NIH Public Access
Author Manuscript
Behav Brain Res. Author manuscript; available in PMC 2009 November 21.

Published in final edited form as:
Behav Brain Res. 2008 November 21; 193(2): 192–196. doi:10.1016/j.bbr.2008.05.010.

Knockout of the gene encoding the K_{2P} channel KCNK7 does not alter volatile anesthetic sensitivity

C. Spencer Yost*, **Irene Oh**, **Edmond I. Eger II**, and **James M. Sonner**
Department of Anesthesia and Perioperative Care, Medical Sciences Building, 513 Parnassus Avenue, University of California, San Francisco, CA 94143

Abstract

The molecular site of action for volatile anesthetics remains unknown despite many years of study. Members of the K_{2P} potassium channel family, whose currents are potentiated by volatile anesthetics have emerged as possible anesthetic targets. In fact, a mouse model in which the gene for TREK-1 (KCNK2) has been inactivated shows resistance to volatile anesthetics. In this study we tested whether inactivation of another member of this ion channel family, KCNK7, in a knockout mouse displayed altered sensitivity to the anesthetizing effect of volatile anesthetics.

KCNK7 knockout mice were produced by standard gene inactivation methods. Heterozygous breeding pairs produced animals that were homozygous, heterozygous or wildtype for the inactivated gene. Knockout animals were tested for movement in response to noxious stimulus (tail clamp) under varying concentrations of isoflurane, sevoflurane and desflurane to define the minimum alveolar concentration (MAC) preventing movement.

Mice homozygous for inactivated KCNK7 were viable and indistinguishable in weight, general development and behavior from heterozygotes or wildtype littermates. Knockout mice (KCNK7 −/−) displayed no difference in MAC for the three volatile anesthetics compared to heterozygous (+/−) or wildtype (+/+) littermates.

Because inactivation of KCNK7 does not alter MAC, KCNK7 may play only a minor role in normal CNS function or may have had its function compensated for by other inhibitory mechanisms. Additional studies with transgenic animals will help define the overall role of the K_{2P} channels in normal neurophysiology and in volatile anesthetic mechanisms.

Keywords

anesthesia; potassium channel; two-pore; tandem pore; volatile anesthetic

Background

Ion channels are integral membrane proteins that mediate rapid ion transfer across cellular membranes, providing important control of cellular excitability. Potassium (K) channels, which selectively pass K ions across membranes, are perhaps the largest class of ion channels

Corresponding author: C. Spencer Yost, Department of Anesthesia and Perioperative Care, 513 Parnassus Avenue, Room S-261, Box 0542, University of California, San Francisco, CA 94143, tel: (415) 476-5200, fax: (415) 476-8841, email: E-mail: yosts@anesthesia.ucsf.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with over seventy identified [27]. The most diverse family of K channels is the tandem pore (K_{2P}) family [7]. These K channels are also known as background, baseline or leak K channels because they are responsible for a resting K conductance in certain excitable cells [5,9,26]. Fifteen unique K_{2P} channel sequences exist in the human genome, organized into six subfamilies based on sequence and functional homology [2].

In recent years, members of this family have emerged as potential targets for the action of volatile and gaseous anesthetics (volatile anesthetics are in liquid form at room temperature while gaseous anesthetics are in gas form). *In vitro* studies have identified a unique effect of anesthetics on some family members. In heterologous expression systems, volatile anesthetics potentiate the currents passed by TREK-1 (KCNK2), TASK-1 (KCNK3), TASK-2 (KCNK5), TASK-3 (KCNK9), TREK-2 (KCNK10) and TRESK (KCNK18) [8,13,16,19]. A role for TREK-1 in mediating the effect of volatile anesthetics has been supported by the finding that TREK-1 knockout mice are relatively resistant to the effect of volatile anesthetics. Whether other K_{2P} channels are necessary to produce the state of anesthesia is not clear at present.

KCNK7 belongs to the inward rectifier subfamily of K_{2P} channels. It is most homologous to two other K_{2P} channels, TWIK-1 and TWIK-2, that are expressed in the cerebral circulation [1] cortex and hippocampus [3,12]. KCNK7 is also expressed in relatively high abundance in the cerebral cortex, hippocampus, nucleus accumbens and spinal cord [18]. However, its sensitivity to the effects of anesthetics has not been determined because it has not been functionally expressed in heterologous expression systems [20]. In this report we have studied a knockout mouse in which the gene coding for KCNK7 has been inactivated. Our goal was to examine the phenotypic consequences of KCNK7 inactivation and determine the potency of inhaled anesthetics in these mice.

Methods

Production of knockout mice

Mice in which the KCNK7 gene was inactivated were produced by Deltagen, Inc (San Carlos, CA) according to the following general outline: (a) introduction of a targeting vector to disrupt the KCNK7 gene in a mouse embryonic stem (ES) cell; (b) selection of an ES cell clone whose genome has incorporated the disrupted KCNK7 gene; (c) introduction of the ES cell of step (b) into a blastocyst; (d) implantation of the resulting blastocyst into a pseudopregnant mouse, i.e. a female in natural estrus that has been mated with a vasectomized male to give birth to a chimeric mouse; (e) breeding of the chimeric mouse to produce the transgenic mouse comprising a heterozygous disruption in the KCNK7 gene and (f) breeding the transgenic mouse of step (e) to produce a transgenic mouse whose genome comprises a homozygous disruption of the KCNK7 gene. Construction of the targeting vector, electroporation of the targeting vector into embryonic stem cells and selection of the targeted clones were carried out by standard procedures [10]. Southern blotting was accomplished by isolating genomic DNA from transfected and parental ES cells, digesting with enzymes (BamHI and EcoRI) that cut outside the construct arms separating the DNA fragments by agarose gel electrophoresis. The digests were transferred to nitrocellulose membranes and probed with a radiolabled fragment that hybridized outside of and adjacent to the construct arm. Bands were visualized by autoradiography exposure to film.

ES cells derived from the 129/OlaHsd mouse substrain were used to generate chimeric mice by the above stated steps. F1 mice were generated by breeding with C57BL/6 females. The resultant F1N0 heterozygotes were backcrossed to C57BL/6 mice to generate F1N1 heterozygotes. F2N1 homozygous mutant mice were produced by intercrossing F1N1 heterozygous males and females. Two breeding pairs served as the founder animals.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Animals

All experiments were performed in accordance with protocols approved by the Animal Care and Use Committees either of Deltagen, Inc. or of the University of California San Francisco. Animals were group housed under standard conditions on a 12-h light/dark cycle and given *ad lib* access to food and water. In these experiments we used male and female homozygous (KCNK7−/−) mice, their control wildtype (KCNK7+/+) and heterozygous (KCNK7+/−) littermates (body weight 16–32 g), derived from the crossing of heterozygous breeding pairs.

Genotyping Sample Preparation

A polymerase chain reaction (PCR) was used to genotype bred animals and to confirm the inactivation of the KCNK7 gene using specific primers. The positions of the PCR primers are shown in Figure 1 (arrows). Briefly, genomic DNA was extracted from bred mice tail snips using Qiagen (Valencia, CA) DNeasy genomic DNA purification kit following the manufacturer's instructions. Approximately 100ng of genomic DNA was used per reaction. A 25 μl reaction volume was prepared on ice with 1 μl template DNA and 24 μl of master mix containing Expand PCR buffer (Roche), 10 mM dNTP, primer mix (mix of primers 1, 2 and 3 at 10 μM), 0.5 unit Expand Taq polymerase (Roche), and H_2O to bring volume to 25 μl. PCR was performed with initial heating to 94°C for 3 minutes, then 40 cycles of 30 seconds at 94° C, 30 seconds at 60°C, 90 seconds at 68°C and final extension for seven minute at 72°C. PCR products were separated by electrophoresis on 1% agarose gel. The expected sizes of bands were: knockout - 517 basepair (bp) band; Wildtype - 256 bp band; Heterozygote - 517 bp and 256 bp bands. The primers used were:

Primer 1: 5′-GCT GAA CTG GCT AGC TTC CAG GCA G-3′

Primer 2: 5′-AAG GGC TTG CAC CCT AGA GCT CTT G-3′

Primer 3: 5′-GGG TGG GAT TAG ATA AAT GCC TGC TCT-3′

Sequence analysis

The protein sequences for human TWIK-1 (KCNK1), TWIK-2 (KCNK6) and human and mouse KCNK7 were downloaded from GenBank. The following accession numbers identify the sequences: TWIK-1 – NM002245; TWIK-2 –NM004823; hKCNK7 variant A – NM033347; mKCNK7 – NM010609. The sequences were imported into DNA Star MegAlign software module and then aligned using the Clustal W method [25].

MAC studies

MAC (the minimum alveolar concentration of desflurane, halothane and isoflurane that produced immobility in mice in response to a tail clamp) was determined in groups of 4 to 8 mice enclosed individually in clear plastic cylinders using techniques previously described [22,23]. Mouse body weights and body lengths were measured before MAC testing. Rectal temperatures were monitored and maintained between 35 and 39°C (range for all mice). Anesthetic concentrations were monitored with an RGM monitor (Datex Ohmeda, Louisville, CO). A low initial target concentration was chosen to ensure response to tail clamp and was imposed for a 40-minute equilibration period. We then increased the anesthetic concentration in steps of 15–20% of the preceding concentration, holding each step for at least 30 minutes before again applying the tail clamp. This continued until all mice ceased moving in response to the tail clamp. At the end of each step (after application of the tail clamp), a sample of gas was taken from one of the cylinders for anesthetic concentration analysis by gas chromatography. This concentration, rather than the RGM concentration was used in the calculation of MAC. MAC testing was done prior to genotyping so that the examiners were unaware of the genotypes of the mice at the time of testing. For each phenotypic group

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

(knockout, wild-type and heterozygotes) the mean and standard deviation of the concentrations at which the mouse failed to move were calculated.

We used a Gow-Mac gas chromatograph (Gow-Mac Instrument Corp., Bridgewater, NJ) equipped with a flame ionization detector to measure inhaled anesthetic concentrations. The 4.6 meter-long, 0.22 cm (ID) column was packed with SF-96. The column temperature was 100°C. The detector was maintained at temperatures approximately 50°C warmer than the column. The carrier gas flow was nitrogen at a flow of 15–20 mL/min. The detector received 35–38 mL/min hydrogen and 240–320 mL/min air. Primary standards were prepared for each compound, and the linearity of the response of the chromatograph was determined. We also commonly used secondary (cylinder) standards referenced to primary standards.

Statistical tests

All animals were tested for anesthetic sensitivity before their genotype was determined. For each mouse, MAC of an agent was taken as the greatest inspired concentration that permitted movement in response to tail clamp. The mean and standard deviation of the concentrations at which mice of a given genotype failed to move were calculated. Differences in MAC between genotypes were determined using analysis of variance for the three anesthetics. A significant difference was defined at a p value < 0.05. For analysis of animal mortality, data were arrayed by contingency table (3 groups × 2 outcomes) and analyzed by Chi square test.

Results

General

The mouse KCNK7 gene was disrupted by replacing exon 1 with a neo cassette (Figure 1A) by homologous recombination in transfected mouse embryonic stem cells (ES cells). The exon 1 of KCNK7 encodes the 5′-untranslated region (including the essential translation initiation consensus “Kozak” sequence) and the N-terminal 106 amino acid residues (including the translation start codon, the intracellular N-terminus, the first extracellular loop with potential glycosylation site, and half of the first pore-forming domain). This exon as a whole possesses no homology to any sequences in the mouse genome, and its coding region has very low homology to some members of the K_{2P} family (the closest is TWIK-1, 38% identity) and no homology to any other proteins. The exon 1 is essential in the expression and function of KCNK7. Therefore, it was expected that the deletion of exon 1 could completely eliminate the functional expression of KCNK7. DNA isolated from parental or knockout ES cells was digested with the indicated restriction enzymes and analyzed by Southern hybridization (Figure 1B) and indicated successful targeting of the KCNK7 wildtype allele. Individual animals derived from these ES cells were genotyped by PCR employing primers that overlapped the substituted region of the KCNK7 gene. PCR products of predicted size (256 bp: wildtype, 517 bp: targeted) were obtained using tail snip DNA from all animals from five different litters (Figure 1C). The expression of KCNK7 mRNA and protein was completely eliminated in the KCNK7−/− mice and was significantly reduced in the KCNK7+/− mice (analyses performed by Deltagen).

Both homozygous mutant males and females were fertile. Their progeny were viable through weaning. The genotypic distribution of the animals is shown in Table 1 and followed the expected 1:2:1 pattern. Homozygous mutant mice did not significantly differ from age- and gender-matched wildtype littermate mice in general appearance, gross anatomy, locomotion or overt behavior. Differences in body weight were present between individual mice but the variability among mice fell within historical reference ranges and was not correlated with genotype. Animals were also tested in the following behavior tests - tail suspension test, rotarod

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

test, hot plate test, startle test, and metrazol test. There were no differences between knockout and heterozygote or wildtype mice in the response to these tests.

MAC Testing

Volatile anesthetic sensitivity testing was done on groups of mice from the five different litters from the breeding pairs. MAC testing was done prior to genotyping so that the examiners were unaware of the genotypes of the mice at the time of testing. Forty-two offspring were tested for sensitivity to isoflurane while thirty-eight offspring were tested with halothane and desflurane. Four animals died in the time interval between isoflurane testing and the other anesthetic testing but the statistical significance of this distribution could not be determined due to insufficient power.

Table 2 shows the MAC values determined for the three genotypes with the three volatile anesthetics. There were no significant differences between the groups in anesthetic sensitivity to halothane, isoflurane or desflurane and the values for MAC that were obtained did not differ significantly from those previously reported for C57BL/6 mice [24]. Although the distribution of gender was unequal within groups, the effect of gender on MAC could not be determined due to insufficient power.

Discussion

The results described above indicate that deletion of the KCNK7 gene results in a mouse that not only is indistinguishable from wildtype or heterozygous animals but also had unaltered response to the three volatile anesthetics tested compared to wildtypes and heterozygotes. These findings most likely indicate that KCNK7 does not play a dominant role in the mechanism of action of volatile anesthetics. These studies cannot however, rule out the possibility of compensatory changes in ion channel gene expression in these mice to mask the effect of KCNK7 knockout.

KCNK7 is grouped with two other members of the weak inward rectifier K_{2P} subfamily based on sequence homology and similarity of genomic structure. KCNK7 is located on mouse chromosome 19 and like TWIK-1 and TWIK-2, the coding sequence is contained on three exons. An exon-intron boundary is also conserved among the three genes within the first GYG pore sequence after the first nucleotide of the second codon. This conservation suggests that members of this group arose by duplication and radiation type of mechanism. Overall, the sequence homology among the weak inward rectifier K_{2P} family is low. Table 3 displays an alignment of representative regions of KCNK7, TWIK-1 and TWIK-2 (Clustal W method) and shows that while in some conserved regions the percentage of identical amino acid residues may reach 50%, the overall sequence identity is in the range of only 30–40% (TWIK-1 v. TWIK-2 41% identify; TWIK-1 v. KCNK7 35% identity; TWIK-2 v. KCNK7 30% identity). This sequence divergence, which is also found within other K_{2P} channel subfamilies, may indicate that these channels are rapidly evolving into specialized cellular roles, as has been suggested for the plethora of K_{2P} channels in the *C. elegans* genome [21].

Of the fifteen mammalian K_{2P} channels, only three (TASK-5, TALK-1 and KCNK7) do not produce functional channels when expressed in heterologous expression systems such as *Xenopus* oocytes or transfected cultured cells. Therefore it has remained unclear whether these are truly non-functional *in vivo*, whether they are missing essential co-factors or whether they need to be paired with other subunits to achieve functional expression [20]. Extensive expression studies by Salinas et al. discovered that KCNK7 is retained intracellularly yet could not identify a retention signal sequence either by site-directed mutagenasis or by fusion protein strategies. The negative results suggest that either the KCNK7 channel is not a significant target for the volatile anesthetics, or the effect is compensated for by other K_{2P} channels.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Nevertheless, based on the preservation of basic gene structure for KCNK7, it is also unlikely that KCNK7 represents a pseudogene. There are two main types of pseudogenes, processed and duplicated. Processed pseudogenes arise by reverse transcription of processed mRNA and thus lack intronic sequences. Duplicated pseudogenes arise from errant genomic DNA replication and thus have similar genomic structure to homologous genes. Even though we believe that KCNK7 arose by gene duplication, based on the similarity of its genomic structure to its K_{2P} homologues, it has maintained an intact open reading frame while diverging significantly in primary sequence from its relatives. This observation implies that it provides some function and has not been released from selection pressure to accumulate random stop codons.

Thus it remains unclear why the KCNK7 knockout mouse displays little difference from the wildtype phenotype. KCNK7 transcript has been detected by RT-PCR in many areas of the human central nervous system including cerebellum, cortex, hippocampus and spinal cord [18]. It was also identified in mouse brain by the Allen Brain Atlas (http://www.brain-map.org/welcome.do); KCNK7 transcript can be seen at low to intermediate levels in choroid plexus within the lateral ventricle, third ventricle and lateral recess of the fourth ventricle. It is a formal possibility that low level expression of KCNK7 in these regions may contribute in some way to CSF production to indirectly affect CNS function.

This report adds to the evidence that the contribution of K_{2P} channel family to the mechanism of action of volatile and gaseous anesthetics may be restricted to a few family members. Previous work in knockout mice found that TASK-2 (KCNK5) does not mediate the action of volatile agents [6]. Other animal studies using blockers of TASK-1 (KCNK3) and TASK-3 (KCNK9) suggest that they also do not play a major role as well [4], although TASK-1 and TASK-3 knockout mice display a small resistance to halothane and isoflurane [14,15]. The inactivation of one K_{2P} channel gene, TREK-1, produced a mouse that showed a variable increase in MAC (7%–48%) that depended on the agent [11]. Studies of mice multiple K_{2P} channel knockouts or conditional knockouts will be needed to fully understand their role in the mechanism of action of volatile anesthetics.

References

1. Bryan RM Jr, You J, Phillips SC, Andresen JJ, Lloyd EE, Rogers PA, Dryer SE, Marrelli SP. Evidence for two-pore domain potassium channels in rat cerebral arteries. Am J Physiol Heart Circ Physiol 2006;291:H770–80. [PubMed: 16565299]
2. Buckingham SD, Kidd JF, Law RJ, Franks CJ, Sattelle DB. Structure and function of two-pore-domain K^+ channels: contributions from genetic model organisms. Trends Pharmacol Sci 2005;26:361–7. [PubMed: 15939489]
3. Chavez RA, Gray AT, Zhao BB, Kindler CH, Mazurek MJ, Mehta Y, Forsayeth JR, Yost CS. TWIK-2, a new weak inward rectifying member of the tandem pore domain potassium channel family. J Biol Chem 1999;274:7887–92. [PubMed: 10075682]
4. Cotten JF, Keshavaprasad B, Laster MJ, Eger EI 2nd, Yost CS. The ventilatory stimulant doxapram inhibits TASK tandem pore (K_{2P}) potassium channel function but does not affect minimum alveolar anesthetic concentration. Anesth Analg 2006;102:779–85. [PubMed: 16492828]
5. Cotten JF, Zou HL, Liu C, Au JD, Yost CS. Identification of native cerebellar granule cell currents due to background K channel KCNK5 (TASK-2). Molecular Brain Research 2004;128:112–120. [PubMed: 15363886]
6. Gerstin KM, Gong DH, Abdallah M, Winegar BD, Eger EI 2nd, Gray AT. Mutation of KCNK5 or Kir3.2 potassium channels in mice does not change minimum alveolar anesthetic concentration. Anesth Analg 2003;96:1345–9. [PubMed: 12707131]
7. Goldstein SA, Bockenhauer D, O'Kelly I, Zilberberg N. Potassium leak channels and the KCNK family of two-P-domain subunits. Nat Rev Neurosci 2001;2:175–84. [PubMed: 11256078]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

8. Gray AT, Zhao BB, Kindler CH, Winegar BD, Mazurek MJ, Xu J, Chavez RA, Forsayeth JR, Yost CS. Volatile anesthetics activate the human tandem pore domain baseline K^+ channel KCNK5. Anesthesiology 2000;92:1722–30. [PubMed: 10839924]
9. Han J, Truell J, Gnatenco C, Kim D. Characterization of four types of background potassium channels in rat cerebellar granule neurons. J Physiol 2002;542:431–44. [PubMed: 12122143]
10. Hasty P, Bradley A, Morris JH, Edmondson DG, Venuti JM, Olson EN, Klein WH. Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. Nature 1993;364:501–6. [PubMed: 8393145]
11. Heurteaux C, Guy N, Laigle C, Blondeau N, Duprat F, Mazzuca M, Lang-Lazdunski L, Widmann C, Zanzouri M, Romey G, Lazdunski M. TREK-1, a K^+ channel involved in neuroprotection and general anesthesia. EMBO J 2004;23:2684–95. [PubMed: 15175651]
12. Lesage F, Lauritzen I, Duprat F, Reyes R, Fink M, Heurteaux C, Lazdunski M. The structure, function and distribution of the mouse TWIK-1 K^+ channel. FEBS Lett 1997;402:28–32. [PubMed: 9013852]
13. Lesage F, Terrenoire C, Romey G, Lazdunski M. Human TREK2, a 2P domain mechano-sensitive K^+ channel with multiple regulations by polyunsaturated fatty acids, lysophospholipids, and Gs, Gi, and Gq protein-coupled receptors. J Biol Chem 2000;275:28398–405. [PubMed: 10880510]
14. Linden AM, Aller MI, Leppa E, Vekovischeva O, Aitta-Aho T, Veale EL, Mathie A, Rosenberg P, Wisden W, Korpi ER. The in vivo contributions of TASK-1-containing channels to the actions of inhalation anesthetics, the alpha(2) adrenergic sedative dexmedetomidine, and cannabinoid agonists. J Pharmacol Exp Ther 2006;317:615–26. [PubMed: 16397088]
15. Linden AM, Sandu C, Aller MI, Vekovischeva OY, Rosenberg PH, Wisden W, Korpi ER. TASK-3 knockout mice exhibit exaggerated nocturnal activity, impairments in cognitive functions and reduced sensitivity to inhalation anesthetics. J Pharmacol Exp Ther 2007;323:924–34. [PubMed: 17875609]
16. Liu C, Au JD, Zou HL, Cotten JF, Yost CS. Potent activation of the human tandem pore domain K channel TRESK with clinical concentrations of volatile anesthetics. Anesth Analg 2004;99:1715–22. [PubMed: 15562060]
17. Meadows HJ, Randall AD. Functional characterisation of human TASK-3, an acid-sensitive two-pore domain potassium channel. Neuropharmacology 2001;40:551–9. [PubMed: 11249964]
18. Medhurst AD, Rennie G, Chapman CG, Meadows H, Duckworth MD, Kelsell RE, Gloger II, Pangalos MN. Distribution analysis of human two pore domain potassium channels in tissues of the central nervous system and periphery. Mol Br Res 2001;86:101–14.
19. Patel AJ, Honore E, Lesage F, Fink M, Romey G, Lazdunski M. Inhalational anesthetics activate two-pore-domain background K^+ channels. Nat Neurosci 1999;2:422–6. [PubMed: 10321245]
20. Salinas M, Reyes R, Lesage F, Fosset M, Heurteaux C, Romey G, Lazdunski M. Cloning of a new mouse two-P domain channel subunit and a human homologue with a unique pore structure. J Biol Chem 1999;274:11751–60. [PubMed: 10206991]
21. Salkoff L, Butler A, Fawcett G, Kunkel M, McArdle C, Paz-y-Mino G, Nonet M, Walton N, Wang ZW, Yuan A, Wei A. Evolution tunes the excitability of individual neurons. Neuroscience 2001;103:853–9. [PubMed: 11301195]
22. Sonner JM, Gong D, Eger EI 2nd. Naturally occurring variability in anesthetic potency among inbred mouse strains. Anesth Analg 2000;91:720–6. [PubMed: 10960407]
23. Sonner JM, Gong D, Li J, Eger EI 2nd, Laster MJ. Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. Anesth Analg 1999;89:1030–4. [PubMed: 10512285]
24. Sonner JM, Vissel B, Royle G, Maurer A, Gong D, Baron NV, Harrison N, Fanselow M, Eger EI 2nd. The effect of three inhaled anesthetics in mice harboring mutations in the GluR6 (kainate) receptor gene. Anesth Analg 2005;101:143–8. [PubMed: 15976221]
25. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–80. [PubMed: 7984417]
26. Washburn CP, Sirois JE, Talley EM, Guyenet PG, Bayliss DA. Serotonergic raphe neurons express TASK channel transcripts and a TASK-like pH- and halothane-sensitive K^+ conductance. J Neurosci 2002;22:1256–65. [PubMed: 11850453]

27. Yu FH, Yarov-Yarovoy V, Gutman GA, Catterall WA. Overview of molecular relationships in the voltage-gated ion channel superfamily. Pharmacol Rev 2005;57:387–95. [PubMed: 16382097]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

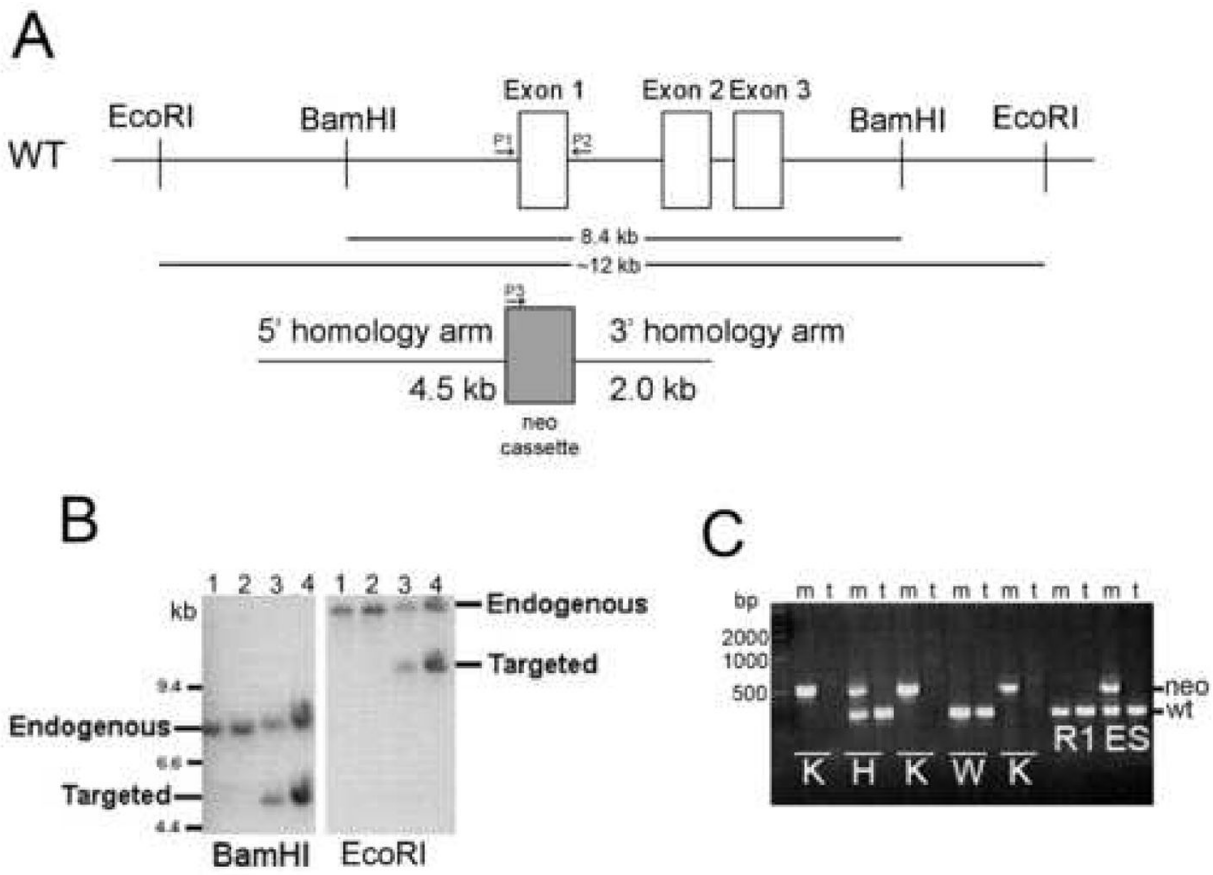

Figure 1.

Disruption of KCNK7 gene. (A) Native (WT) allele and targeting vector. Arrowheads designated as P1, P2 and P3 display locations of PCR primers used to characterize bred mice. Restriction sites (EcoRI and BamHI) and expected sizes of restriction fragments for Southern analysis shown. (B) 3′ Southern blot confirmation: genomic DNA isolated from ES lines were digested with BamHI or EcoRI restriction enzymes. The DNA was analyzed by Southern hybridization, probing with a radiolabeled DNA fragment hybridizing out and adjacent to targeting construct. The parent ES lines (lanes 1 and 2 of each blot) showed single bands representing the targeted allele. The selected ES line (lanes 3 and 4) showed an additional band representing the targeted allele with the expected homologous recombination event. (C) PCR genotyping with genomic DNA. Reactions from five littermates are shown starting from left. Reactions were run with either all three primers (P1 + P2 +P3=multiplex − lanes m) or single wild-type primer pair (P1 + P2=target − lanes t). Bands represent amplification of sequence from neo cassette (neo - 517 bp) or wild-type allele (wt - 256 bp). Abbreviations: K=knockout, H=heterozygote, W=wildtype, R1=parental ES cells, ES=targeted ES cells.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Numbers of animals of each genotype tested for MAC (#male:#female)

	Wildtype	Heterozygous	Knockout
Isoflurane	9 (3m:6f)	20 (11m:9f)	13 (4m:9f)
Halothane	8 (3m:5f)	20 (11m:9f)	10 (4m:6f)
Desflurane	8 (3m:5f)	20 (11m:9f)	10 (4m:6f)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

MAC values

	Wildtype	Heterozygous	Knockout
Isoflurane	1.50±0.02	1.52±0.03	1.53±0.02
Halothane	1.23±0.04	1.16±0.03	1.18±0.05
Desflurane	7.80±0.3	7.44±0.2	7.48±0.2

Table 3

Protein sequence alignment (single letter amino acid code) of regions of four closely related K_{2P} channels. Shaded letters indicate areas of sequence identity among the four sequences.

	First Extracellular loop	First pore	Intracellular loop	Second pore
TWIK-1	S S V E L P Y E D L L R Q E L	T T G Y G H T V P L S D G G K A F	T V H V T R R P V L Y F H I R	W Y F C F I S L S T I G L G D
TWIK-2	A R L E G P H E A R L R A E L	T V G Y G Y T T P L T D A G K A F	S L L L T H V P L S W L S M R	W Y F C F I S L S T I G L G D
hKCNK7	Q A L E G P P A C R L Q A E L	T T G Y G H M A P L S P G G K A F	L L P V L S R P R A W V A V H	W Y F C F S S L S T I G L E D
mKCNK7	Q A L E G P P A R H L Q A Q V	T T G Y G H M A P L S S G G K A F	L L P V F S R P G D W V A I R	W Y F C F G S L S T I G L G D

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript