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Expression and localization of K^+ channels KCNQ2 and KCNQ3 in the mammalian cochlea

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Abstract

KCNQ1 and KCNQ4 voltage-gated potassium channel subunits play key roles in hearing. Other members of the *KCNQ* family also encode slow, low voltage-activated K^+ M currents. We have previously reported the presence of M-like K^+ currents in sensory hair cells, and expression of *Kcnq* family genes in the cochlea. Here, we describe *Kcnq2/3* gene expression and distribution of M channel subunits KCNQ2 and 3 in the cochlea. By using RT-PCR, we found expression of *Kcnq2* in modiolus and organ of Corti, while *Kcnq3* expression was also detected in the cochlear lateral wall. Five alternative splice variants of the *Kcnq2* gene, one of which has not been reported previously, were identified in the rat cochlea. KCNQ2 and KCNQ3 immunoreactivities were observed in spiral ganglion auditory neurons. In addition, the unmyelinated parts of the nerve fibers innervating hair cells and synaptic regions under hair cells showed KCNQ2 immunoreactivity. KCNQ3 immunoreactivity was also prominent in spiral ganglion satellite cells. These findings suggest that cochlear M channels play important roles in regulation of cellular excitability and maintenance of cochlear K^+ homeostasis in the auditory system.

Keywords

Alternative splicing; Cochlea; Gene expression; Immunohistochemistry; Voltage-gated K^+ channel KCNQ2; Voltage-gated K^+ channel KCNQ3

Introduction

Ion channels are vitally important for the excitability, electrical activity and signaling properties of most cell types in the body; this is perhaps most obvious in cells of the nervous system, particularly neurons. Voltage-gated K^+ (K_V) channels belong to the structurally and functionally highly diverse K^+ channel gene super-family, which comprises more than 70 genes in the human, with splice variants adding further diversity [Yellen 2002]. K_V channels are the molecular brakes of neuronal signaling; they sense depolarization and act to

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terminate action potentials. By limiting neuronal excitability, they also have a fundamental influence on neuronal coding.

M currents are low-threshold, slowly activating/inactivating, voltage- and time-dependent potassium currents that show no inactivation. The currents were named M for muscarinic due to their suppression by muscarinic receptors activation in bullfrog sympathetic ganglion neurons [Brown and Adams 1980]. M currents have been recorded in a number of preparations from central and peripheral nervous tissue, e.g. in hippocampus [Moore et al. 1988], and in visual system [Beech and Barnes 1989]. In superior sympathetic ganglia and some hippocampal neurons, M channels consist of tetrameric assemblies of KCNQ2 and KCNQ3 subunits [Wang et al. 1998]. Such M channels regulate membrane potential, neuronal excitability and limit repetitive firing, thus opposing epileptic activity. Gene mutations of either *KCNQ2* or *KCNQ3* lead to inherited forms of epilepsy – benign familial neonatal convulsions, BFNC1 [Singh et al. 1998] and BFNC2 [Charlier et al. 1998], respectively. KCNQ5 [Lerche et al. 2000; Schroeder et al. 2000] add to the M channel subunit repertoire, and isoforms resulting from alternative splicing also contribute to M current diversity (see review by Jentsch [Jentsch 2000]).

An array of ion channels and transport proteins permeable to potassium ions (K^+) are known to exert a major influence on several processes in cochlear physiology and auditory function [Wangemann 2002]. Electrochemical gradients of K^+ ions and transmembrane K^+ ion channels are essential for sound transduction by sensory hair cells and neuronal signaling. Hearing disorders specifically associated with *KCNQ* genes include *KCNQ1* and *KCNQ4*. Mutations in *KCNQ1* (as well as *KCNE1*) can lead to Jervell and Lange-Nielsen syndrome with congenital deafness resulting from faulty K^+ recycling via I_{sK} current in marginal cells of stria vascularis [Neyroud et al. 1997]. Mutations in *KCNQ4* which is mainly expressed in outer hair cells lead to autosomal dominant progressive hearing loss, DFNA2 [Kubisch et al. 1999]; alternatively, inner hair cell expression and role of KCNQ4 in regulation of the resting potential and intracellular calcium concentration may underlie this inherited deafness [Oliver et al. 2003]. Kubisch et al [Kubisch et al. 1999] detected weak expression of KCNQ3 in mouse cochlea and vestibule. However, pathophysiological roles of KCNQ2 or KCNQ3 in the inner ear are as yet unknown.

We have recently recorded M-like currents from outer hair cells isolated from guinea pig cochlea, and confirmed that M channel subunit genes *Kcnq2* and *Kcnq3* are expressed in the cochlea [Liang et al. 2005]. Following that observation, we have identified expression of KCNQ1-5 subunit mRNAs in rat and guinea pig hearing organs [Liang et al. 2006]. The present study extends our work on the voltage-gated K^+ channel KCNQ subfamily ($K_V7.x$), with the identification of *Kcnq2* and *Kcnq3* gene expression in micro-dissected cochlear sub-regions, with the detection of *Kcnq2* alternative splice variants in the rat cochlea, and with the immunolocalization of M channel subunits in cryo-sectioned cochlea using specific antibodies directed against KCNQ2 and KCNQ3.

Materials and Methods

Animal use and care

The animals (adult Sprague-Dawley rats, pigmented guinea pigs, and C57 BL/6 mice) and acute procedures used in this study were approved by the Karolinska Institutet ethics committee (N146/99, N10/01).

One-step RT-PCR

The cochleas were quickly dissected out, and then further micro-dissected in RNAlater RNA stabilization reagent (Qiagen) under a dissection microscope. Cochlear sub-regions

(modiolus, organ of Corti, and lateral wall) were immediately put in TRIzol (Invitrogen Corporation, Carlsbad, California, USA) and homogenized well. Total RNA was isolated from the cochlear fractions using the TRIzol and RNeasy MinElute Cleanup kits (Qiagen GmbH, Hilden, Germany) according to the manufacturers' instructions. The concentration and quality of total RNA was measured on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, California, USA).

cDNA synthesis and PCR were performed using the SuperScript One-Step RT-PCR system (Invitrogen) in an MJ Research gradient thermocycler (PTC-200, Bio-Rad Laboratories, Inc., Waltham, Massachusetts, USA). PCR primers were purchased from Invitrogen: *Kcnq1*, forward 5'-GGATGGAGATTGTCCTGGTG-3', reverse 5'-CCTGGCGATGGATGAAGA-3'; *Kcnq2*, forward 5'-CGTTCATCTACCACGCCTAC-3', reverse 5'-TACTCCACGCCAAACACCAC-3'; *Kcnq2_sv*, forward 5'-TCACGCACCGACCTGCACTC-3', reverse 5'-CCTTCCCAAGCCGTCCCATC-3'; *Kcnq2_sv_new*, forward 5'-TCCCCATCTACAGACTCATTCC-3', reverse 5'-CCTCGGGGGCTTGGTGAT-3'; *Kcnq3*, forward 5'-CCAAGGAATGAACCATATGTAGCC-3', reverse 5'-CAGAAGAGTCAAGATGGGCAGGAC-3' [Kubisch et al. 1999]; *Myo7a*, forward 5'-GCCGATTCCATCTTCCACTA-3'; reverse 5'-TCCGTAGTTTGCTTCACCTC-3'; *Gapdh*, forward 5'-GCCAACATCAAGTGGGGTGATG-3', reverse 5'-GTCTTCTGGGTGGCAGTGATG-3'. The optimal annealing temperature and cycle number for each primer pair are: *Kcnq1* (62°C, 34 cycles), *Kcnq2* (60°C, 32 cycles), *Kcnq2_sv* (64°C, 34 cycles), *Kcnq2_sv_new* (56°C, 34 cycles), *Kcnq3* (50°C, 30 cycles), *Myo7a* (50°C, 34 cycles), *Gapdh* (60°C, 28 cycles). PCR products were separated by agarose gel electrophoresis, and directly sequenced after gel extraction with QIAquick Gel Extraction kit (Qiagen), using AmershamPharmacia Biotech DYEnamic ET terminator cycle sequencing kit (US81050), and an ABI100 model 377 sequencer (KIseq, the DNA sequencing core at Karolinska Institutet).

Antibodies

The rabbit anti-KCNQ2N antibody is directly against residues from the N-terminal region of KCNQ2 (GEKKLKVGFVGLDPGAPDSTRDC), which are conserved in mammals [Cooper et al. 2001]. The guinea pig anti-KCNQ3N antibody is developed against residues from the long N-terminal splice variant of KCNQ3 (AGDEERKVGLAPGDVEQVTLAL), which is also fully conserved in mouse, rat and human. The antiserum was affinity-purified against the immunogenic peptide. The specificity of KCNQ3N antibody was examined by immunoblotting of cell lysates from human embryonic kidney (HEK) cells transfected with *KCNQ2* or *KCNQ3* cDNA, following the protocols as previously described [Cooper et al. 2001]. Mouse anti-specific Class III β-tubulin (βIII-tubulin) (TUJ1) antibody was used to label neuronal cells (Covance). The rabbit anti-KCNJ10 polyclonal antibody was purchased from Alomone Labs (Jerusalem, Israel).

Tissue preparation and immunohistochemistry

Animals were deeply anaesthetized with an overdose sodium pentobarbital and transcardially perfused with normal saline (0.9%) followed by ice-cold 4% formaldehyde fixative freshly prepared from paraformaldehyde. The cochleas were dissected, locally perfused and immersed in the same fixative at 4°C overnight, then decalcified in 0.1 M EDTA. Mouse brain was used as positive control for the KCNQ antibodies. The tissues were cryo-protected by submerging in sucrose solutions (10% and 30% sucrose in PBS, 24 h each), and finally embedded in O.C.T. compound. Tissue blocks were stored at −80°C and sectioned at a 12-μm thickness using a cryostat (Leica).

Cryosections were processed for immunohistochemistry using immunofluorescence or the Avidin: Biotinylated enzyme Complex (ABC) method for detection. Sections for ABC staining were pre-treated with 0.3% H_2O_2 for 5 minutes to inactivate endogenous peroxidase. All sections were blocked with 5% goat serum, 5% BSA and 0.4% Triton X-100 in 0.1 M PBS for 1 h at room temperature, and further incubated overnight at 4°C with rabbit anti-KCNQ2N (1:100) or guinea pig anti-KCNQ3N (1:100) antibody. Mouse anti-TUJ1 (1:250) and rabbit anti-KCNJ10 (1:400) antibodies were applied in double fluorescent labelling. For immunofluorescence, the sections were incubated with fluorophore-conjugated secondary antibodies (Jackson Immunoresearch) and subsequently stained with DAPI (Molecular Probe) to visualize the nuclei. The sections were observed using a fluorescence microscope (Zeiss) or a confocal laser scanning microscope system (Zeiss LSM 510). For ABC staining, the sections were then incubated with biotinylated secondary antibody for 1 h, and ABC complex (Vector Labs) for 40 minutes at room temperature. Immunolabeling was visualized with di-amino-benzidine (DAB) substrate (Vector Labs) and the nuclei were stained with Gill's haematoxylin. The sections were observed and photographed in a light microscope (Zeiss) equipped with a digital camera (Nikon Coolpix 990). Primary antibodies were omitted from slides used as negative controls.

Results

Differential expression of Kcnq2 and Kcnq3 mRNA transcripts in the mammalian cochlea

To compare the relative expression of *Kcnq2* and *Kcnq3* mRNA transcripts in the sub-regions of micro-dissected adult guinea pig cochlea, RT-PCR conditions were optimized within the linear amplification range. The primers were designed to span a region of transcript common to all the splice forms. As shown in Figure 1, strong expression of *Kcnq2* was found in modiolus (containing the spiral ganglion), with lower levels in the organ of Corti, and absence in the cochlear lateral wall. In contrast, *Kcnq3* was expressed in all cochlear sub-fractions, although the expression was lowest in the lateral wall. *Myo7a* and *Kcnq1* primers were run in parallel to assess potential template contamination during the micro-dissection procedure, as *Myo7a* and *Kcnq1* were exclusively detected in the organ of Corti and the cochlear lateral wall, respectively. Omission of RNA template or reverse transcriptase served as negative controls. Expression of the reference gene *Gapdh* functioned as positive loading control and confirmed the quality of the RNA. All PCR products were sequence-verified. The *Kcnq2* and *Kcnq3* expression pattern was found to resemble that of RNA samples from adult rat and mouse cochlear sub-fractions.

Multiple alternative splice variants of the *Kcnq2* gene have been identified in neuronal tissues, in which the major splicing events occurred in the long intracellular C-terminus. We designed a primer pair (*Kcnq2_sv*) in order to amplify all the different C-terminal splice variants. Five *Kcnq2* alternative splicing isoforms were detected in the adult rat cochlea (Fig. 2): four isoforms (A, C, E, and H) which were also identified in rat brain and superior cervical ganglia (SCG) [Pan et al. 2001], and a new (the shortest) isoform with exons 8, 11, 12a and 15a spliced out. All the cochlear splice variants contained the alternative 5' splice site in exon 13 (13a shown in Fig. 2) but lacking exon 15a. We have also designed a primer pair (*Kcnq2_sv_new*) which specifically amplified only the new *Kcnq2* isoform. The new *Kcnq2* splice variant was abundantly expressed in several brain regions (e.g. hippocampus, cerebellum, cerebellar cortex) (data not shown).

KCNQ2 localization in the mammalian cochlea

The specificity of anti-KCNQ2N antibody was first confirmed in cryosections of mouse hippocampus (data not shown). It was consistent with previously reported intense staining in

hippocampal mossy fiber pathways in the hilus of the dentate gyrus (DG) and stratum lucidum in CA3 [Cooper et al. 2001].

KCNQ2 immunoreactivity in guinea pig, rat (Fig. 3A, B, and C), and mouse (data not shown) cochlea was observed in the cell bodies of primary auditory neurons of the spiral ganglion and their afferent projections at the base of inner hair cells, which were also immunolabeled by the anti-KCNQ2N antibody. KCNQ2 antibodies also labeled the nerve fibers after entering through *habenula perforata* (where auditory afferents lose their myelin sheath). In the organ of Corti, there was also pronounced staining beneath outer hair cells, possibly relating to afferent or efferent synapses, as well as weaker staining in the outer hair cells themselves. KCNQ2 immunoreactivity was also prominent in pillar cells. No KCNQ2 immunoreactivity was noticed in the cochlear lateral wall. Negative control sections with primary antibody omitted did not exhibit any significant immunolabeling (Fig. 3D).

Characterization of KCNQ3N antibodies and KCNQ3 localization in the mammalian cochlea

The anti-KCNQ3N immune serum was tested by using HEK cells transfected with *KCNQ3* cDNA. The KCNQ3 band (≈90 kDa) was absent in pre-immune serum, while immune serum detected the KCNQ3 protein. After affinity purification, the anti-KCNQ3N antibody recognized a single predominant band of ≈90 kDa; this was abolished by pre-incubation of the antibodies with the KCNQ3 immunogenic peptide, whereas KCNQ3N antibodies still recognized the KCNQ3 band in the presence of KCNQ2 peptide (Fig. 4A).

Using the ABC immunoperoxidase staining method, KCNQ3 immunoreactivity in rat (Fig. 4B) and mouse (data not shown) cochlea was observed in the spiral ganglion, both in neurons and, more pronounced, in the satellite cells surrounding the spiral ganglion neurons. KCNQ3 immunoreactivity was not present either in the organ of Corti or in the lateral wall (supplemental figure). However, the subcellular localization of KCNQ3 in the spiral ganglion was not readily resolved at the current magnification level. Negative control sections with primary antibody omitted were virtually devoid of immunostaining (Fig. 4C). To determine what cell types in the spiral ganglion express KCNQ3 subunit proteins, double immunofluorescence staining of KCNQ3 with either a neuronal marker, class III β-tubulin (TUJ1) or a specific satellite cell marker in the spiral ganglion, the KCNJ10 K^+ channel [Hibino et al. 1999;Jin et al. 2006], were carried out (Figs. 4D–I). KCNQ3 immunostaining was clearly observed in the somata of spiral ganglion neurons (Figs. 4D and G), while it appeared more intensely as “punctate rings” around the neurons, as seen in the overlay image of KCNQ3 and TUJ1 immunoreactivities (Fig. 4F). In addition, complete overlap of KCNQ3 and KCNJ10 immunoreactivities around the cell bodies of spiral ganglion neurons (Fig. 4I), further demonstrated that KCNQ3 channel proteins were indeed expressed in the satellite cells.

Discussion

Following our recent reports on M currents in cochlear outer hair cells, and expression of *Kcnq* genes in the cochlea [Liang et al. 2005; Liang et al. 2006], we here provide further evidence that the molecular correlates of M currents, KCNQ2 and KCNQ3 M channel subunits, are distributed in the cochlea.

By RT-PCR analysis, *Kcnq2* and *Kcnq3* mRNA transcripts were detected in cochlear sensorineural sub-regions (organ of Corti and spiral ganglion). Within these sub-regions, KCNQ3 is most likely coassembling with KCNQ2 or KCNQ4 to form functional heteromultimers. In addition, *Kcnq3* transcripts were expressed in the cochlear lateral wall. Additional experiments, e.g. single-cell RT-PCR and RNA *in situ* hybridization, can be performed to further elaborate on the spatial localization of *Kcnq2/Kcnq3* mRNA in the

cochlea. A recent study showed expression of several spliced forms of *Kcnq4* gene in the mouse cochlear lateral wall [Beisel et al. 2005]. These results suggest that KCNQ3/KCNQ4 channel subunits may play a role in the cochlear K^+ recycling pathway.

The alternative splicing of the *Kcnq2* gene has been previously described, producing several differentially spliced transcripts, predominantly in the C-terminal region [Nakamura et al. 1998; Pan et al. 2001]. These splice variants encode channel proteins which display distinct functional properties [Pan et al. 2001; Smith et al. 2001; Tinel et al. 1998]. Splicing mutations in *KCNQ2* have been reported in patients with BFNC [de Haan et al. 2006; Lee et al. 2000].We have detected five *Kcnq2* splice isoforms in the rat cochlea, of which four isoforms (A, C, E and H) were also present in rat brain and SCG [Pan et al. 2001]. However, the splice isoforms which contained exon 15a, partially accounting for the slow deactivation-activation kinetics of M-currents [Pan et al. 2001], were not expressed in the rat cochlea. The new *Kcnq2* isoform identified in the rat cochlea also existed in rat brain sub-regions, but the functional properties of channel encoded by this new isoform remain to be determined in future studies.

The KCNQ2 antibodies used in this study were previously shown to exhibit widespread specific immunoreactivity in mouse brain, mapping in particular to key sites for control of rhythmic neuronal activity and synchronization [Cooper et al. 2001]. In the cochlea, KCNQ2 immunoreactivity also localized to important sensorineural structures: spiral ganglion neurons and their afferent projections towards inner hair cells, which also stained for KCNQ2. There was significant immunolabeling beneath outer hair cells, while those sensory cells had lower levels of KCNQ2 expression. A recent report [Kharkovets et al. 2006] using immunofluorescent labeling, showed similar KCNQ2 immunoreactivity in unmyelinated axon fibers under hair cells in mouse cochlea. For KCNQ3, immunolabeling was less intense in the spiral ganglion neurons. In contrast, the perineuronal satellite cells were distinctly immunolabeled with the KCNQ3 antiserum. This was somewhat unexpected, however, satellite cell KCNQ3 subunits may form functional channels with an as yet uncharacterized subunit in these cells, and the functional significance may involve removal of potassium from the spiral ganglion neurons. Analogous to the control of intracellular calcium ion concentration ($[Ca^{2+}]_i$) by KCNQ4 in inner hair cells [Oliver et al. 2003], the satellite cell KCNQ3 subunits may participate in release of trophic support factors to spiral ganglion neurons via regulation of $[Ca^{2+}]_i$. KCNQ3 channel proteins were not detected in the organ of Corti and cochlear lateral wall by immunohistochemistry, whereas RT-PCR showed that *Kcnq3* mRNA transcripts were present in both cochlear sub-regions. It is reasonable to assume that in these cochlear partitions KCNQ3 channel subunits exist at a low level which cannot be easily detected by an immunohistochemical approach, which is inferior to RT-PCR in sensitivity and specificity.

KCNQs are clearly involved in auditory function as evidenced by deafness resulting from mutations in KCNQ1 and 4. Here, we provide further support for involvement of KCNQ2 and/or KCNQ3 in M channels with a cochlear distribution, which clearly indicates important roles such as control of auditory sensorineural excitability, possibly synchronization of auditory signaling, and potassium recycling. A potential role in neuronglia interaction is further suggested. As previously described, other *Kcnq* genes, e.g. *Kcnq5*, and alternatively spliced forms of *Kcnq2*, expressed in the cochlea, should contribute to functional diversity of cochlear M currents [Liang et al. 2005; Liang et al. 2006].

As one of the most common clinical auditory symptoms, tinnitus has been hypothesized as a form of “sensory epilepsy” [Shea and Harell 1978], although the molecular mechanisms underlying tinnitus remain unclear. However, hyper-excitability in the peripheral as well as central auditory system, may contribute to the generation of tinnitus [Bauer 2004].

Immunohistochemical analysis of KCNQ2 and 3 channel subunits showed strong somatic staining in the relay sites of the central auditory pathway, e.g. cochlear nucleus and superior olivary nucleus ([Cooper et al. 2001], and unpublished data). Taken together with our data, it is assumed that dysfunction of M channels, KCNQ2 and 3 in particular, may also be involved in tinnitus pathophysiology. Thus, this work may provide a basis for exciting opportunities for novel therapeutic avenues, e.g. utilizing M channels openers to alleviate tinnitus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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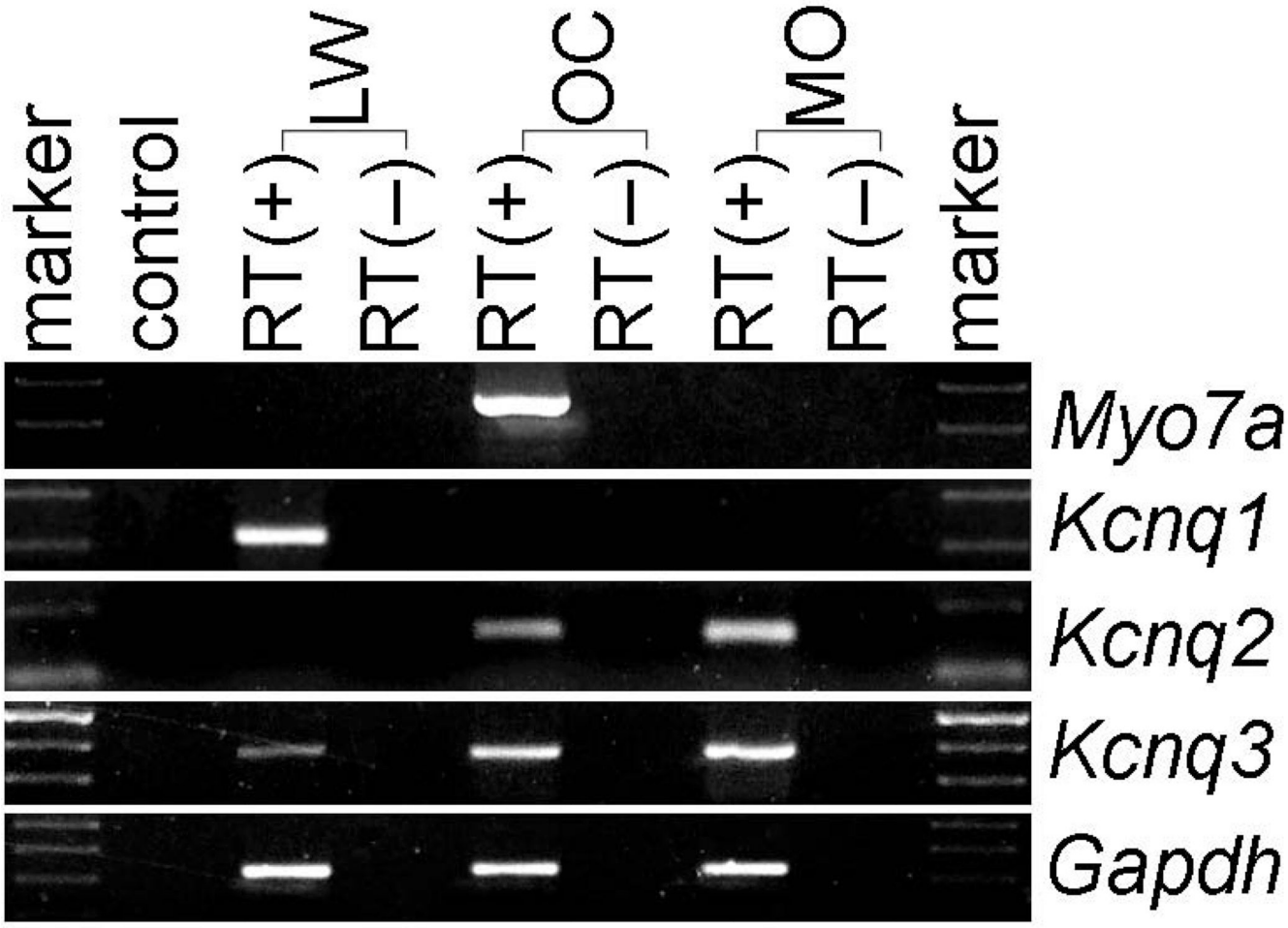

Fig. 1.

RT-PCR expression analyses for *Kcnq2* and *Kcnq3* mRNA in micro-dissected guinea pig cochlea. *Myo7a* and *Kcnq1* served as controls for detecting template contamination. *Gapdh* acted as positive loading controls. In negative controls, where template RNA or reverse transcriptase was omitted, no PCR products were observed. LW: lateral wall; OC: organ of Corti; MO: modiolus. PCR product size: *Kcnq1*, 309 bp; *Kcnq2*, 148 bp; *Kcnq3*, 462 bp; *Myo7a*, 341 bp; *Gapdh*, 310 bp. Three independent cochlear RNA preparations were tested; 10 μl PCR product was loaded in each lane.

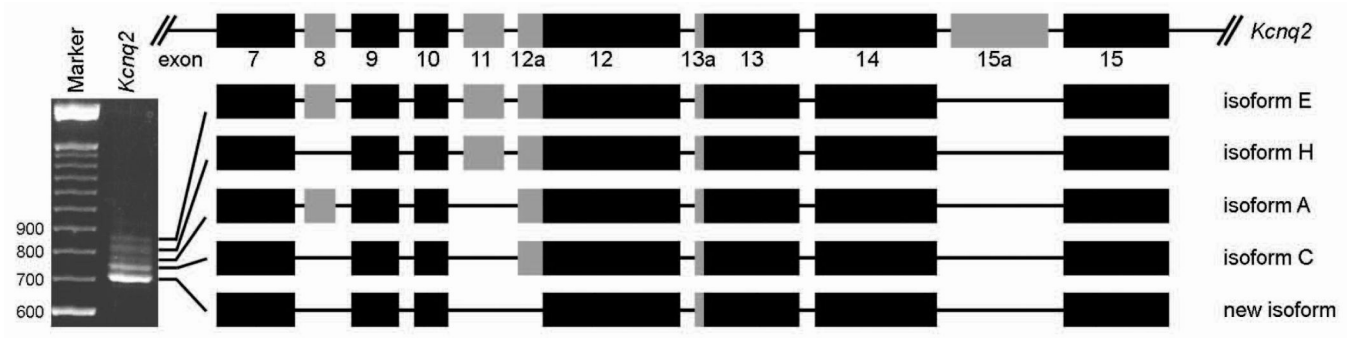

Fig. 2.

Alternative splice variants of *Kcnq2* transcripts expressed in the adult rat cochlea. (Left) Five *Kcnq2* splicing isoforms were identified in the rat cochlea by RT-PCR. (Right) A schematic view of the *Kcnq2* gene structure with alternative splicing region and display of cochlear *Kcnq2* splice variants. Grey boxes indicate exons that are alternatively spliced.

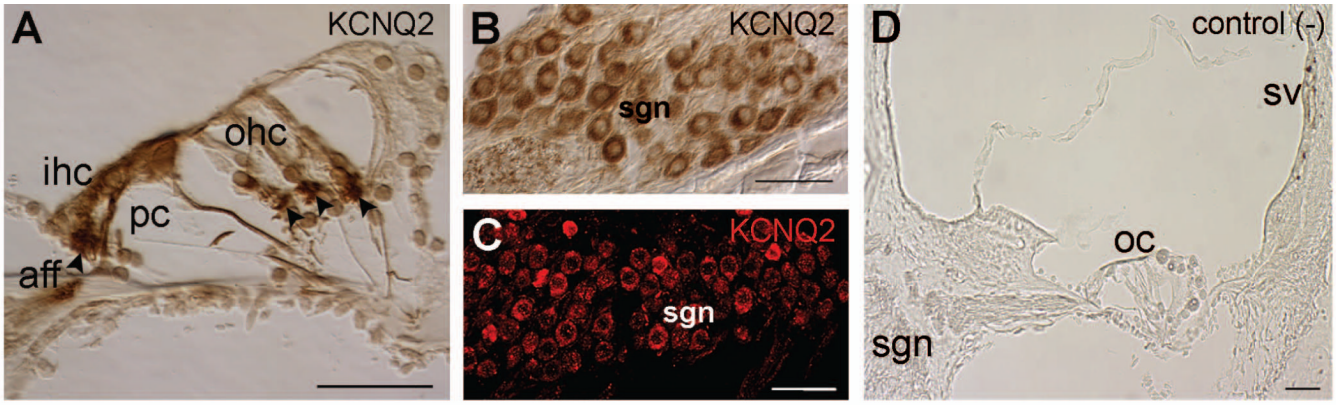

Fig. 3.

Immunolocalization of KCNQ2 in guinea pig (A,B) and rat (C) cochleas. (A) Immunoperoxidase staining for anti-KCNQ2N antiserum in the organ of Corti showing immunoreactivity in synaptic subregions (arrowheads) under inner (ihc) and outer hair cells (ohc). Also, pillar cells (pc) as well as afferent fibers (aff) after passing *habenula perforata* exhibit KCNQ2 immunoreactivity. (B) Immunoperoxidase labeling for KCNQ2 in spiral ganglion neurons (sgn) of the guinea pig cochlea. (C) Rat spiral ganglion neurons immunoreactive to anti-KCNQ2N antiserum. (D) Guinea pig cochlear control section with primary antibody omitted, no staining is observed in spiral ganglion neurons (sgn) and organ of Corti (OC), while weak background staining is observed in red blood cells of stria vascularis (SV). Scale bars = 20 μm.

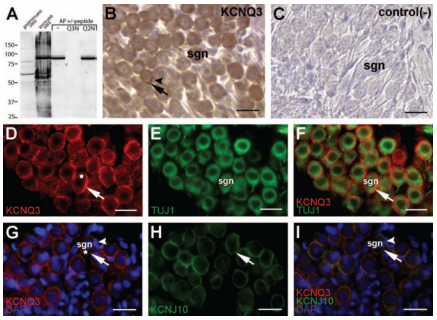

Fig. 4.

(A) Characterization of anti-KCNQ3N antiserum and affinity-purified antibody (AP) by Western blot using pre-immune and immune sera against KCNQ3 expressed in HEK cells. Immunoreactivity against the ≈90 kDa KCNQ3 band is absent using pre-immune serum, while immune serum detects the KCNQ3 protein. After affinity purification, the anti-KCNQ3N antibody recognizes a specific band of ≈90 kDa; this is abolished by pre-incubation of the antibodies with the KCNQ3 immunogenic peptide (Q3N), whereas KCNQ3N antibodies still recognize the KCNQ3 band in the presence of KCNQ2 peptide (Q2N). (B) KCNQ3 subunit immunoreactivity in rat spiral ganglion neurons (sgn) and perineuronal satellite cells (black arrow) with clear nuclei (black arrowhead) was detected by immunoperoxidase (DAB) staining. (C) Control section with primary antibody (anti-KCNQ3) omitted. (D–I) Double immunostaining of KCNQ3 (red in D and G) and TUJ1 (green in E) or KCNJ10 (green in H) in the rat spiral ganglion. KCNQ3 immunoreactivity was observed in the somata of spiral ganglion neurons (sgn, indicated by white star in D and G) and appeared more pronounced in the surrounding satellite cells (white arrow). In the overlay image (I), KCNQ3 and KCNJ10 immunoreactivities were co-localized in the satellite cells (white arrowhead indicating DAPI-stained nuclei). Scale bars = 20 μm.