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Potassium Channel Gene Expression in the Rat Cochlear Nucleus

David R. Friedland, Rebecca Eernisse, and Paul Popper

Department of Otolaryngology & Communication Sciences Medical College of Wisconsin, Milwaukee, WI

Abstract

Potassium channels play a critical role in defining the electrophysiological properties accounting for the unique response patterns of auditory neurons. Serial analysis of gene expression (SAGE), microarrays, RT-PCR, and real-time RT-PCR were used to generate a broad profile of potassium channel expression in the rat cochlear nucleus. This study identified mRNAs for 51 different potassium channel subunits or channel interacting proteins. The relative expression levels of 27 of these transcripts among the AVCN, PVCN, and DCN were determined by real-time RT-PCR. Four potassium channel transcripts showed substantial levels of differential expression. *Kcnc2* was expressed more than 15-fold higher in the DCN as compared to AVCN and PVCN. In contrast, *Kcnj13* had an approximate 10-fold higher expression in AVCN and PVCN than in DCN. Two subunits that modify the activity of other channels were inversely expressed between ventral and dorsal divisions. *Kcns1* was over 15-fold higher in DCN than AVCN or PVCN, while *Kcns3* was about 25-fold higher in AVCN than in DCN. The expression patterns of potassium channels in the subdivisions of the cochlear nucleus provide a basis for understanding the electrophysiological mechanisms which sub-serve central auditory processing and provide targets for further investigations into neural plastic changes that occur with hearing loss.

Keywords

cochlear nucleus; gene expression; SAGE; potassium channels; Kv3.2; Kir7.1

1. INTRODUCTION

The cochlear nucleus is the only central nervous system region to receive direct innervation from the auditory nerve. The auditory nerve fibers branch and distribute among the three subdivisions of the cochlear nucleus duplicating the tonotopic map in each locale. Within the anterior ventral (AVCN), posterior ventral (PVCN), and dorsal (DCN) cochlear nuclei the primary auditory signal is recoded and distributed to higher brain stem centers. The response to primary input, and thus the output to other auditory nuclei, is determined by the electrophysiological properties of distinct classes of cochlear nucleus neurons found in each subdivision. These electrophysiological properties are governed by the neuronal circuitry, cellular morphology, and patterns of potassium channel subunit expression among the classes of cochlear nucleus neurons.

Cochlear nucleus neurons have been studied and characterized, electrophysiologically, with regards to their currents and conductances. For example, octopus cells show high voltage-

Correspondence: David R. Friedland, MD, PhD Medical College of Wisconsin Dept of Otolaryngology and Communication Sciences 9200 W Wisconsin Ave Milwaukee, WI 53226 414-805-5626 (office) 414-805-7936 (fax) dfriedla@mcw.edu

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activated potassium currents (I_{KH}), low voltage-activated potassium currents (I_{KL}) and hyperpolarization-activated currents (I_H) (Cao and Oertel, 2005, Bal and Oertel, 2001). Bushy cells show low-threshold non-inactivating currents (I_{LT}) and both bushy and stellate cells demonstrate high-threshold delayed-rectifier-like currents (I_{HT}) (Rothman and Manis, 2003a). In some cases these currents are strongly correlated with specific potassium channels such as I_{HT} and Kv3.1 (Perney and Kaczmarek, 1997, Rothman and Manis, 2003a). For other currents the correlation is not as clear as in the postulated association of I_{KL} with several potential members of the Kv1 group of channels (Cao and Oertel, 2005). Identification of the regional distribution of potassium channel subunits, and their levels of expression, will allow better correlation between observed currents and the specific underlying potassium channels accounting for that physiology.

In addition to the role a single channel may play in defining conductance, channel modifiers and interactions between different channel subunits likely account for subtle differences in electrophysiological properties that may have significant functional implications. For example, the high fidelity and rapid firing rate seen among bushy cells appears to be dependent upon the kinetics of the Kv3.1 channel (Perney and Kaczmarek, 1997). Yet, bushy cells having normal Kv3.1 but lacking Kv1.1 show increased latencies and are unable to follow high frequency stimuli (Kopp-Scheinflug et al., 2003). Additionally, expression of Kv3.1b isoforms and their phosphorylation alters conductances and changes the neuronal response (Macica et al., 2003). As the potassium channel superfamily consists of over 100 different channel subunits and channel associated proteins (for review see (Coetzee et al., 1999, Miller, 2000)) it is impractical to individually identify those subunits and modifiers that may contribute to auditory neuronal physiology.

The principal aim of this study was to provide a broad profile of potassium channel expression in the cochlear nucleus for use in further investigations of central auditory neuronal function. As such, we used two methods of high throughput gene expression analyses, and PCR-based techniques, to identify transcripts for 51 potassium channels or interacting proteins in the cochlear nucleus. These analyses were performed independently in the AVCN, PVCN, and DCN to allow a comparison of potassium channel RNA levels among subdivisions. Real-time RT-PCR was used to further define the degree of differential expression of 27 of these channel subunits in the cochlear nucleus. Four genes were found to have substantial levels of differential expression and many others showed preferential expression in one or two subdivisions. Characterization of expression patterns of potassium channels in the auditory brain stem will better enable modeling of neuronal responses to acoustic stimuli. These findings will also direct attention to less abundant channels and modifiers and their potential roles in establishing the unique response properties of cochlear nucleus neurons.

2. METHODS

2.1 Potassium Channel Nomenclature

Potassium channel subunits have been extensively studied on molecular biological and protein levels. As such, nomenclature for these channels is often complex with imperfect agreement among names for genes, RNA sequences and proteins. Most auditory scientists are familiar with channel subunit names using the protein nomenclature (e.g., Kv3.1, TASK5) yet some genes have multiple common protein names associated with them. This manuscript will use gene names, as this is a molecular study of RNA transcripts, but attempt is made to indicate the more familiar protein names with a backslash where possible.

2.2 RNA Acquisition

RNA for the SAGE, microarray, RT-PCR, and real-time RT-PCR experiments was acquired from cochlear nuclei of female Brown Norway rats. A single gender and strain was used to reduce genetic variation. Rats at approximately 6 weeks of age were used to ensure auditory maturity and minimize potential age-related degenerative changes. Rats were anesthetized with Nembutal (50mg/kg IP) and decapitated after cessation of the paw-pinch reflex. Brains were dissected onto RNase-free Petri dishes for removal and subdivision of the cochlear nucleus. The nucleus was divided into anterior-ventral, posterior-ventral, and dorsal divisions under a dissecting microscope. The DCN was dissected from the ventral divisions at a consistent indentation along the rostral cochlear nucleus at the granule cell domain. The ventral divisions were divided into anterior and posterior at the entry zone for the auditory nerve. Overlap of cochlear nucleus subdivisions was minimized by pooling tissue from both sides of 20 animals. Two such pools of tissue were used for generating the SAGE libraries and for microarray evaluation. Additional pools were used for the RT-PCR and real-time RT-PCR experiments.

Total RNA was extracted from the dissected tissue using the TRIzol protocol (Invitrogen, Carlsbad, CA). The RNA was precipitated in isopropyl alcohol, washed with 75% ethanol and dissolved in RNase-free water. The RNA was analyzed by gel electrophoresis for appearance of distinct 28S and 18S RNA bands. Spectrophotometry was performed to determine RNA concentration and purity of the sample (i.e., A260/A280 ratio of greater than 1.9).

2.3 SAGE Library Generation

We have previously reported our SAGE protocol and the general process has been well-documented (Halum et al., 2004, Velculescu et al., 1995). A commercial kit was used for the generation of the libraries (Invitrogen, Carlsbad, CA). We utilized the *Nla*III and *Bsm*FI restriction endonucleases to form 10 base pair “tags” immediately downstream of CATG sites in cDNA. Tags were amplified, concatenated into long chains and cloned into plasmid for sequencing. We retrieved an average of 20-25 tags per sequencing reaction (Seqwright, Houston, TX). Sequencing reactions were run through PHRED base calling software to ensure high quality sequences (i.e. PHRED > 20) and the SAGE tags automatically extracted and compiled using eSAGE software (Margulies and Innis, 2000). Libraries were quality checked by confirming a low number of duplicate ditags and assessing that the GC content in each library was approximately 45% (Margulies et al., 2001). SAGE tags were mapped to known genes and transcribed sequences (i.e., ESTs) using the reliable tag database from SAGEMap (<http://www.ncbi.nlm.nih.gov/SAGE/>). The initial database used was from the November 2004 build, but tags of interest were re-queried individually in SAGEMap for potential new matches in January-February of 2006. Tag frequency was compared in pair-wise fashion (i.e., AVCN to PVCN, AVCN to DCN, and PVCN to DCN) using eSAGE software to determine p-values of differential expression (Audic and Claverie, 1997, Margulies and Innis, 2000). The libraries were subsequently maintained in an Excel spreadsheet and queried for “potassium” and “channel” to identify relevant transcripts. The entire AVCN, PVCN and DCN libraries have been made publicly available (NCBI Gene Expression Omnibus (GEO): AVCN: GSM83999; PVCN: GSM83400; DCN GSM83401; Rat Cochlear Nucleus Series: GSE3628).

2.4 Microarray Experiments

We used microarray as a supporting scaffold for our SAGE data. Microarrays can provide better expression information for transcripts with potentially low expression levels as may be the case for some potassium channel genes. Approximately 12-15ug of total RNA from each subdivision was submitted to GenUs Biosystems (Chicago, Ill.) for microarray analysis. This RNA represented a portion of one pool of total RNA harvested from 20 rats. The RNA was subjected to two cycles of linear amplification prior to hybridization to the CodeLink Rat Whole Genome Bioarray (GE Healthcare). Each subdivision was run in duplicate and raw data

processed through Codelink Expression Analysis software. Each hybridization spot was analyzed by the software for quality and we accepted only “good calls”, which are those significantly above background fluorescence levels around each probe. Hybridization intensities were normalized to the median for each chip and processed with GeneSpring software (Silicon Genetics, Redwood, CA) for comparison of replicates and generation of gene lists. The expression levels for each gene were taken as the average intensity of the two arrays performed for each subdivision. Those probes with average intensities above the normalized array median (i.e., > 1.0) were used for further analyses as comparison of the replicates showed poor correlation at expression levels below the median. Hybridization intensities less than 1.0 were reported if SAGE tags were found for the same transcript.

2.5 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Confirmation of the presence of select potassium channels was performed by reverse transcriptase polymerase chain reaction (RT-PCR). Primer sets were designed using NCBI mRNA sequences for the potassium channel transcript of interest and publicly available Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Where possible, forward and reverse primers were designed to lie in different exons as a control for genomic contamination. Additionally, all samples were DNase treated prior to RT-PCR reactions. RNA was acquired from differing pools of 20 rats than those used for the SAGE or microarray experiments. Approximately 0.5 to 2.0 µg aliquots of total RNA were treated with 1U DNase I, Amp Grade (Invitrogen, Carlsbad, CA) in 1X DNase I Reaction Buffer (Invitrogen, Carlsbad, CA) and incubated for 15 minutes at room temperature. DNase I was inactivated by adding 1 µl of 25 mM EDTA and heating for 20 minutes at 70°C. RNA was reverse transcribed using oligo dT primers (Invitrogen, Carlsbad, CA) and the Omniscript® Reverse Transcription kit (Qiagen Inc., Valencia, CA). The following primer sets for potassium channel transcripts were used:

GENE	DIRECTION	PRIMER SEQUENCE	EXPECTED AMPLICON SIZE (bp)
Kcnb1	For	5'-CACAGCAATAGCATTCAACTTC	589
	Rev	5'-GCTAGGTCTTAGTGGTGGCTG	
Kcnc2	For	5'-GAGTCTTCGCCTATGTGCTCAAC	513
	Rev	5'-GATCCGTTTCGATTTCATACTGG	
Kcnc3	For	5'-CTTTTGTAGGACCCCTACTCGTC	517
	Rev	5'-GTAACAAGAACTCGTTGGTGCTG	
Kcnip1	For	5'-GCACAGACGAACTTCACCAAG	489
	Rev	5'-CATGATGTTGTCATCCTCCTGACAG	
Kcnip2	For	5'-CTGGAACAACCTCCAGGAACAGACC	531
	Rev	5'-GACATTATCAAAGAGCTGCATG	
Kcnj6	For	5'-ATGGGAAACTATGCCTGATGTTT	540
	Rev	5'-GTTCTGCATGTTGGTTTCACTTTG	
Kcnj13	For	5'-GCTGATCCCAAAAAGAACTGAG	594
	Rev	5'-TTAGGTTTGCCATCTTTGTGAGC	
Kcnk12	For	5'-ACTTCCCTGGAGCCTTCTACTTC	508
	Rev	5'-ATGAAAAGGAAGTTGCCAGAC	
Kcnk15	For	5'-GCGAGTTCCGCAGAAAGTACC	580
	Rev	5'-AGGAAAAGCACCAATGACTGTGAG	
Kcns3	For	5'-TTTGTAGCATCTGCTTTGACTCG	547
	Rev	5'-ATCTCCTGGCAAAAGGAGAAGAC	
Kcna3	For	5'-TCAGGGAGGCTGTGTCAGTGGC	618
	Rev	5'-TTGTGGCAGCCTCAAACACGTC	
Kcna4	For	5'-CATGGGGATGGGTGTTCCTAC	415
	Rev	5'-GAGCCCTGTCTCCTCTTCTCT	
Kcna6	For	5'-AGACCTAGCCTGGCAGAGAGAC	620
	Rev	5'-CTTCTCATCCTCACCACCTTCGG	
Kcnk9	For	5'-TGATCGCCTGTACCTTCACCTAC	455
	Rev	5'-GACGGTCACCATGTTCTCCATAG	
Kcnq4	For	5'-GCTGAGGAGAAGAGCTACCACTG	476
	Rev	5'-CTGTGGTAGTCCGAGGTGATGTC	

2.6 Real-Time Reverse Transcriptase PCR

Real-time reverse transcriptase PCR (real-time RT-PCR) was used to confirm the comparative expression levels of select potassium channels detected by SAGE and microarray. Channels were selected for further analysis if they had significant expression levels in SAGE or microarray or have been previously reported as important in the auditory system. Hypoxanthine guanine phosphoribosyl transferase (HPRT) was used as a reference gene for all real-time RT-PCR experiments as it showed similar expression levels among the cochlear nucleus subdivisions in both the SAGE and microarray experiments. Other commonly used reference genes such as GAPDH and beta-actin showed differential expression by SAGE and microarray among AVCN, PVCN and DCN and were therefore not used.

Taqman® Gene Expression Assays were purchased from Applied Biosystems (ABI, Foster City, CA) for HPRT (ABI# Rn01527838), Kcna2 (ABI# Rn00564239_m1), Kcnc1 (ABI# Rn00563433_m1), Kcnc2 (ABI# Rn00594923_m1), Kcnc3 (ABI# Rn00588870_m1), Kcnd3 (ABI# Rn00709608_m1), Kcnh4 (ABI# Rn00586212_m1), Kcnip1 (ABI# Rn01470997_m1), Kcnip2 (ABI# Rn01411445_g1), Kcnj5 (ABI# Rn01789221_mH), Kcnj6 (ABI# Rn00755103_m1), Kcnj9 (ABI# Rn00587665_m1), Kcnj10 (ABI# Rn00581058_m1), Kcnj13 (ABI# Rn00586020_m1), Kcnj14 (ABI# Rn00821873_m1), Kcnk1 (ABI# Rn00572452_m1), Kcnk12 (ABI# Rn02132664_s1), Kcnk15 (ABI# Rn01776009_s1), Kcnma1 (ABI# Rn00582881_m1), Kcnn2 (ABI# Rn00570910_m1), Kcnn3 (ABI# Rn00570912_m1), Kcnq3 (ABI# Rn00580995_m1), Kcns1 (ABI# Rn00588597_m1), Kcns3 (ABI# Rn00696209_m1), Kcnv1 (ABI# Rn00572555_m1), Kcna1 (ABI# Rn00597355_sl), Kcna3 (ABI# Rn00570552_sl), and Kcnb2 (ABI# Rn00588883_m1). Real-time PCR was performed on the iCycler iQ Multicolor Real-Time Detection System (Bio Rad Laboratories, Hercules, CA). Real-time reactions contained 10µl 2x Taqman® Universal PCR Master Mix (ABI, Foster City, CA), 1µl of 20x Taqman® Gene Expression Assay (ABI, Foster City, CA), and 9µl cDNA in RNase-free water. The thermal cycling conditions were as follows: 50°C hold for 2 min, 95°C hold for 10 min, followed by two-step PCR for 45 cycles of 95°C for 15 sec and 60°C for 1min. An RT negative control was run for each channel and samples for each subdivision were run in triplicate.

Within each experiment, the cycle threshold (C_T) for each of the triplicates for the potassium gene of interest and for the HPRT reference, in each subdivision, was calculated and averaged. If the standard deviation of cycle threshold was greater than one cycle, that experiment was discarded. The C_T of the potassium channel was subtracted from the C_T of the HPRT to generate ΔC_T . Each experiment was run a total of three times and ΔC_T determined for each of the three runs. The ΔC_T from each of the three runs was averaged and the standard deviation calculated. This process was repeated for each potassium channel for each subdivision. The relative levels of transcript in each subdivision were compared by determining the differences in ΔC_T . This difference is termed $\Delta\Delta C_T$ and a fold-change in expression was calculated as $2^{-(\Delta\Delta C_T)}$. For example, the fold difference for a single potassium channel between AVCN and PVCN would be calculated as follows:

		AVCN				PVCN			
Run 1	KCN HPRT	a	b	c	Avg	a	b	c	Avg
		C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T1avgKCN}$ $C_{T1avgHPRT}$	C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T1avgKCN}$ $C_{T1avgHPRT}$
		$C_{T1avgKCN} - C_{T1avgHPRT} =$				$C_{T1avgKCN} - C_{T1avgHPRT} =$			
		ΔC_{T1}				ΔC_{T1}			
Run 2	KCN HPRT	C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T2avgKCN}$ $C_{T2avgHPRT}$	C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T2avgKCN}$ $C_{T2avgHPRT}$
		$C_{T1avgKCN} - C_{T1avgHPRT} =$				$C_{T1avgKCN} - C_{T1avgHPRT} =$			
		ΔC_{T2}				ΔC_{T2}			
Run 3	KCN HPRT	C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T3avgKCN}$ $C_{T3avgHPRT}$	C_{Ta} C_{TaHPRT}	C_{Tb} C_{TbHPRT}	C_{Tc} C_{TcHPRT}	$C_{T3avgKCN}$ $C_{T3avgHPRT}$

AVCN				PVCN			
a	b	c	Avg	a	b	c	Avg
$C_{T1avgKCN} - C_{T1avgHPRT}$			ΔC_{T3}	$C_{T1avgKCN} - C_{T1avgHPRT}$			ΔC_{T3}
Average ($\Delta C_{T1}, \Delta C_{T2}, \Delta C_{T3}$) = $\Delta C_{Tavcn} \pm SD$				Average ($\Delta C_{T1}, \Delta C_{T2}, \Delta C_{T3}$) = $\Delta C_{Tpvcn} \pm SD$			
$\Delta \Delta C_T = \Delta C_{Tavcn} - \Delta C_{Tpvcn}$							

$$\text{Fold Difference} = 2^{-(\Delta \Delta C_T)}$$

This method of calculating fold difference implies an amplification efficiency of 1.0 which is the reported efficiency of TaqMan primer sets (ABI, Foster City, CA) and is methodologically consistent with that described by Muller and colleagues (Muller et al., 2002). A one-tailed Student paired t-test was performed between subdivisions on the $\Delta \Delta C_T$ values for all potassium channel genes to assess differential expression. We report significance at levels of $p < 0.05$.

3. RESULTS

3.1 Serial Analysis of Gene Expression (SAGE)

SAGE libraries were generated from each of the subdivisions of the cochlear nucleus and mapped to potential genes through the NCBI SAGEMap database. The libraries were maintained in an Excel spreadsheet which was filtered and searched for the terms “potassium” or “channel” among the gene descriptions. This process identified 40 tags expressed in at least one subdivision that represented 31 potassium channel genes in the reliable tag database (Table 1). The majority of channels were represented by tags expressed in only one copy and in 21 of these cases the tag was only in a single subdivision. There were 7 channel genes represented by more than one tag copy and these included Kcnc1, Kcnc2, Kcnc3, Kcnk1, Kcnk15, Kcnv1 and Hcn2. For Kcnc1, Kcnc3, Kcnk15, Kcnv1, Kcnj6 and Hcn2 there was more than one tag sequence mapping to that transcript and the table reports the sum of these tags. There was only one SAGE tag sequence representing Kcnc2 and Kcnk1 which, for the latter, was expressed in greater than one copy in all three subdivisions. Statistically significant differences in SAGE tag counts were seen between the DCN and PVCN for Kcnc3 ($p < 0.05$) and Kcnk1 ($p < 0.01$). No other differences in SAGE tag frequency reached significant levels.

3.2 Microarray Experiments

Probes for 40 potassium channel or interacting protein transcripts were present on the gene chip. As noted in the Methods, normalized hybridization intensities below 1.0 were considered unreliable due to divergence in signal intensity in replicate arrays. Of the 40 probes, 28 had normalized intensities equal to or greater than 1.0 in all three subdivisions (Table 1). The most highly expressed transcripts by microarray in descending order were Kcnc1, Kcnk1, Kcna2, Kcns3 and Kcnk15. Three of these (i.e., Kcnc1, Kcnk1 and Kcnk15) were also among the transcripts most highly represented in the SAGE library. Of the remaining two, Kcna2 was not found at all by SAGE and Kcns3 was represented by 1 tag in the PVCN. The 12 channel transcripts with less than median levels of hybridization on the microarray were all represented by at least one tag in the SAGE library strongly suggesting their presence in the tissue. Data from SAGE and microarray were combined to provide a comprehensive profile of potassium channel expression in the cochlear nucleus (Figure 1).

Relative levels of the 10 most highly expressed transcripts by microarray in each subdivision were compared (Figure 2). Kcnc1 which encodes channel Kv3.1 was the most prevalent in each subdivision followed by Kcnk1/TWIK-1 in the AVCN and PVCN but by Kcna2/Kv1.2 in the DCN. Kcna2 was slightly more represented in DCN than Kcnk1 which was the third most prevalent in this subdivision. The most noticeable difference between subdivisions was the prevalence of Kcns3/Kv9.3 in the AVCN and PVCN, where it was fourth most prevalent, but the absence of this transcript in the top ten most expressed in the DCN. Other differences

included the appearance of Kcnh8/Kv12.1 in the top channels of the PVCN and DCN but not in AVCN. Similarly, Kcnip1 was one of the top ten channels in AVCN and DCN but not PVCN.

3.3 Confirmation of Expression by Reverse-transcriptase PCR

Ten potassium channels were tested to confirm their expression in each subdivision using RT-PCR. Nine channels were chosen from among the 46 identified by SAGE and microarray. These nine channels represented a variety of SAGE and microarray findings including those: not in SAGE but high on the microarray; those prominent in SAGE but low or not present on the chip; those high in both modalities; and those low in SAGE with various levels on the microarray. The tenth channel, Kcnb1, was neither in SAGE nor present on the microarray but was chosen because its product, Kv2.1, associates with α -subunit modifier Kv9.3 which was quite robust by gene chip in the AVCN and PVCN (see figure 2). Figure 3 demonstrates the expression of these transcripts in all three subdivisions as determined by RT-PCR. Control experiments are not shown but confirmed that expression represented messenger RNA and not genomic DNA contamination. These findings validated combining the SAGE and microarray results to generate a profile of potassium channel expression.

3.4 Quantitative Assessment of Expression by Real-time RT-PCR

Quantitative evaluation of expression differences among the three subdivisions could not be achieved by SAGE due to the low tag frequency for most transcripts. The microarray provided quantitative data for some channels but many had low hybridization intensities and thus comparisons were not reliable. Therefore, real-time quantitative RT-PCR was performed on 27 channels to investigate their expression patterns in the cochlear nucleus. Channels were chosen if they showed notable levels of expression in the SAGE or microarray experiments or if they represented a channel of interest due to previous reports of expression in auditory nuclei (e.g., Kcnc1/Kv3.1 or Kcnk15/TASK-5). The primer-probe set for Kcnj11/Kir6.2 failed to amplify reliably and thus no data were obtained for this gene.

The relative fold difference in potassium channel expression for 27 genes is depicted in Figures 4 and 5. The fold differences presented are relative to the subdivision with lowest expression of that channel transcript. These figures do not represent absolute levels of the transcript and thus comparison of levels between genes should not be performed. Statistical analysis of fold differences was performed and significant differences are reported at $p < 0.05$ for a one-tailed test. Eight of the 27 transcripts demonstrated statistically significant differences in expression among the three subdivisions ($p < 0.05$) although several other transcripts trended toward significance ($p < 0.10$). Among the latter was Kcna1/Kv1.1 with higher levels in the AVCN than DCN and Kcna3/Kv1.3 with higher levels in the DCN than AVCN. Preferentially expression (e.g., > 2 -fold) was noted in the AVCN for Kcnc1, Kcnc3, Kcnip1, Kcnj5, Kcns3 and Kcnk1 as compared to at least one of the other two subdivisions. The PVCN did not manifest any unique preferential expression when compared to AVCN and DCN and, in fact, the PVCN had the lowest level of transcript in 14 of the 27 potassium channels studied. Combining the AVCN and PVCN data to compare the ventral cochlear nucleus to the DCN showed higher levels of Kcnk12, Kcnj13 and Kcns3 in the ventral region. In contrast, the DCN had uniquely higher levels of expression of Kcnd3, Kcnv1, Kcnc2 and Kcns1. Several other transcripts including Kcnj6, Kcnk15, Kcna3, Kcnb2 and Kcnq3 had preferentially higher levels in the DCN but did not reach statistical significance.

3.5 Evaluation of Expression of Non-Identified Potassium Channels

Potassium channel subunits Kcna3, Kcna4, Kcna6, Kcnk9 and Kcnq4 have been reported in the cochlear nucleus but were not found by either SAGE or microarray in the present work. The expression of these channels was investigated using RT-PCR in the three subdivisions of the cochlear nucleus as well as in a whole brain cDNA library serving as a positive control

(Figure 6). This experiment was performed semi-quantitatively with equal amounts of starting RNA used for each subdivision. Transcript was amplified for Kcna6, Kcnk9 and Kcnq4 in all subdivisions of the cochlear nucleus. The semi-quantitative nature of this experiment suggested low levels of Kcnq4 in the DCN as compared to AVCN and PVCN for this channel. Kcna4 had minimal amplification in the PVCN and was not amplified from other subdivisions. Kcna3 was not amplified from any cochlear nucleus subdivision; however, Kcna3 was detected by real-time RT-PCR with preferential expression noted in the DCN (Figure 4).

4. DISCUSSION

The results of this study provide a broad profile of potassium gene expression in the cochlear nucleus and its anatomical subdivisions. These data can provide a useful template for interpreting neurophysiological experiments and for modeling the responses of auditory neurons to acoustic stimuli.

4.1 Potassium Channel Expression Patterns in the Cochlear Nucleus

Several potassium channels were found to be expressed in the cochlear nucleus that have not been previously reported or well studied in this region (Figure 1). The largest group of these consisted of the Kcnj (i.e., Kir) channels for which we found SAGE or microarray evidence of expression for 9 different subunits: Kcnj4, 5, 6, 9, 10, 11, 13, 14 and 16. Among these, Kcnj10 and Kcnj16/Kir5.1 expression has been reported in the mammalian cochlear nucleus using *in situ* hybridization (Wu et al., 2004). The same study failed to find expression above baseline for Kcnj1/Kir1.1 and Kcnj4/Kir2.3, the latter of which we did find in this study. We also identified members of the ether-à-go-go (i.e., eag) class of potassium channels including all known subunits of the elk group (Kcnh3, Kcnh4, Kcnh8) and 2 members of the erg group (Kcnh2, Kcnh6). Kcnh4 was several-fold lower in AVCN than DCN but this did not reach statistical significance. Expression of the elk class has been previously reported in the pons by real-time RT-PCR but there are no direct observations of expression in the cochlear nuclei (Zou et al., 2003).

Expression of the auditory pathway specific channel Kcnk15/TASK-5 was represented by 5 SAGE tags in the AVCN placing it among only 7 channels indicated by multiple copies of a tag. The real-time RT-PCR results, although not statistically significant, showed higher expression in the DCN and this is consistent with observations using *in situ* hybridization (Karschin et al., 2001). Other two pore potassium channels such as Kcnk1/TWIK-1, Kcnk3/TASK-1, Kcnk9/TASK-3 and Kcnk12/THIK-2 have been reported in the cochlear nucleus (Talley et al., 2001, Karschin et al., 2001, Rajan et al., 2001, Holt et al., 2006). We confirm those reports identifying Kcnk1 and Kcnk3 by microarray and Kcnk9 by RT-PCR. Kcnk12 was preferentially expressed in the ventral divisions by real time RT-PCR consistent with other studies (Rajan et al., 2001).

Four channels, Kcnk2, Kcnk10, Kcnk4 and Kcnk13 (i.e., TREK-1, TREK-2, TRAAK and THIK-1, respectively), have been previously reported as *not* having appreciable expression by *in situ* hybridization in the cochlear nucleus (Rajan et al., 2001, Talley et al., 2001). We, however, report the presence of Kcnk4 and Kcnk2, the former by SAGE and the latter by microarray (see Table 2). This may reflect differences in thresholds for detection of transcript between high throughput gene expression methods and *in situ* hybridization. That is, staining at or near background in an *in situ* hybridization experiment may not be sufficient to confirm expression whereas a single SAGE tag would suffice. Kcnk10 or Kcnk13, consistent with prior reports of low expression, were not found by SAGE or microarray in this study. Many of these channels (i.e., Kcnk2, Kcnk4, Kcnk10 and Kcnk13) have since been identified in cochlear nucleus homogenates by real-time RT-PCR (Holt et al., 2006).

4.2 Limitations in Identifying Expressed Channels and Neuron Specific Channels

There are likely other potassium channel genes expressed in the cochlear nucleus that were not identified by SAGE or microarray. For example, many potassium channel subunits were not represented on the microarray and thus were not tested by that modality. This is one limitation of using a closed-set system such as gene chips for gene detection. SAGE, which is open-set and can potentially identify any transcript may fail to produce tags for all expressed transcripts. Specifically, a transcript in low copy number may fall below the threshold of identification in moderately sized SAGE libraries. One example of the limitations of the techniques used in this study is our failure to identify transcript for Kcnq4/Kv7.4. Kcnq4 has been of particular interest as it is expressed in cochlea hair cells and maps to the DFNA2 locus of hereditary deafness (Kubisch et al., 1999). Kcnq4 expression has been shown in the AVCN and PVCN but not in the DCN (Kharkovets et al., 2000). Our RT-PCR experiment noted expression in all three subdivisions despite not finding this channel by SAGE or microarray. Despite these limitation the use of SAGE and microarray did identify 46 potassium channels, some of which have not been previously reported.

As detailed below, we noted significant differential expression of several channels and were able to postulate their localization to bushy cell, stellate cell or DCN interneuron populations. We did not, however, find evidence for localization to octopus cells. The principal issue was the relative low levels of expression seen in our experiments for octopus cell associated channels such as Kcna1/Kv1.1, Kcna2/Kv1.2 or the HCN subunits (Cao and Oertel, 2005, Bal and Oertel, 2001). This is likely secondary to our anatomic dissection of the nucleus using traditional anatomic conventions such that the octopus cell domain was divided among the PVCN and DCN pools of tissue and thus diluted. In contrast, RNA from spherical bushy cells or DCN specific neurons was concentrated in one subdivision and was more likely to be detected by the high through-put methods and to be differentially expressed. Current experiments using single cell capture techniques should allow identification of octopus cell specific gene expression patterns.

4.3 Differential Expression in the Cochlear Nucleus

Real-time RT-PCR demonstrated notable differential expression of transcript for potassium channel α -subunits Kcnj13/Kir7.1 and Kcnc2/Kv3.2 and subunit modifiers Kcns1/Kv9.1 and Kcns3/Kv9.3. Kcnj13 was found by real-time RT-PCR to be highly expressed in AVCN and PVCN as compared to DCN. Kcnj13 encodes for channel subunit Kir7.1 which is highly expressed in kidney and retinal pigmented epithelium (Shimura et al., 2001, Yang et al., 2003, Suzuki et al., 2003). Kir 7.1 is an inward-rectifying channel with low conductance and may allow precise regulation of the cellular resting membrane potential (Krapivinsky et al., 1998). Studies in the brain have noted robust expression in the choroid plexus but not in neuronal populations (Hasselblatt et al., 2006, Nakamura et al., 1999). The origin of this transcript in the AVCN and PVCN, given these prior reports, is unclear but does not likely represent choroid plexus contamination as this structure is easily dissected away from the cochlear nucleus. Our findings may represent expression by glial or endothelial cells but there is little evidence for inherent differences between supporting and vascular cells in the cochlear nucleus. Ongoing investigations using *in situ* hybridization and immunohistochemistry may confirm our hypothesis that Kcnj13/Kir7.1 differential expression represents differences at the neuronal level and is associated with specific cell types.

The DCN was found by real time RT-PCR to have significantly higher expression of Kcnc2/Kv3.2. Kv3.2 is a Shaw-related potassium channel subunit that has demonstrated differential expression between brain regions and among various populations of neurons (McDonald and Mascagni, 2006, Weiser et al., 1994). This subunit appears to be associated with interneurons and preferential expression to the DCN over the VCN has been seen by *in situ* hybridization

(Lau et al., 2000, Weiser et al., 1994). The Kv3.2 containing channel allows high frequency firing rates due to its rapid activation and deactivation (Hernandez-Pineda et al., 1999, Lien et al., 2002). Absence of the Kv3.2 subunit prevents interneurons from exhibiting normal rapid firing patterns and causes dis-inhibition of target neurons such as seen in epilepsy (Lau et al., 2000). In analogous fashion, a DCN interneuron, the cartwheel cell, may modulate fusiform cell activity and be the source of high Kv3.2 transcript. A reduction in Kv3.2 subunit expression within cartwheel cells may therefore lead to abnormal fusiform cell activity as has been associated with tinnitus (Brozoski et al., 2002). Interestingly, Kv3.2 activity is suppressed by PKA and cAMP second messenger pathways (Lien et al., 2002, Atzori et al., 2000) and is thus linked to cellular components of neural plasticity thought to produce tinnitus. Down-regulation of Kv3.2 transcription or activity in the DCN after tinnitus inducing insults has not been investigated.

Two α -subunit modifier genes, Kcns1/Kv9.1 and Kcns3/Kv9.3, were significantly differentially expressed between the ventral and dorsal subdivisions. Both are modulatory alpha-subunits that stabilize Kv2 subunits and promote closed-state inactivation which attenuates membrane excitability (Kerschensteiner et al., 2003, Shepard and Rae, 1999). The Kv2.1-Kv9.3 heteromer may prevent spontaneous or premature cellular discharge as seen in cardiac arrhythmias (Kerschensteiner and Stocker, 1999). We found over 20-fold higher levels of Kcns3/Kv9.3 in the AVCN suggesting an association with spherical bushy cells. This may stabilize Kv currents enabling the cell to remain in a highly excitable and responsive state without undue spontaneous discharges. Analysis of gene expression for Kcns3/Kv9.3 and its obligate heteromer Kcnc1/Kv2.1 in isolated bushy cell populations may confirm localization to these neurons.

Kcns1/Kv9.1 has been less well studied than Kv9.3 and is most strongly expressed in the brain although has shown low levels of expression in other tissues (Stocker and Kerschensteiner, 1998, Shepard and Rae, 1999, Salinas et al., 1997). In contrast to Kv9.3, we found high levels of Kcns1/Kv9.1 in the DCN relative to both ventral subdivisions. Given the similar physiological properties, such differential expression may not indicate functional differences but may reflect differences in phylogenetic and embryologic origin between the DCN and VCN. Alternatively, its expression in the DCN may afford some flexibility to the heavily synaptically regulated neurons of the DCN as Kv9.1 has been modeled to have different physiologic effects based upon underlying neuronal currents (Richardson and Kaczmarek, 2000).

4.4 Channel Expression and Electrophysiological Models

The separation of the cochlear nucleus into anatomic subdivisions most clearly isolates VCN stellate and bushy cells from neurons of the DCN. Rutherford and Manis have described at least three types of currents, I_A , I_{LT} , I_{HT} , in these cells (Rothman and Manis, 2003c, Rothman and Manis, 2003b, Rothman and Manis, 2003a). They noted that rapidly inactivating I_A currents have properties consistent with Kcna4, Kcnc3, Kcnc4, Kcnd1, Kcnd2 or Kcnd3 (Rothman and Manis, 2003b). We found only low levels, and a lack of differential expression, of Kcnd2 and did not detect Kcnc4 by SAGE or microarray. Kcnd3 had significantly higher levels in the DCN rather than in bushy and stellate cell regions. Of the remaining channels potentially underlying ventral cochlear nucleus I_A kinetics we identified significantly higher levels of Kcnc3 (Kv3.3) in the AVCN than in the DCN. Rothman and Manis have indicated that such I_A currents appear to be associated with stellate cells only (Rothman and Manis, 2003c).

The non-inactivating high threshold I_{HT} current was found by Rothman and Manis in all VCN neurons but was considered most highly associated with bushy cells (Rothman and Manis, 2003c, Rothman and Manis, 2003a). The channel likely contributing to this current was suggested as Kcnc1 (Kv3.1) which has been shown to be highly expressed in bushy cells and

in neurons with similar firing rates (Li et al., 2001, Perney and Kaczmarek, 1997). We found significantly elevated levels of Kcnc1/Kv3.1 in the AVCN which contains the spherical bushy cells.

The slowly-inactivating low-threshold I_{LT} currents, probably associated with globular bushy cells, are most consistent with channels Kcna1/Kv1.1 or Kcna2/Kv1.2 (Rothman and Manis, 2003c, Rothman and Manis, 2003b). We found higher levels of Kcna2 in the AVCN than both other subdivisions and Kcna1 was slightly higher in both AVCN and PVCN than in DCN. These results may reflect incorporation of globular bushy cells near the nerve root in our dissections. The Rothman and Manis model indicated that small changes in I_{LT} activation levels could dramatically affect temporal acuity and suggested that modifiers may be present to better tune the neuron as a coincidence detector. Indeed, modifiers of α -subunit activity were quite prevalent among the potassium channel subunits identified by SAGE and microarray. These included several β -subunits (e.g., Kcnab1, 2 and 3), interacting proteins such as Kcnp1 and Kcnp2 and the previously mentioned modifiers Kv9.1 and Kv9.3. Localization of the potassium channels and modifiers identified in this study to individual cell populations in the cochlear nucleus is an important focus of our future studies to better characterize the expressed genes sub-serving the unique electrophysiological properties of auditory neurons.

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Figure 1.

Graphic depiction of known potassium channels organized by number of transmembrane spanning regions and subclass of channel. Classes are arranged in phylogenetic trees based upon sequence homology (<http://www.receptors.org/KCN/index.html>). Boxed channel names represent 49 potassium channel subunit genes found in this study to be transcribed in the cochlear nucleus. Channel names with an asterisk represent those not found by SAGE or microarray but confirmed as present in at least one subdivision by RT- or real-time PCR. Unmarked channels were not identified in the cochlear nucleus by either of two high-throughput methods. An additional two interacting proteins, Kcnip1 and Kcnip2, were identified but do not fall into the depicted subunit classes.

Figure 2.

Relative levels of the 10 most abundant potassium channels in each cochlear nucleus subdivision as determined by microarray hybridization intensity. Normalized hybridization intensities ranged from 112.86 for Kv3.1 to 4.07 for Kv12.1 (median intensity across entire array equals 1.0). The majority of channels were present in all regions and Kcnc1/Kv3.1 transcript was the most prevalent in each. Notable differences include Kcns3/Kv9.3 which was the fourth most highly expressed transcript in the ventral subdivisions but was not among the top 10 transcripts in the DCN. Kv9.3 is a modifier subunit for Kcnb1/Kv2.1 which attenuates channel activity.

Figure 3.

Gel electrophoresis of RT-PCR experiment to confirm the presence of a subset of potassium channels identified by SAGE and microarray. This gel is not quantitative and all channels were run between 35 and 40 cycles. These data demonstrate the specificity of the SAGE and microarray experiments; that is, channel transcripts found by the high-throughput methods are valid and present in the tissue. *Kcnb1* (lane 1) was not found by SAGE, nor was it probed on the microarray; however the presence of its modifier *Kcns3/Kv9.3* suggested that it should be present. This experiment confirmed that it is indeed expressed in all subdivisions of the cochlear nucleus.

Figure 4.

Graph of fold difference in potassium channel expression among the three cochlear nucleus subdivisions as determined by real-time RT-PCR. Significant differences in expression were seen with Kcnc1, Kcnc3, Kcnd3 and Kcnj5 ($p < .05$). Three of these were most highly expressed in the AVCN and one (e.g., Kcnd3) was highest in the DCN. Greater than two-fold differential expression was also noted for 13 additional channels although these did not reach statistical significance. Upper limit of fold-difference error bars are depicted and arrow tops indicate ranges beyond that of the graph. Those transcripts with large errors are likely expressed at low absolute copy number leading to the observed variability.

Figure 5.

Graph of fold difference in potassium channel expression among the three cochlear nucleus subdivisions as determined by real-time RT-PCR for 4 genes with high levels of differential expression. Kcnc2/Kv3.2 was approximately 15-fold higher in the DCN than in the AVCN or PVCN. Kcnj13/Kir7.1, in contrast, showed significantly higher levels in the ventral divisions than the DCN. The modifier subunits Kcns1/Kv9.1 and Kcns3/Kv9.3 had inverse patterns of expression with the former being relatively high in the DCN and the latter showing more robust expression in the AVCN and PVCN. All differences were statistically significant at $p < 0.05$. Upper limit of fold-difference error bars are depicted and arrow tops indicate ranges beyond that of the graph.

Figure 6.

Gel electrophoresis of RT-PCR experiment to investigate the expression of 5 potassium channels not identified in this study but previously reported as being expressed in the cochlear nucleus. Equal amounts of starting RNA were used for all subdivisions and a brain cDNA library was used as a positive control. Kcna6, Kcnk9 and Kcnq4 were able to be amplified from all three subdivisions after 40 cycles of RT-PCR. Kcna3 was not present in any subdivision by RT-PCR but was identified by real-time RT-PCR (Figure 4). Kcna4 showed faint banding in the PVCN but no expression in the AVCN or DCN. These data show that some channel transcripts in low copy number may not appear in the SAGE library but are present in the cochlear nucleus. (Abbreviations: A: AVCN; D: DCN; P: PVCN; B: brain cDNA library (i.e., positive control))

Table 1

List of 46 potassium channels and interacting proteins identified in the cochlear nucleus by SAGE or microarray. SAGE data indicate the number of tags representing each channel found in each subdivision. Channels represented by more than one tag are denoted by an asterisk and all tags are indicated. Several channels were represented by more than one probe on the microarray and data presented indicate the mean of hybridization intensity for those probes (denoted by °). All hybridization data is normalized with the median hybridization for the entire chip equal to 1.0.

Gene	Channel	SAGE				Microarray			
		AVCN	PVCN	DCN	Tag	AVCN	PVCN	DCN	
Hcn1	Hcn1	-	-	-	-	2.20	1.74	1.86	
Hcn2*	Hcn2	8	6	3	Sum of Tags	0.15	0.16	0.14	
Hcn2	Hcn2	6	6	3	AATGTACTGA	0.15	0.16	0.14	
Hcn2	Hcn2	1	0	0	ATGCTGCTGC	0.15	0.16	0.14	
Hcn2	Hcn2	1	0	0	CGGGGACAG	0.15	0.16	0.14	
Hcn3	Hcn3	0	0	1	GTGGTGAGAA	0.50	0.41	0.46	
Hcn4	Hcn4	-	-	-	-	1.94	1.86	1.57	
Kcna1°	Kv1.1	-	-	-	-	13.21	17.58	11.00	
Kcna2°	Kv1.2	-	-	-	-	35.30	34.42	27.95	
Kenab1°	KvBeta1	-	-	-	-	1.99	1.39	1.17	
Kenab2	KvBeta2	-	-	-	-	1.14	1.11	1.00	
Kenab3	KvBeta3	-	-	-	-	1.48	1.26	3.26	
Kenb2°	Kv2.2	-	-	-	-	2.71	2.43	2.36	
Kencl*	Kv3.1	3	3	4	Sum of Tags	112.86	83.90	66.86	
Kencl	Kv3.1	2	3	2	AAATAAATT	112.86	83.90	66.86	
Kencl	Kv3.1	0	0	1	AAATTAACA	112.86	83.90	66.86	
Kencl	Kv3.1	1	0	0	GATTTCCAA	112.86	83.90	66.86	
Kencl	Kv3.1	0	0	1	GCTAAGCAGA	112.86	83.90	66.86	
Kencl	Kv3.1	1	1	3	TACTAACTG	0.22	0.21	0.49	
Kencl*	Kv3.2	10	14	5	Sum of Tags	-	-	-	
Kencl	Kv3.3	9	13	3	CCCTCCCCA	-	-	-	
Kencl	Kv3.3	1	1	2	GACTTGCCC	-	-	-	
Kencl	Kv3.3	0	0	1	TGCTCTGATC	-	-	-	
Kencl	Kv4.1	0	0	0	-	1.19	1.11	1.31	
Kencl	Kv4.2	0	0	0	-	2.12	1.91	3.06	
Kencl	Kv4.3	0	0	0	-	1.57	1.64	1.68	
Kencl	Kv11.1	0	0	0	-	0.66	0.55	0.58	
Kencl	Kv12.2	0	0	1	TTTTTTATAT	6.76	5.89	5.41	
Kencl	Kv12.3	0	1	0	GTCTCGACCT	2.80	2.84	2.67	
Kencl	Kv11.2	1	0	0	CGCACCCCA	4.07	4.80	4.56	
Kencl	Kv12.1	-	-	-	-	5.10	2.60	5.25	
Kencl	Kenip1	1	0	0	-	0.11	0.05	0.08	
Kencl	Kenip1	1	0	0	GTGACTGCT	-	-	-	
Kencl	Kenip1	1	0	0	GGGTCTGGG	-	-	-	
Kencl	Kir2.3	0	1	0	TTGTGATGAC	3.48	3.51	3.23	
Kencl	Kir3.4	0	1	0	Sum of Tags	0.51	0.48	0.45	
Kencl	Kir3.2b	1	1	0	CTTGATGCCA	0.51	0.48	0.45	
Kencl	Kir3.2b	0	1	0	GCCTTGTC	0.51	0.48	0.45	
Kencl	Kir3.3	1	0	0	GGAGGGGTG	0.49	0.40	0.54	
Kencl	Kir4.1	0	1	0	TGATAGATGA	1.97	2.25	1.83	
Kencl	Kir6.2	1	1	0	TGATGGGAAA	9.32	8.36	7.13	
Kencl	Kir7.1	1	0	0	CTAGAGGCT	1.06	1.95	1.11	
Kencl	Kir2.4	1	1	1	TTGGCTAGAG	2.83	3.13	2.85	
Kencl	Kir5.1	1	0	0	CTTTGGCACG	-	-	-	
Kencl	K2p1.1	6	15	3	GCAGATTGCA	42.75	45.97	27.54	
Kencl	K2p2.1	-	-	-	-	1.32	2.43	2.52	

Gene	Channel	SAGE				Microarray			
		AVCN	PVCN	DCN	Tag	AVCN	PVCN	DCN	
Kenk3	K2p3.1	-	-	-	-	2.35	2.35	1.31	
Kenk4	K2p4.1	0	0	1	CTTAGCTTAT	-	-	-	
Kenk12	K2p12.1	0	0	1	AACAACCGGC	3.18	2.61	1.70	
Kenk15 *	K2p15.1	5	1	0	Sum of Tags	25.89	9.36	11.69	
Kenk15	K2p15.1	3	0	0	TGACCCGCTC	25.89	9.36	11.69	
Kenk15	K2p15.1	2	1	0	TGGGAAATTA	25.89	9.36	11.69	
Kenma1	KCa1.1	0	1	0	CTGAATCACT	0.57	0.74	1.19	
Kenmb2	BK-Beta2	0	1	0	TTTGAATGA	-	-	-	
Kenm2	KCa2.2	-	-	-	-	6.41	5.20	6.00	
Kenm3	KCa2.3	0	0	1	TATGACTTGA	1.01	1.02	0.79	
Kenq3	Kv7.3	0	1	0	GTTAAATTTT	0.48	0.38	0.37	
Kens1	Kv9.1	0	1	0	GTCTCTGCTT	0.39	0.15	0.60	
Kens3	Kv9.3	0	1	0	TTCCAGACAC	31.64	19.33	3.20	
Kenv1 *	Kv8.1	2	0	0	Sum of Tags	0.88	0.94	1.06	
Kenv1	Kv8.1	1	0	0	AATATGGAAA	0.88	0.94	1.06	
Kenv1	Kv8.1	1	0	0	TCTTCAACGT	0.88	0.94	1.06	

* gene is represented by more than one SAGE tag
° gene is represented by more than one spot on the chip

Table 2

Two-pore potassium channels investigated for expression in this study and prior reports of their presence or absence in the cochlear nucleus. We found evidence by SAGE, microarray or RT-PCR for expression of 7 of these channels. Two channels not found have also been previously reported as not prominent in the cochlear nucleus. All channels in the table have been amplified by real-time RT-PCR (Holt et al., 2006) and thus the latter may be expressed in very low transcript numbers.

Channel Gene	Common Name	Identified?	Prior Reports
Kcnk1	TWIK-1	Yes	++ in cochlear nucleus ¹
Kcnk2	TREK-1	Yes	absent in cochlear nucleus ¹
Kcnk3	TASK-1	Yes	+ / ++ in cochlear nucleus ^{1,2}
Kcnk4	TRAAK	Yes	absent in cochlear nucleus ¹
Kcnk9	TASK-3	Yes	+ / ++ in cochlear nucleus ^{1,2}
Kcnk10	TREK-2	No	absent in cochlear nucleus ¹
Kcnk12	THIK-2	Yes	+ in DCN; +++ VCN ³
Kcnk13	THIK-1	No	absent DCN, PVCN; only in AVCN ³
Kcnk15	TASK-5	Yes	++ / +++ in cochlear nucleus ²

all were found by Holt et al., 2006

¹Talley et al., 2001

²Karschin et al., 2001

³Rajan et al., 2001