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Deafness associated changes in expression of two-pore domain potassium channels in the rat cochlear nucleus

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Abstract

Two-pore domain potassium channels (K_{2PD}^+) play an important role in setting resting membrane potential by regulating background leakage of potassium ions, which in turn controls neuronal excitability. To determine whether these channels contribute to activity-dependent plasticity following deafness, we used quantitative real-time PCR to examine the expression of 10 K_{2PD}^+ subunits in the rat cochlear nucleus at 3 days, 3 weeks and 3 months after bilateral cochlear ablation. There was a large sustained decrease in the expression of TASK-5, a subunit that is predominantly expressed in auditory brain stem neurons, and in the TASK-1 subunit which is highly expressed in several types of cochlear nucleus neurons. TWIK-1 and THIK-2 also showed significant decreases in expression that were maintained across all time points. TWIK-2, TREK-1 and TREK-2 showed no significant change in expression at 3 days but showed large decreases at 3 weeks and 3 months following deafness. TRAAK and TASK-3 subunits showed significant decreases at 3 days and 3 weeks following deafness, but these differences were no longer significant at 3 months. Dramatic changes in expression of K_{2PD}^+ subunits suggest these channels may play a role in deafness-associated changes in the excitability of cochlear nucleus neurons.

Keywords

Auditory; Deafness; Potassium channels; Plasticity; Cochlear nucleus

1. Introduction

Two-pore domain potassium channels (K_{2PD}^+) play an important role in setting resting membrane potential. These channels are voltage-insensitive and potassium selective, thereby contributing “leak” currents that drive the resting potential toward the equilibrium potential

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for potassium. Activation of K_{2PD}^{+} through biochemical or mechanical mechanisms produces a hyperpolarized membrane potential and suppresses neuronal excitability (see Plant et al., 2005 for review).

To date, the two-pore domain potassium channel family comprises 18 members and 6 different classes. The distribution of several K_{2PD}^{+} subunits have been mapped throughout the brain using in situ hybridization (Karschin et al., 2001; Rajan et al., 2001; Talley et al., 2001). The TASK-5 subunit is selectively expressed at high levels in auditory brain stem neurons and Purkinje cells of the cerebellum, with no detection in other labeled brain regions except for a few neurons in the spinal trigeminal nucleus, the mammillary nucleus and the olfactory bulb. In contrast, TASK-1 and TASK-3 are widely expressed throughout the brain but with moderate expression in the auditory brain stem (Karschin et al., 2001). Recently, high levels of TASK-1 expression have been localized specifically to spherical bushy cells of the rat cochlear nucleus (Pal et al., 2005).

The expression of two-pore domain K^{+} channels has been shown to be influenced by activity as well as biochemical and physical cues (Enyeart et al., 2003; Kang et al., 2004; Li et al., 2005; Liu and Saint, 2004; Xu et al., 2004; Yeom et al., 2005). Therefore, these channels may play a role in synaptic plasticity, where activity-dependent signaling can alter the inherent excitability of target neurons. Changes in auditory nerve activity following deafness have been shown to induce changes in excitability and response properties in the cochlear nucleus (Francis and Manis, 2000; Kaltenbach and Afman, 2000; Kanold and Manis, 2005; Wang and Manis, 2005) and inferior colliculus (Bledsoe et al., 1995, 1997; Mossop et al., 2000; Salvi et al., 2000; Syka and Rybalko, 2000; Vale and Sanes, 2002; Vale et al., 2004) (for reviews Moller, 2005; Syka, 2002). While the acoustic environment has been shown to influence auditory brain stem responses through modulation of voltage-gated potassium channels, this is achieved largely through phosphorylation rather than chronic alterations in gene expression (Chambard and Ashmore, 2005; Kaczmarek et al., 2005; Macica et al., 2003; Song et al., 2005). Studies in the avian cochlear nucleus (nucleus magnocellularis) have shown large deafness-related changes in Kv1.1 and Kv3.1 expression following cochlear ablation (Lu et al., 2004; von Hehn et al., 2004). However, these changes are transient (Lu et al., 2004). Similarly, the expression of Kv1.1 and Kv1.2 in the rat cochlear nucleus is unchanged 10 days after cochlear ablation (Camino et al., 2005).

Given the selective and high expression of TASK-5 in auditory brain stem neurons, we sought to determine if the expression of TASK-5 is regulated by changes in auditory activity. Therefore, the present study used quantitative real-time PCR to examine changes in expression of TASK-5 and other K_{2PD}^{+} subunits in the rat cochlear nucleus at 3 days, 3 weeks and 3 months following bilateral cochlear ablation. We examined 10 members from 4 of the K_{2PD}^{+} classes (Table 1; acid sensitive: TASK-1, TASK-3, TASK-5; weak inward rectifiers: TWIK-1, TWIK-2; unsaturated fatty acid and stretch-activated: TREK-1, TREK-2, TRAAK; and halothane sensitive: THIK-1, THIK-2).

2. Methods

All studies were approved by the University of Michigan Committee on the Use and Care of Animals. Male Sprague-Dawley rats, 200–300 g, were obtained from Charles River Laboratories. Hearing was assessed by auditory brain stem responses (ABR) recorded from all animals prior to the beginning of the study and normal hearing thresholds were required for inclusion in the study. Animals were then randomly assigned to four groups, each containing 12 rats: Normal hearing age matched controls, rats bilaterally deafened by cochlear ablation and assessed either at 3 days, 3 weeks, or 3 months following deafening. For RNA isolation, each group of 12 rats was randomly divided into four sub-groups each comprising three rats resulting in four different pools of cochlear nucleus mRNA for each condition ($n = 48$).

2.1. Cochlear ablation

Rats in the cochlear ablation groups were deafened by mechanically ablating the cochlea bilaterally. Each rat was anesthetized with an i.m. injection of xylazine (8 mg/kg) and ketamine (75 mg/kg). Local injections of 1% lidocaine-HCl solution were made at the site of each surgical incision. Surgical procedures were performed under aseptic conditions. A skin incision was made through the postauricular region and the bulla exposed without resection of any major muscles. Cochlear ablation, by mechanical dissection, was performed through the lateral wall of the bulla under a dissection microscope. The skin incision then was closed with sutures. Following surgery, animals were injected with sterile 0.9% sodium chloride-HCl solution and allowed to recover under a heating lamp.

2.2. RNA isolation

Rats were heavily anesthetized with 0.8 cc of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI), decapitated and the brain removed and placed in ice cold DEPC-treated PBS. The entire cochlear nucleus from both hemispheres was rapidly removed using fine tipped-forceps and placed in microcentrifuge tubes containing RNAlater (Ambion Inc., Austin, TX). Tissue from three animals (six CN) was combined into a pool and homogenized in Trizol (Invitrogen) for 10 s. RNA was then isolated in Trizol. Chloroform was added to the homogenate, which was briefly vortexed and then centrifuged at 14,000g. The aqueous phase was transferred to a Phase Lock Gel (Heavy) tube (Eppendorf) and extracted with acid phenol and chloroform (1:1). The aqueous phase was then decanted and RNA precipitated with isopropanol. The pellet was washed in 75% ethanol, air dried and re-suspended in DEPC-treated water. The RNA quality was assessed using an RNA 6000 Nano LabChip (Agilent, Palo Alto, CA). The Bioanalyzer separates nucleic acids by capillary electrophoresis. This provides information on the integrity of the 18S and 28S ribosomal RNA bands and the integrity of these bands was necessary for inclusion of the pool in the study. RNA concentrations were measured based on the reading of the absorbance at 260 nm (A_{260}) with a spectrophotometer.

2.3. Quantitative RT-PCR (Q RT-PCR)

First strand cDNA was synthesized from total RNA (2 μ g) using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) that utilizes 250 units of MultiScribe

reverse transcriptase and random primers in the presence of RNasin (20 units; Invitrogen, Carlsbad, CA) in a total reaction volume of 100 μ l. The reactions were incubated at 25 °C for 10 min followed by 37 °C for 2 h. The PCR primer sequence for TASK 5 (Forward Primer Sequence-GGCTCTTTCTACTTCGCCATCAC; Reverse Primer Sequence-GGTGCCTGGAGCAGCAT; Probe Sequence-ATCACCACCATCGGGTATG) was designed such that the primer crossed the boundary between exons 1 and 2 near the 5' end of the gene sequence (Assays-by-Design, Applied Biosystems). The primer probe pairs for the other nine genes of interest were acquired through Assays-on-Demand (TaqMan probes, Applied Biosystems).

For each gene assayed, quantitative real-time PCR (qRT-PCR) was performed on four cDNA samples in a Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). To minimize experimental variability among multiple pools of RNA samples, the expression levels of each gene was assayed across each experimental group simultaneously, i.e. in the same PCR plate. The expression levels of the genes of interest were normalized using a house-keeping gene encoding ribosomal protein *S16*. Levels of *S16* mRNA remained relatively constant among experimental groups. The threshold cycle (C_T), is defined as the PCR cycle number at which the fluorescence intensity crosses a manually determined threshold value, at a level where the fluorescent signal is appreciably above the background level but is still in the early exponential phase of amplification. Each sample was assayed in triplicate and the resulting C_T value was averaged and the standard deviation calculated. If the standard deviation of the triplicate values was more than 0.3 then the value for that sample was not used in the final analysis. Next, for each pool of RNA the difference in the averaged C_T values (C_{Tavg}) for a gene of interest and *S16* for a given experimental group was calculated and defined as C_T for each gene of interest. The C_T value for each of four pools for each experimental group was then averaged and the standard deviation (SD) calculated. For each gene, the average C_T value for the normal, non-deafened control was subtracted from the average C_T value of the deafened group resulting in the C_T . The error associated with C_T was equivalent to the standard deviation of the mean of the C_T for the experimental group. The data are reported as fold change, which was calculated as 2^{-C_T} . This assumes doubling of products every amplification cycle or 100% amplification efficiency (the Applied Biosystem user's guide states that the amplification efficiency for primers in their TaqMan assays is close to 1.0). The error is represented as $2^{-(C_T \pm SD)}$. This results in an asymmetric distribution of the error across the bar graph (Livak and Schmittgen, 2001). The significance of the C_T values was evaluated by performing a student *T*-test (StatView, SAS Institute, Cary, NC) with a *p* value of *p* < 0.05 considered significant.

3. Results

3.1. Acid sensitive channels

Expression in the rat cochlear nucleus was found for all three of the 2-pore domain acid sensitive potassium channel subunits assessed, TASK-1, TASK-3 and TASK-5. This is consistent with previous studies in the cochlear nucleus using in situ hybridization (Karschin et al., 2001; Pal et al., 2005; Talley et al., 2001).

In the current study, TASK-1 and TASK-5 subunits showed significant and sustained decreases in expression in the cochlear nucleus following deafening at all three times assessed (Fig. 1). TASK-5, the subunit most selective for auditory neurons, also showed the greatest overall decrease across the ten 2-pore domain potassium channel subunits assessed in the present study. The level of TASK-5 expression decreased by 84% (0.16 ± 0.17) at 3 days following deafness. This decrease was statistically significant ($p = 0.05$). Expression of TASK-5 at 3 weeks post-deafness was undetectable (0.00 ± 0.001 ; $p = 0.05$) with a 98% decrease in expression after 3 months of deafness when compared to undeafened controls (0.02 ± 0.003 ; $p = 0.05$). The TASK-1 subunit decreased by 60% (0.41 ± 0.45 ; $p = 0.05$) at 3 days following deafness with a further decrease to undetectable expression levels at 3 weeks (0.01 ± 0.001 ; $p = 0.05$) maintaining very low expression levels (0.01 ± 0.002 ; $p = 0.05$) after 3 months of deafness, compared to normal expression. The TASK-3 subunit showed significant decreases at 3 days and 3 weeks following deafening. TASK-3 expression was decreased by 35% at the 3 day (0.65 ± 0.70 ; $p = 0.05$) and 38% at the 3 week time point, (0.63 ± 0.65 ; $p = 0.05$). By 3 months after deafening, however, TASK-3 expression showed a decrease of 22% that was no longer statistically significant, indicating a trend towards recovery of TASK-3 expression.

3.2. Weak inward rectifiers

Expression in the rat cochlear nucleus was found for both of the 2-pore domain weak inward rectifier potassium channel subunits that were evaluated, TWIK-1 and TWIK-2 (Fig. 1). While TWIK-1 expression has been previously shown in the cochlear nucleus using in situ hybridization (Talley et al., 2001) the results from the current study are the first report of TWIK-2 expression in the CN.

TWIK-1 was significantly decreased at all three times following deafness. At three days following deafness there was a 44% decrease in TWIK-1 expression with decreases to 78% and 63% below normal at 3 weeks and 3 months after deafness, respectively ($p = 0.05$ for all groups). Alteration in TWIK-2 expression was delayed compared to TWIK-1. There was no significant decrease in expression of the TWIK-2 at 3 days following deafening (1.25 ± 1.35 ; $p > 0.05$), but by 3 weeks and 3 months following deafening there were significant reductions in TWIK-2 transcripts to 56% and 45% of undeafened controls, respectively ($p = 0.05$).

3.3. Unsaturated fatty acid and stretch-activated

Expression in the rat cochlear nucleus was found for three of the 2-pore domain unsaturated fatty acid and stretch-activated potassium channel subunits that were assessed, TREK-1, TREK-2 and TRAAK (Fig. 2). Previous studies using in situ hybridization reported labeling for TREK-1, TREK-2 and TRAAK in the cochlear nucleus similar to background levels (Talley et al., 2001).

TREK-1 and TREK-2 had similar patterns of deafness-associated changes with significant decreases in expression occurring at both later time points (3 week and 3 months), with no significant differential expression at the 3 day time point. TREK-1 expression showed a significant deafness-associated decrease of 96% at 3 weeks and an 85% decrease three

months after deafening. TREK-2 expression had a significant 88% decrease at 3 weeks after deafening with a 76% decrease at the 3 month time point ($p = 0.05$).

TRAAK showed changes occurring earlier than either TREK-1 or TREK-2 with a significant decrease of 30% at 3 days following deafness ($p = 0.05$). There was also a significant 41% decrease at 3 weeks of deafness ($p = 0.05$). Although there was an average decrease of 30% at the 3 month time point, this change in expression was not significant due to increased variability in this group.

3.4. Halothane sensitive channels

There was expression of both the THIK-1 and THIK-2 halothane sensitive 2-pore domain potassium channels in the rat cochlear nucleus. (Fig. 2). Both THIK-1 and THIK-2 have previously been shown in the rat cochlear nucleus using in situ hybridization (Rajan et al., 2001).

The THIK-2 subunit showed deafness-associated decreases in expression at all three times assayed following deafness with a 52% decrease at 3 days, a 58% decrease at 3 weeks and a 38% decrease at 3 months ($p = 0.05$ for all groups). The THIK-1 subunit, on the other hand, was the only one of the 2-pore domain potassium channel subunits assessed which did not show significant deafness-associated decreases at any of the times assessed, perhaps because of the limited distribution of this subunit in the CN (Rajan et al., 2001).

4. Discussion

With one exception, all of the K_{2PD}^+ subunits examined in this study exhibited deafness-associated changes in expression. In each of the nine affected transcripts, expression decreased. This uniformity leads one to speculate that in the absence of auditory input to the cochlear nucleus, a global regulatory mechanism designed to reduce leak conductance may exist. However, the general differences in the magnitude and timing of altered expression suggest multiple regulatory controls for different K_{2PD}^+ channels comprising various subunits. Of note is the fact that the data reflect averaged effects across multiple cells types (RNA assayed was from the entire cochlear nucleus), thereby potentially masking even more dramatic reductions in gene expression in certain cells or sub-regions of the CN.

Two predominant patterns of altered gene expression were identified. In the more common pattern, the reduction in expression became greater at 3 weeks of deafness compared to 3 days. TASK-1, TASK-5, TWIK-1, TREK-1, and TREK-2 had an initial decrease seen at 3 days following deafening that became greater at 3 weeks and was maintained at 3 months, while TWIK-2 showed no decrease at three days and then decreased in expression at 3 weeks and 3 months. The other general pattern revealed decreases in expression at the 3 day and 3 week times, remaining significantly decreased at 3 months for THIK2 or showing a trend towards long-term recovery for TASK-3 and TRAAK subunits. Both patterns were found across the four different classes of subunits, suggesting that the two types of deafness-associated changes are not likely correlated with specific sets of K_{2PD}^+ response properties (Table 1).

The K_{2PD}^{+} subunits are divided into six groups or classes based on different sensitivities to various modulators. To date, attempts at functional expression (Ashmole et al., 2001; Rajan et al., 2001) of TASK-5 and THIK-2 have failed so their biophysical properties are undefined. However, sequence homology and similarities in topology with other K_{2PD}^{+} family members strongly suggests conserved function. Physiological roles for these channels extend beyond mere regulation of resting potential to include metabolic signaling, cell volume regulation, pain and heat sensation, G protein signaling, and potassium homeostasis (Kim, 2005). These functions are mediated by sensitivities to endogenous and exogenous factors, including activation by pH, unsaturated fatty acids, and membrane stretch, and inhibition by anesthetics, neurotransmitter-receptor signaling, and alterations in cytosolic calcium. Thus far, gene expression for K_{2PD}^{+} from four of the six different classes have been identified in the cochlear nucleus suggesting that there are cochlear nucleus cells containing K_{2PD}^{+} that are sensitive to these associated modalities. However, specific neurons or glial cells in the cochlear nucleus may be more or less responsive to select modalities. There is limited information regarding leak conductances in the each of the cell types found within the cochlear nucleus. Where information does exist, we briefly illustrate below the functional significance of decreased expression following deafness.

4.1. Neuronal excitability

The decrease in expression of K_{2PD}^{+} in the cochlear nucleus following deafness may be a consequence of the decreased activity in cochlear neurons following bilateral cochlear ablation. The responses of cochlear nucleus neurons to losing their dominant excitatory input may be to lower their threshold for activation through a downregulation of K_{2PD}^{+} channels, which function to reduce excitability when they are active. If this is the case, the increase in neuronal excitability might be associated with the increased neuronal activity in the CN following complete or partial deafness found in central tinnitus (Brozoski et al., 2002; Imig and Durham, 2005; Kaltenbach et al., 2004; Zhang and Kaltenbach, 1998).

There is evidence from empirical observations and computational modeling that K_{2PD}^{+} channels are in a position to influence neural excitability in cochlear nucleus neurons (Fujino and Oertel, 2001; Rothman and Manis, 2003). In the ventral cochlear nucleus, muscarinic receptor activation excites stellate cells by blocking potassium leak currents (Fujino and Oertel, 2001). The authors concluded that cholinergic muscarinic inhibition could excite stellate cells in response to sound, enhancing sensitivity to spectral peaks in noise. Muscarinic inhibition of potassium leak conductance has been observed in other brain regions (Boyd et al., 2000; Millar et al., 2000; Womble and Moises, 1992). The mechanism for inhibition is poorly understood but may include signaling through phospholipase C (Czirjak et al., 2001) or altered cytosolic free Ca^{2+} (Boyd et al., 2000) and involves K_{2PD}^{+} channels (Czirjak et al., 2001).

Computational modeling also supports the importance of leak conductances in stellate (Type I) cells in the cochlear nucleus (Rothman and Manis, 2003). The leak conductance in Type II neurons appears to be small relative to other potassium conductances. Therefore, modulation

of K_{2PD}^{+} channels in these neurons would have little impact on resting membrane potential and excitability. In contrast, the resting potential of Type I neurons is regulated by leak conductance since other relevant potassium conductances are inactive at rest. Decreased K_{2PD}^{+} expression in these neurons would lead to a depolarized resting potential, an increase in input resistance, and an increase in membrane time constant. In the absence of changes to other ion channels, affected neurons would exhibit decreased threshold for activation as well as temporal changes in firing behavior. Therefore, identifying the specific neurons undergoing altered K_{2PD}^{+} expression following deafness is critically important. If stellate cells contribute to the observed changes, hyperexcitable Type I responses might be expected. Altered excitability in cochlear nucleus neurons has important implications for understanding coding after cochlear implantation and pathophysiological phenomena like central tinnitus.

4.2. Glial expression

Variations in K_{2PD}^{+} expression may also reflect changes in glial cells. Multiple members of the K_{2PD}^{+} family have been identified in astrocytes (Gnatenco et al., 2002; Rusznak et al., 2004), where they are likely involved in ionic homeostasis. Decreased K_{2PD}^{+} expression in cochlear nucleus glia may result in ionic imbalance and further pathology within the nucleus. Alternatively, changes in K_{2PD}^{+} expression may reflect an attempt by glial cells to restore homeostasis in response to reduced auditory signaling.

4.3. Cell volume regulation

There have been numerous reports of deafness-associated decreases in cell size in the cochlear nucleus (Kawano et al., 1997; Lesperance et al., 1995; Lustig et al., 1994; Niparko, 1999; Niparko and Finger, 1997; Sie and Rubel, 1992; Willott et al., 1994). One modality for the activation of TREK K_{2PD}^{+} is through mechanical gating via stretching the cell membrane (Kim et al., 2005). A decrease in cell size following deafness may lead to an inactivation of K_{2PD}^{+} channels and an activity-dependent loss in expression. Alternatively, a decrease in TREK expression may be causative, initiating volume regulation signaling that results in cell shrinkage.

The implications of altered K_{2PD}^{+} expression are broad, underscoring the need to identify the cell types contributing to the current results. Without Western blotting data we cannot assume that these dramatic changes in gene expression reflect changes in protein levels. However, if these decreases in gene expression translate into changes in protein production, then using both in situ hybridization and immunocytochemistry to determine the localization of the K_{2PD}^{+} subunits that we have identified as “activity dependent” is a critical next step in future studies and would allow for further differentiation between cell types in the cochlear nucleus leading to a better understanding of the role of these channels in auditory processing.

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Fig. 1.

Changes in the gene expression of acid sensitive and weak inward rectifier 2-pore domain potassium channel subunits following deafness. TASK-1, TASK-5, and TWIK-1 show sustained significant decreases at all times assessed following bilateral cochlear ablation (3 days, 3 weeks, and 3 months). While the average change in TASK-3 expression is decreased across all time points the change is no longer significant by 3 months of deafness. There is no significant change in the level of TWIK-2 expression 3 days following the onset of deafness, but significant decreases are observed after 3 weeks and 3 months of deafness. Asterisks indicated statistically significant differences when compared to normal expression levels. Error bars indicated standard deviation. $n = 48$ (3 animals per pool and a total of 4 pools per experimental group).

Fig. 2.

Deafness decreases the expression levels of unsaturated fatty acid and stretch-activated as well as halothane sensitive 2-pore domain potassium channel subunits following deafness. The THIK-2 and the TRAAK 2-pore domain potassium channel subunits showed an average decrease in expression that was sustained across all time assessed following deafness (3 days, 3 weeks, and 3 months). However, the change in the TRAAK subunit expression level was no longer significant by 3 months. The TREK-1 and TREK-2 subunits did not show significant decreases in expression at the 3 day time point, but were significantly decreased in expression at the 3 week and 3 month time points. Asterisks indicated statistically significant differences when compared to normal expression levels. Error bars indicated standard deviation. $n = 48$ (3 animals per pool and a total of 4 pools per experimental group).

Table 1

Classification of K_{2PD}^{+} subunits

Gene name	Common name	Classification	Reported function
Kenk1	TWIK1	Weak inward rectifier	Activated by desmoylation; upregulated by PKC activation; downregulated by intracellular acidosis
Kenk2	TREK1, rTREK1d	Unsaturated fatty acid and stretch-activated	Activated by increasing mechanical pressure applied to cell membrane
Kenk3	TASK1, rTASK	Acid sensitive channel	Inhibition by acidic external pH; voltage and [K+] dependant
Kenk4	TRAAK, KT4.1	Unsaturated fatty acid and stretch-activated	Activity elicited by increasing mechanical pressure applied to cell membrane
Kenk6	TWIK2	Weak inward rectifier	Inhibited by intracellular but not extracellular acidosis
Kenk9	TASK3	Acid sensitive channel	Inhibited reversibly by acidic extracellular pH
Kenk10	TREK2	Unsaturated fatty acid and stretch-activated	Activity elicited by fatty acid-stimulation and increased mechanical pressure applied to cell membrane
Kenk12	THIK2	Halothane sensitive channels	Tandem pore domain halothane inhibited $K^{(+)}$ channel
Kenk13	THIK1, prdx1	Halothane sensitive channels	Tandem pore domain halothane inhibited $K^{(+)}$ channel
Kenk15	TASK5	Acid sensitive channel	TWIK-related acid-sensitive potassium channel; Sensitive to changes in pH