked suppression without obvious changes in the distribution of efferent terminals in the OHC area. However, in contrast to the \(\alpha9\) overexpresser, SK2 overexpressers do not show enhanced resistance to acoustic injury. Results are consistent with the view that protective effects of MOC activation are mediated via downstream actions of Ca\(^{2+}\) entry other than activation of SK2 channels.

Materials and Methods

**Mutant animals:** SK2 overexpresser mice were created as described in Hammond et al. (2006). Overexpresser mice were created by inserting the tetracycline regulatory cassette 5' of the initiating methionine codon such that the native SK2 promoter drives expression of the tetracycline transactivator (tTA) protein, which in turn induces the transcription of the SK2 gene by binding to the minimal cytomegalovirus (CMV\(_{\text{min}}\)) promoter. Male heterozygous SK2
overexpresser mice, maintained as a congenic C57BL/6 strain, were genotyped by PCR for the presence of the tetracycline promoter: TET F: CAGCGCATAGAGCTGCT and TET off R3: AATGCCACAGCGCTGAG. The homozygous SK2 overexpresser is embryonic lethal (Hammond et al. 2006), thus wildtype and heterozygous littermates were compared in the present study. All animals procedures were approved by the IACUC of the Massachusetts Eye and Ear Infirmary.

**ABR and DPOAE Measurements:** Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured in each animal at 7 log-spaced frequencies (half octave steps from 5.6 to 45.2 kHz) before and after acoustic overexposure. Mice at age 8-10 wks were anesthetized with xylazine (20 mg/kg i.p.) and ketamine (100 mg/kg i.p.). Needle electrodes were inserted at vertex and pinna, with a ground near the tail. ABRs were evoked with 5-ms tone pips (0.5-ms rise-fall with a cos² onset envelope delivered at 35/sec). The response was amplified, filtered and averaged in a LabVIEW-driven data-acquisition system. Sound level was raised in 5 dB steps from at least 10 dB below threshold up to 80 dB SPL (mean data suggest the start level – if threshold is overestimated, the run is aborted and re-started at a lower SPL). At each sound level, 1024 responses were averaged (with stimulus polarity alternated), using an "artifact reject," whereby response waveforms were discarded when peak-to-peak amplitude exceeded 15 µV. On visual inspection of stacked waveforms, "ABR threshold" was defined as the lowest SPL level at which any wave could be detected, usually corresponding to the level step just below that at which the peak-to-peak response amplitude rose significantly above the noise floor (approximately 0.25 µV). For amplitude versus level functions, the wave I peak was identified by visual inspection at each sound level and the peak-to-peak amplitude computed. DPOAEs at 2f₂-f₁ were recorded in response to primary tones: f₁ and f₂ with f₂/f₁ = 1.2 and f₂ level at 10 dB < f₁ level. Ear-canal sound pressure was amplified and digitally sampled at 4 µs intervals. Fast Fourier Transform were computed and averaged over 5 consecutive waveform traces, and 2f₁-f₂ DPOAE amplitude and surrounding noise floor (-10 to -20 dB SPL depending on frequency) were extracted. Iso-response contours were interpolated from plots of amplitude vs. sound level, performed in 5 dB steps of f₁ level. "DPOAE threshold" is defined as the f₁ level required to produce a DPOAE at 0 dB SPL.

**Medial Olivocochlear Assay:** A craniotomy and cerebellar aspiration exposed the floor of the IVth ventricle. Shocks were applied through a pair of silver wires placed at the brainstem midline, at an appropriate rostro-caudal location based on surface landmarks. Shock threshold for facial twitches was determined, then paralysis induced with α-D-tubocurarine (1.25 mg/kg i.p.), and the animal connected to a respirator. Shock levels were raised 6 dB above twitch threshold. During the MOC suppression assay, f₂ level was set to produce a DPOAE ~10-15 dB > noise floor. The primary tones were presented continuously, and DPOAE amplitudes were measured roughly every 5 seconds, before, during and after a 70-second period during which a shock train (150 µsec monophasic pulses at 300/sec) was delivered to the brainstem electrodes. Magnitude of MOC-mediated suppression was defined as the difference in dB between the mean pre-shock DPOAE amplitude and the mean of the amplitudes obtained during the first 3 during-shocks measures.

**Acoustic Overexposure:** Animals were exposed free-field, awake and unrestrained, in a small reverberant chamber. Acoustic trauma consisted of a 2-hr exposure to an 8-16 kHz octave band
noise presented at 100 dB SPL. The exposure stimulus was generated by a custom white-noise source, filtered (Brickwall Filter with a 60 dB/octave slope), amplified (Crown power amplifier), and delivered (JBL compression driver) through an exponential horn fitted securely to a hole in the top of a reverberant box. Sound exposure levels were measured at 4 positions within each cage using a 0.25" Bruel and Kjaer condenser microphone: sound pressure was found to vary by <0.5 dB across these measurement positions.

**Histological analysis:** Cochlear morphology was assessed in 4-8 wk old mice via serial sections of either osmium-stained plastic sections or haemotoxylin/eosin-stained paraffin sections. Tissue was fixed by intracardial perfusion with 0.01 M phosphate-buffered saline (PBS) followed by either 4% paraformaldehyde (paraffin sections) or 2.5% glutaraldehyde with 1.25% paraformaldehyde (plastic sections). For both procedures, cochleas were post-fixed at 4 °C overnight, then decalcified in 120 mM EDTA for 3–7 days, depending on the age. Ears to be plastic-embedded were then osmicated for 1 hr in 1% OsO₄. All ears were then dehydrated in ethanol, embedded and sectioned at either 12 or 40 mm (in paraffin or plastic, respectively). For paraffin sections, deparaffinized slides were exposed to hematoxylin (ThermoShandon), acid ethanol, ammonium water and eosin (ThermoShandon) before dehydration and coverslipping.

**Cochlear Immunohistochemistry:** After intracardial perfusion buffered 4% paraformaldehyde, cochleas were decalcified as described above and dissected into 6 or 7 segments for subsequent immunostaining. Tissue was permeabilized with TBS containing Triton X-100 overnight, then blocked in TBS containing 1% BSA, 3% normal horse serum for 30 min at room temperature. Tissue was then incubated in TBS containing one or more of the following primary antibodies: mouse anti-synaptophysin (1:2500, Chemicon), rabbit anti-neurofilament-200 (1:1000, Sigma), or vesicular acetylcholine transporter (1:2000; Chemicon), followed by fluorescent-conjugated secondary antibody (1:200, species appropriate, Alexafluor 488 or 594, Molecular Probes, Eugene, OR). Tissue was slide-mounted in gelvatol or vectastain, coverslipped and examined with high-NA immersion objectives in a laser confocal microscope.

**RT-PCR:** Gene specific primers and probes were designed using Primer Express (see Table 1). RNA isolation was performed using the Versagene tissue kit as described by manufacturer. Cochleae from adult mice (age 3-6 wks), harvested immediately after anesthesia and stored at -70°C, were placed in lysis buffer containing Tris (2-carboxyethyl) phosphine (TCEP). Cochleae were homogenized and lysates were passaged through two columns and washed until RNA was isolated. Individual RNA samples (~2µg) were then reverse transcribed to generate cDNA using the cDNA High Fidelity Archive kit (Applied Biosystems). Quantitative RT-PCR was performed using a 7900HT Applied Biosystems machine. Experiments were performed in triplicate for each sample including a standard curve. At least two mice per genotype were used. The mRNA in each sample was normalized to murine-specific GAPDH (Applied Biosystems).

### Results

The SK channel family consists of four members: SK1, 2, 3 and 4 (Hammond et al. 2006). SK2 is the only member of this family highly expressed in the cochlea, where it appears at the basal pole of OHCs, opposite the cholinergic synapses of MOC fibers (Dulon et al. 1998; Oliver
et al. 2000). Although SK2 overexpression in this mutant line did not result in the up-regulation of other SK channels in the brain (Hammond et al. 2006); we confirmed in the cochlea, by quantitative RT-PCR, that the 8-fold up-regulation of SK2 was not accompanied by changes in either SK1 or SK3 (Fig. 1). Additional RT-PCR results also suggest little change in the expression of nAChR α9 and α10 subunits (Fig. 1), which co-localize with SK2 at the efferent synapses on OHCs.

To assess baseline cochlear function, auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured in age-matched (8-10 wks) wildtype and SK2 overexpressers. There was no difference in mean threshold values between the two groups via either cochlear-function test (Fig. 2 A,B). Suprathreshold response amplitudes at each of the test frequencies were also unaffected by genotype: data from one test frequency are shown (Fig. 2C,D). The absolute thresholds in both wildtypes and transgenic animals are poor for frequencies at 32 kHz and above: such a pattern of high-frequency loss is not unexpected given the C57Bl/6 background of the animals (see (Hequembourg and Liberman 2001; Zheng et al. 1999).

Cochlear morphology was assessed by light-microscopic evaluation of serial sections through a number of wildtype and transgenic cochleas, stained with either haematoxylin/eosin (3 wildtypes, 7 overexpressers) or osmium (2 wildtypes; 2 overexpressers). Consistent with the normal cochlear responses, no abnormalities were visible in hair cells, supporting cells, neurons or other structures of the cochlear duct in the overexpresser ears (Fig. 3).

To evaluate effects of SK2 overexpression on the size, number or distribution of afferent and efferent terminals in the sensory epithelium, we double-immunostained cochlear whole mounts for neurofilament (NF-200: to stain all axonal processes) and vesicular acetylcholine transporter (VAT: to stain all cholinergic efferent terminals). Since the density of both afferent and efferent innervation changes systematically from the base to the apex (the gradient of SK2 expression has not been described), it is critical to compare place-matched regions along the cochlear spiral. As documented by the confocal images from Figure 4, the efferent innervation of OHCs appeared unaffected by the SK2 overexpression. In middle to basal cochlear regions (e.g. 24 kHz) of both genotypes, there is a rosette of 1-3 VAT-positive terminals under virtually every OHC in each of the three rows; these terminals are positioned exclusively in the sub-nuclear regions of the OHCs (see y-z projections in Fig. 4). In contrast, in the more apical regions (e.g. 8 kHz), the cholinergic efferent innervation is sparser, the terminals are smaller and they are sometimes found in supranuclear locations along the sides of the OHCs in both genotypes. Similar results were obtained when synaptophysin and NF200 were used for efferent (data not shown).

The most striking phenotype of the SK2 overexpressers was a significant enhancement in magnitude of efferent-mediated cochlear suppression. To assess the magnitude of efferent-mediated effects on cochlear responses, we measured DPOAEs before, during and after a 70-sec epoch of electrical stimulation of the OC bundle. In normal ears (Fig. 5A, filled circles), this paradigm of efferent stimulation elicits a fast suppression of DPOAE amplitude, which decays during the continued period of shock-evoked efferent activation. After termination of the shock train, DPOAE amplitudes often "overshoot" the mean pre-shock value, in what appears to be a slow response enhancement that eventually recovers back to baseline over tens of seconds. The magnitude of fast suppression and slow enhancement vary with the frequency of the DPOAE-evoking stimuli: both effects tend to be largest in the mid-basal turn (16 - 32 kHz), where the density of efferent innervation is greatest. The decrease of effect-size at 32 and 45 kHz in the wildtype animals seen here is due to the relatively poor DPOAE thresholds at these high
frequencies: if OHC function is compromised, there is little further suppression that can be elicited by efferent stimulation. As shown in Figure 5B, the magnitude of fast suppression was significantly enhanced in the SK2 overexpressers (F(1,8)=6.029, p=0.04 by two-way ANOVA), however, the small changes in magnitude of post-shock enhancements were not significant (F(1,8)=0.003, p>0.05 by two-way ANOVA).

Previous studies have shown that sound or shock-evoked activation of the OHC efferent innervation can reduce the vulnerability of the ear to temporary or permanent noise-induced injury (e.g. Rajan 1991). To assess whether overexpression of the SK2 channel, and the associated magnification of shock-evoked efferent suppression, leads to enhanced resistance to acoustic injury we exposed matched groups of transgenic overexpressers and wildtype littermates to a noise band designed to produce roughly 30 dB of permanent threshold shifts, as measured 1-wk post-exposure. The noise vulnerability of the two genotypes was virtually identical (Fig. 6), whether measured by ABR or DPOAE metrics: group differences were not significant by two-way ANOVA (ABR: F(1,12)=0.085, p>0.05; DPOAE: F(1,20)=0.689, P>0.05).

Discussion

A. Cholinergic actions at the OHC: mechanisms of slow vs. fast effects

The binding of ACh to its cochlear receptors sets in motion a complex set of events within the OHCs, which are visible in vivo as suppression of cochlear responses on both fast (tens of msec) and slow (tens of seconds) time scales (Sridhar et al. 1997; Sridhar et al. 1995). In vitro work suggests that the cholinergic synapses on hair cells couple ACh-gated Ca\(^{2+}\) entry through \(\alpha_9/\alpha_{10}\) nAChRs to a Ca\(^{2+}\)-activated K\(^+\) channel, namely the SK2 (Elgoyhen et al. 1994a; Elgoyhen et al. 2001; Fuchs and Murrow 1992; Oliver et al. 2000). As described below, the in vivo fast effect is probably associated with SK2 activation, whereas the slow effects are associated with other downstream effects of Ca\(^{2+}\) entry into the OHC.

In vivo, this fast-onset suppression (\(\tau \sim 100\) msec) can be measured in the sound-evoked vibration amplitudes of the cochlear duct (Cooper and Guinan 2003), as well as all other "downstream" measures of the cochlear transduction cascade from hair-cell receptor potentials (Brown and Nuttall 1984) to sound-evoked discharge rates in single auditory-nerve fibers (Wiederhold and Kiang 1970). In the present experiment, fast suppression is seen as the precipitous drop in DPOAE amplitude visible in the first during-shocks point of the MOC effect assay (Fig. 5A). This "classic" fast effect is 1) potently blocked by strychnine (Kujawa et al. 1994), 2) completely abolished by targeted deletion of the \(\alpha_9\) nAChR (Vetter et al. 1999), the \(\alpha_{10}\) nAChR (Vetter et al. 2005b) or the SK2 channel (Vetter et al. 2005), and 3) enhanced by overexpression of the \(\alpha_9\) subunit (Maison et al. 2002b) or the SK2 channel (present results). These data are consistent with immunohistochemical and in situ hybridization studies suggesting that \(\alpha_9\) (Elgoyhen et al. 1994a), \(\alpha_{10}\) (Elgoyhen et al. 2001) and SK2 (Oliver et al. 2000) are all expressed by OHCs, and are also consistent with the known channel properties and pharmacology of \(\alpha_9\) and/or \(\alpha_{10}\) receptors expressed in vitro (Elgoyhen et al. 1994b; Elgoyhen et al. 2001).

Slower suppressive effects (\(\tau \sim 10\) sec) are also evoked, in vivo, by efferent-mediated ACh release (Sridhar et al. 1995). These slow suppressive effects are harder to study in mouse, because they are often masked by a slow efferent-mediated enhancement of response, which, as
we have recently shown, is independent of the OHCs nAChRs (Maison and Liberman 2006). In guinea pigs, both slow and fast suppressive effects are blocked by the same palette of cholinergic antagonists, suggesting that both require initial Ca\(^{2+}\) entry through \(\alpha 9/\alpha 10\) nAChRs (Sridhar et al. 1995); and both effects are eliminated by targeted deletion of \(\alpha 9\) subunits in mouse (Vetter et al. 1999). However, slow suppression can be selectively enhanced by antagonists of pumps mediating Ca\(^{2+}\) re-uptake into intracellular stores (e.g., thapsigargin and cyclopiazonic acid), suggesting the involvement of a wave of Ca\(^{2+}\)-induced Ca\(^{2+}\) release propagated along the subsurface cisternae within the OHCs, both opposite the efferent terminals as well as along much of the cell’s basolateral membrane (Sridhar et al. 1997).

In non-mammalian vertebrates, efferent activation in vivo hyperpolarizes the target hair cell and reduces receptor potentials on a time scale consistent with fast effects (Art et al. 1984). Intracellular recordings from mammalian OHCs in vivo during efferent stimulation have not been reported. However, if OHC hyperpolarization and receptor-potential reduction also occur, this would decrease OHC electromotility (Santos-Sacchi et al. 1998) and could account for the reduction in basilar membrane motion and all the other consequent reductions in cochlear sound-evoked responses.

ACh application to isolated OHCs increases electromotility in the load-free condition as it decreases the axial stiffness of the OHCs (Dallos et al. 1997). The effect is blocked by strychnine at concentrations similar to those blocking Ca\(^{2+}\) entry into \(\alpha 9/\alpha 10\) heteromers in vitro (Elgoyhen et al. 2001), consistent with the idea that it is mediated by the cochlea's unique nAChRs. Other evidence suggests that this (slow) cholinergic effect on OHCs in vitro involves Ca\(^{2+}\)-mediated phosphorylation of the OHC motor molecular prestin and/or Ca\(^{2+}\)-mediated changes in OHC cytoskeletal elements (Sziklai et al. 2001; Zhang et al. 2003). Other in vitro manipulations, such as diamiide application, reduce OHC stiffness by interfering with actin cross-linking, and also reduce OHC force generation.

Slow effects observed in vivo in the efferent-mediated suppression of basilar membrane motion are associated with phase lags that are consistent with changes in OHC stiffness (Cooper and Guinan 2003). The relations between in vitro electromotility and in vivo amplification of cochlear motion are complex and poorly understood: nonetheless in some micromechanical cochlear models, a decrease in OHC stiffness reduces basilar membrane vibration at best frequency (Allen 1990).

B. Fast vs. slow effects and the protective actions of the efferent pathway

The efferent pathway to the OHCs constitutes the effector arm of a sound-evoked negative feedback loop, which acts to control the masking of transient stimuli by continuous noise backgrounds and also reduces the ear's vulnerability to acoustic injury (Guinan 1996b). In anesthetized animals, electrical stimulation of the efferent bundle reduces the temporary threshold shifts seen after short-duration high-intensity stimulation (Rajan 1988). In awake animals, the strength of the sound-evoked efferent reflex is a strong predictor of vulnerability to permanent acoustic injury (Maison and Liberman 2000), and unilateral surgical section of the efferent bundle significantly increases noise-induced permanent threshold shifts seen in the de-efferented ear (Kujawa and Liberman 1997).

Circumstantial evidence, based largely on the frequency dependence of the effects, has suggested that efferent-mediated protection is associated with slow rather than fast effects of cholinergic transmission (Reiter and Liberman 1995). Results of the present study provide additional evidence, of a more direct nature, for that hypothesis. A previous study (Maison et al.
2002b) showed that transgenic overexpression of α9 nAChRs (1.7 fold increase by Western blot in homozygotes) increased fast effect magnitude by at most ~4 dB (measured at 22 kHz) and decreased permanent noise-induced threshold shift from about 40 dB (in wildtypes) to about 20 dB (in homozygotes). In the present study, SK2 overexpression (almost 8 fold by qRT-PCR: Fig. 1) produced a significant enhancement of efferent-mediated fast effects (by as much as ~7 dB at 22 kHz: Figure 5), yet caused no change in noise vulnerability (Figure 6). The noise exposure band (8-16 kHz), and thus the cochlear regions maximally affected by the trauma (~20 kHz), were identical in the two studies; thus the pre-exposure threshold elevations at frequencies > 32 kHz in the present study are not a significant complication. However, hints that protective effects of cochlear manipulations such as heat shock differ between the extreme base and the rest of the cochlea (Yoshida et al. 1999) suggest that re-evaluation of both SK2 and α9 overexpression in a mouse model with better function at very high frequencies could be informative.

These results are consistent with the notion that the protective effects of efferent cholinergic transmission arise from downstream effects of Ca²⁺ entry other than those involving SK2 activation and the subsequent increase of K⁺ conductance, cell hyperpolarization and the decrease in receptor potential and OHC electromotility that follow from it. It is interesting, in this regard, that the Ca²⁺ permeability of the α9/α10 receptor complex is particularly large, compared with other nAChRs (Weisstaub et al. 2002). Downstream effects of Ca²⁺ entry could include protein phosphorylation, or other structural modifications, that decrease the sound-evoked mechanical motions of the partition. Although efferent suppressive effects tend to be largest at low sound pressure levels, even at high sound levels, efferent activity can decrease vibrations in a manner equivalent to a decrease in input of a few dB (Russell and Murugasu 1997), and relatively small changes in effective sound level can have large effects on noise damage (Yoshida et al. 2000). It is also possible that the protective effects of cholinergic signaling at the cochlear hair cell involve changes in expression of anti-apoptotic pathways, as has been proposed as a downstream effect of nAChR signaling via α7 and other subunits in studies of neural degeneration in vivo and in vitro (for review see (Dajas-Bailador and Wonnacott 2004).

References Cited


Figure 1: Expression levels of SK channels and $\alpha_9/\alpha_{10}$ nAChRs in wildtype and overexpresser animals, as assayed by quantitative RT-PCR of whole cochleas (4 from each genotype) and expressed relative to levels in wildtypes. Only the differences in SK2 expression levels were statistically significant (*$P < 0.05$ by Student’s t test). Means and SEMs are shown.
Figure 2: Cochlear response thresholds, and suprathreshold response amplitudes, were unaffected by SK2 overexpression. A and B show mean thresholds (± SEMs) for the two genotypes, as measured by ABRs and DPOAEs respectively. C and D show mean amplitude-vs.-stimulus-level curves evoked by stimuli at 16 kHz for wave 1 of the ABR (summed activity of cochlear nerve fibers: C), or for the DPOAE (f2 at 16 kHz: D). Numbers of ears represented in each sample are included in the key: keys in A and B apply to C and D respectively. Numbers of ears are larger for DPOAE because, in one group of animals, DPOAEs were recorded from both ears while ABRs were recorded only from one.
Figure 3: Cochlear morphology was normal in the SK2 overexpresser. Each image shows the upper basal turn: inner and outer hair cell areas are indicated in panel A by the open and filled arrows, respectively. Scale bar in A also applies to B.
Figure 4: Immunostaining for an axonal marker (neurofilament 200: red) and a cholinergic marker (vesicular acetylcholine transporter: green) reveals a normal efferent innervation of the cochlea in the overexpresser ears. Representative confocal z-stacks are shown from cochlear whole mounts at two different frequency regions (apical turn: 8 kHz and upper basal turn: 26 kHz). Each z stack is shown as an x-y maximum projection (left image of ear pair) and a y-z maximum projection (right image of each pair), in which the approximate position of each of the three OHC rows is indicated by dashed lines (OHC location is better seen when gamma in the red channel is adjusted to bring up the background staining). Scale bar in the x-y projection of A applies to all images.
Figure 5: Efferent-mediated suppression of cochlear responses is enhanced in the SK2 overexpresser mice. A: Amplitude of the DPOAE evoked by a continuous primaries is repeatedly measured, before during and after a 60-sec shock train delivered to the efferent bundle, and then plotted as deviation from the mean value seen before the shock-train onset. Fast suppressive effects are averaged over the 1st three during-shocks point; post-shock enhancement is averaged over the 7th - 12th post-shocks points, as schematized. Data shown here are means of all runs from wildtypes vs. overexpressers for f2 at 22.6 kHz. B: Mean suppressive (during shocks) and enhancing (post-shocks) effects of efferent stimulation as a function of stimulus frequency for 3 wildtype and 4 overexpresser animals.
Figure 6: Noise vulnerability is not affected by SK2 overexpression. Mean permanent threshold shifts (±SEM), as measured by either ABR (A) or DPOAE (B) are shown. Group sizes are shown in the key: numbers are larger for DPOAE measures, because in one cohort (3 animals of each genotype) DPOAE thresholds were measured in both ears, whereas ABRs were not.
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*Table 1:* Primers used for RT-PCR