

Mutation of KCNK5 or Kir3.2 Potassium Channels in Mice Does Not Change Minimum Alveolar Anesthetic Concentration

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Several reports suggest that clinically used concentrations of inhaled anesthetics can increase conductance through noninactivating potassium channels and that the resulting hyperpolarization might decrease excitability, thereby leading to the anesthetic state. We speculated that animals deficient in such potassium channels might be resistant to the effects of anesthetics. Thus, in the present study, we measured the minimum alveolar anesthetic concentration (MAC) needed to prevent movement in response to a noxious stimulus in 50% of adult mice lacking functional KCNK5 potassium channel subunits and compared these results with those for heterozygous and wild-type mice. We also measured MAC in weaver mice that had a mutation in

the potassium channel Kir3.2 and compared the resulting values with those for wild-type mice. MAC values for desflurane, halothane, and isoflurane for KCNK5-deficient mice and isoflurane MAC values for weaver mice did not differ from MAC values found in control mice. Our results do not support the notion that these potassium channels mediate the capacity of inhaled anesthetics to produce immobility. In addition, we found that the weaver mice did not differ from control mice in their susceptibility to convulsions from the nonimmobilizers flurothyl [di-(2,2,2,-trifluoroethyl)ether] or 2N (1,2-dichlorohexafluorocyclobutane).

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Results from several studies indicate that inhaled anesthetics produce immobility in the face of noxious stimulation through actions at the spinal cord (1,2). Investigators have proposed that potassium channels of the KCNK subfamily might mediate this end-point of inhaled anesthetic administration (3-6). The KCNK subfamily is defined by the presence of two pore domains within their primary amino acid sequence, allowing channels to form as homodimers with four pore domains lining the ion conduction pathway. Two pore domain K⁺ channels are active at all holding potentials and therefore serve as baseline (or background) channels capable of modulating the

resting membrane potential. KCNK channels activated by volatile anesthetics include acid-sensitive channels (TASK-1, TASK-2, and TASK-3) as well as outward rectifiers (TREK-1 and TREK-2). Volatile anesthetics activate KCNK5 (TASK-2) currents in a concentration-dependent fashion (7), leading to hyperpolarization. KCNK5 subunits have been localized to many regions of the central nervous system, including mammalian spinal cord (8).

Recent technology allows the creation and identification of insertion mutations in embryonic stem (ES) cells (9). This technology, referred to as "gene trapping," has, through random insertion, mutated the *KCNK5* (TASK-2) gene. Gene-trap mutations produce (if not a true null) a severely hypomorphic phenotype. Gene-trap phenotypes are functionally identical to many previously established phenotypes resulting from targeted disruption of developmentally expressed genes (10). The mutation in the *KCNK5* (TASK-2) gene offered the opportunity to test the KCNK5 null phenotype with respect to volatile anesthetic potency. We hypothesized that disruption of the *KCNK5* gene would increase the concentration of volatile anesthetic required to prevent movement in response to a tail-clamp stimulus in adult mice.

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Such a finding would demonstrate that potassium channels might be important mediators of the capacity of inhaled anesthetics to produce anesthesia.

In addition, we tested whether the potency of inhaled anesthetics and convulsants is altered in mice with mutation of the Kir3.2 (GIRK2) potassium channel (the weaver mutation). Kir3.2 channels are found mainly in the hippocampus, cerebellum, cortex, thalamus, and brainstem (11). Weaver mice contain a point mutation (G156S) in the pore-forming region of the Kir3.2 potassium channel that renders Kir3.2 channels less selective for potassium. In addition, the weaver Kir3.2 current amplitudes are attenuated and not activated by gamma-aminobutyric acid type B receptors. Seizure activity has been reported in weaver mice, as well as in Kir3.2 null mutant mice (12,13). Because Kir3.2 channels in the weaver mouse contribute less to repolarization than in normal mice, the thresholds for inhaled anesthetic action or convulsions could be altered. These experiments were designed to test the specific hypotheses that the nonimmobilizer 2N (1,2-dichlorohexafluorocyclobutane) produces convulsions by inhibition of Kir3.2 channels and that minimum alveolar anesthetic concentration (MAC) might be increased.

Methods

These studies were approved by the University of California, San Francisco committee on animal research. Disruption of the *KCNK5* gene was produced by gene-trap insertion in the laboratory of William Skarnes at the University of California, Berkeley. Gene trapping in mouse ES cells creates random insertions in genes active in development (9). The secretory gene-trap vector pGT1.8TM, consisting of a mouse splice acceptor sequence upstream of a *lacZ* reporter gene construct, was used to electroporate mouse strain 129/Ola ES cells. This vector targets genes that encode cell-surface molecules by introducing a transmembrane domain that permits cytosolic β -galactosidase activity. Gene-trap vectors are constructed to encode polypeptide fusion proteins with both β -galactosidase and neomycin phosphotransferase activities.

Mouse strain 129/Ola E14 ES cells were electroporated with linearized gene-trap vector pGT1.8TM DNA and placed under Geneticin (Invitrogen Life Technologies, Carlsbad, CA) selection. Cells were double-selected for gene-trap vector insertions by identification of colonies with β -galactosidase activity. An ES cell line containing an insertion of the secretory gene-trap vector pGT1.8TM within an intron of the *KCNK5* (*TASK-2*) gene was identified by 5' rapid amplification of complementary DNA (cDNA) ends-polymerase chain reaction (PCR) on ES cell RNA as previously described (14).

Table 1. The Genotypes and Sex Resulting from Matings of Mice Heterozygous for *KCNK5* Deficiency Follow the Expected 1:2:1 Distribution Except for Homozygous Females

Genotype	Males	Females
Wild-type	8	14
Heterozygous	18	27
Homozygous	9	3

C57BL/6J mouse blastocysts were injected with one of these clones and implanted into pseudopregnant C57BL/6J female mice (The Jackson Laboratory, Bar Harbor, ME). Male offspring with chimeric coats were mated with C57BL/6J females. Resulting heterozygous males carrying the disrupted *KCNK5* allele were backcrossed twice to C57BL/6J females to reduce the potential problem of mutations that arise in 129 ES cell culture and place *KCNK5* deficiency on a more nearly isogenetic background. Resulting *KCNK5* heterozygous offspring were intercrossed to generate the *KCNK5*^{+/+} and *KCNK5*^{-/-} mice.

Genomic DNA was obtained by tail biopsy (5- to 10-mm distal fragment) of 3-wk-old mice. Quantitative dot-blots of genomic DNA from tail-biopsy specimens were used to genotype mice by using *lacZ* probes as previously described (15). Amputation of the tail tip does not affect desflurane, isoflurane, or halothane MAC in B6129F2/J mice (15).

Reverse transcription (RT)-PCR was used to confirm insertion of the gene-trap vector in the *KCNK5* gene. Approximately 50–100 mg of liver was homogenized in 1–2 mL of TRIzol reagent (Invitrogen Life Technologies). Total RNA was isolated per the manufacturer's protocol. Approximately 2 μ g of total RNA was used as template in cDNA synthesis. An oligodeoxythymidylic acid (oligo dT) primer was used to initiate RT. Complementary DNA synthesis was performed by using the manufacturer's protocol of the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen Life Technologies). Sense and antisense primers were designed to the mTASK-2 cDNA (GenBank AF259395) by using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA). A sense primer (corresponding to mouse TASK-2 bases 64–83) and antisense primer (corresponding to mouse TASK-2 bases 393–374) were designed. PCR products were gel-purified and sequenced to confirm product identity.

Methods used to determine MAC have been described (15). Animals were housed two to six per cage in the animal-care facility for at least 1 wk before study, under 12-h cycles of light and dark. Mice received access to food and water *ad libitum*. Mice of either gender were 7 to 9 wk old at the time of study.

MAC values for halothane, isoflurane, and desflurane were determined with at least 1 wk separating

Table 2. Desflurane, Halothane, and Isoflurane MAC Values Among Wild-Type, Heterozygous, and Homozygous (Null) Mutant KCNK5 Mice

Variable	<i>n</i>	MAC (% atm)	SD	95% Confidence interval	<i>P</i> value
Desflurane					
Wild-type (backcross)	5	8.28	0.94	-0.86, 1.30	0.66
Heterozygous (backcross)	6	8.06	0.64		
Homozygous null (intercross)	9	8.63	0.57	-0.53, 0.67	0.81
Heterozygous/wild-type (intercross)	8	8.56	0.59		
Halothane					
Wild-type (backcross)	8	0.98	0.15	-0.12, 0.16	0.76
Heterozygous (backcross)	7	0.96	0.09		
Homozygous null (intercross)	6	1.24	0.11	-0.20, 0.08	0.38
Heterozygous/wild-type (intercross)	8	1.30	0.13		
Isoflurane					
Wild-type (backcross)	7	1.67	0.10	-0.03, 0.21	0.13
Heterozygous (backcross)	6	1.58	0.10		
Homozygous null (intercross)	9	1.58	0.08	-0.10, 0.16	0.64
Heterozygous/wild-type (intercross)	9	1.55	0.17		

Both heterozygous and wild-type littermates were used as controls for null mice generated by intercrossing; 95% confidence intervals for differences and *P* values are shown. No significant differences between control and experimental groups were found. MAC = minimum alveolar anesthetic concentration; atm = atmosphere.

Table 3. MAC Values and Convulsivity Thresholds for Weaver and Control Mice

Variable	<i>n</i>	MAC ^a or ED ₅₀ ^b (% atm)	SD	95% Confidence interval	<i>P</i> value
Isoflurane					
Weaver	4	1.49 ^a	0.12	-0.19, 0.07	0.30
Control	5	1.55 ^a	0.02		
Indoklon					
Weaver	4	0.317 ^b	0.06	-0.07, 0.12	0.56
Control	4	0.293 ^b	0.05		
2N					
Weaver	4	5.92 ^b	1.10	-1.01, 2.69	0.31
Control	4	5.08 ^b	1.04		

The 95% confidence intervals for differences and *P* values are shown. No significant differences between weaver mice and controls were found. MAC = minimum alveolar anesthetic concentration; ED₅₀ = 50% effective dose; 2N = 1,2-dichlorohexafluorocyclobutane; atm = atmosphere.

each measurement. Each animal was placed in an individual plastic chamber connected to a circle system containing a carbon dioxide absorber, fan, and oxygen source. Halothane, isoflurane, and desflurane were delivered from commercial anesthesia vaporizers. The temperature of each mouse was measured rectally and maintained between 36°C and 38°C by using heating pads under the plastic chambers as needed. The anesthetic 50% effective dose (MAC) was measured as the mean of the halothane, isoflurane, and desflurane partial pressures bracketing the animal's response and lack of response to a 1-min tail clamp. If an animal responded to the tail clamp, the anesthetic partial pressure was increased in steps of approximately 20% until no response was obtained. The animals breathed each anesthetic partial pressure at each step for 20 min for desflurane, 30 min for

isoflurane, and 40 min for halothane before application of the tail clamp. For each genotype, MAC was the average of MAC for each mouse, with each animal contributing one value.

Weaver mice were obtained from The Jackson Laboratory. Control mice for these studies were C57BL/6J mice. Responses to isoflurane and the nonimmobilizers 2N and flurothyl [di-(2,2,2-trifluoroethyl)ether] were measured. Weaver mice were approximately 7 wk old at the time of isoflurane MAC studies, 8 wk old at the time of 2N convulsant studies, and 15 wk old at the time of flurothyl convulsant studies. 2N and Indoklon were obtained from the same source (SynQuest Labs., Inc., Alachua, FL).

MAC values and convulsant 50% effective dose values for control versus experimental groups were compared with two-sample unpaired Student's *t*-tests.

Mean values are reported with SD. $P < 0.05$ was considered statistically significant.

Results

Adult KCNK5 knockout mice could be bred, implying that KCNK5 is not critical for development and survival, but mating of heterozygous mice resulted in a small number of female homozygous offspring, suggesting that a subviable phenotype can cause antenatal mortality (Table 1). KCNK5 expression during development could relate to the observed pattern of survival. One female homozygote animal, which did not undergo experimental testing, was found dead in the cage at 12 days of age. No other deaths were observed in heterozygous or wild-type animals before studies.

Homozygous animals were normal in appearance, weight, locomotion, and overt behavior. MAC values for the KCNK5 knockout mice did not differ as a function of genotype (Table 2). Similarly, MAC values for the weaver mice did not differ as a function of genotype (Table 3).

Weaver mice had normal responses to inhaled anesthetics (Table 3). The data show no difference in isoflurane MAC, flurothyl convulsant threshold, or 2N convulsant threshold in comparing wild-type and weaver mutant mice.

Discussion

Neither the KCNK5 mutant nor weaver mice demonstrated an increased resistance to inhaled anesthetics as defined by their MAC values, a finding inconsistent with the notion that potassium channels mediate this anesthetic action. However, several caveats apply to this conclusion. First, multiple KCNK subunits have been identified, and, thus, the contribution of any one subunit to excitability could be small. Second, inhaled anesthetic action is independent of cerebrospinal fluid (CSF) pH over a broad range (CSF pH, 7.5 to 7.1) (16). In contrast, acidosis profoundly affects some KCNK currents *in vitro* (TASK channels are acid sensitive). Thus, the particular potassium channel we tested may not be relevant, but other potassium channels without this pH limitation may be relevant. Third, TASK-2 is less abundant than other KCNK subunits expressed in spinal cord (17,18). TREK-1 and TREK-2 appear to be more highly expressed. Our observations support the conclusion that TASK-2 is not essential for volatile anesthetic inhibition of nocifensive behavior, but this does not exclude a role for other potassium channels.

Background genes from parental strains can compromise the interpretation of mutant phenotypes (19). Our knockout mice, like most current models, used parental strains 129 and C57BL/6J, resulting in a

mixed genetic background. Our heterozygous mice were backcrossed to the parental C57BL/6J strain to reduce the potential problem of mutations that arise in 129 ES cell culture and to place KCNK5 deficiency on a more uniform genetic background, one that approaches C57BL/6J. MAC (tail-clamp) studies assay several components that participate in nocifensive behavior. Although outcrossing of 129 substrains can result in extensive genetic variability (20), measurements of MAC in many of these substrains have been consistent (15). Our estimates of volatile anesthetic potency in both control and experimental animals were consistent with historical measurements in the parental 129 and C57BL/6J strains (15).

We also found that Kir3.2 potassium channel dysfunction by weaver mutation does not alter MAC or predispose to convulsions produced by 2N or flurothyl. Compensatory mechanisms may maintain normal responses of KCNK5 null mice to inhaled anesthetics. However, weaver mice are phenotypically abnormal, indicating that they have not completely compensated for the mutation. Therefore, it is unlikely that compensatory mechanisms preserve responses to inhaled anesthetics or inhaled convulsants in weaver mice. This suggests Kir3.2 does not play a role in immobility or convulsions produced by these inhaled drugs.

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References

1. Antognini JF, Schwartz K. Exaggerated anesthetic requirements in the preferentially anesthetized brain. *Anesthesiology* 1993;79:1244-9.
2. Rampil JJ. Anesthetic potency is not altered after hypothermic spinal cord transection in rats. *Anesthesiology* 1994;80:606-10.
3. Gray AT, Winegar BD, Leonoudakis DJ, et al. TOK1 is a volatile anesthetic stimulated K^+ channel. *Anesthesiology* 1998;88:1076-84.
4. Sirois JE, Pancrazio JJ, Lynch C III, Bayliss DA. Multiple ionic mechanisms mediate inhibition of rat motoneurons by inhalation anaesthetics. *J Physiol* 1998;512:851-62.
5. Patel AJ, Honore E, Lesage F, et al. Inhalational anesthetics activate two-pore-domain background K^+ channels. *Nat Neurosci* 1999;2:422-6.
6. Sirois JE, Lei Q, Talley EM, et al. The TASK-1 two-pore domain K^+ channel is a molecular substrate for neuronal effects of inhalation anesthetics. *J Neurosci* 2000;20:6347-54.
7. Gray AT, Zhao BB, Kindler CH, et al. Volatile anesthetics activate the human tandem pore domain baseline K^+ channel KCNK5. *Anesthesiology* 2000;92:1722-30.
8. Gabriel A, Abdallah M, Yost CS, et al. Localization of the tandem pore domain K^+ channel KCNK5 (TASK-2) in the rat central nervous system. *Mol Brain Res* 2002;98:153-63.
9. Skarnes WC, Moss JE, Hurlley SM, Beddington RS. Capturing genes encoding membrane and secreted proteins important for mouse development. *Proc Natl Acad Sci U S A* 1995;92:6592-6.
10. Skarnes WC. Gene trapping methods for the identification and functional analysis of cell surface proteins in mice. *Methods Enzymol* 2000;328:592-615.

11. Karschin C, Dissmann E, Stuhmer W, Karschin A. IRK(1-3) and GIRK(1-4) inwardly rectifying K⁺ channel mRNAs are differentially expressed in the adult rat brain. *J Neurosci* 1996;16:3559-70.
12. Eisenberg B, Messer A. Tonic/clonic seizures in a mouse mutant carrying the weaver gene. *Neurosci Lett* 1989;96:168-72.
13. Signorini S, Liao YJ, Duncan SA, et al. Normal cerebellar development but susceptibility to seizures in mice lacking G protein-coupled, inwardly rectifying K⁺ channel GIRK2. *Proc Natl Acad Sci U S A* 1997;94:923-7.
14. Townley DJ, Avery BJ, Rosen B, Skarnes WC. Rapid sequence analysis of gene trap integrations to generate a resource of insertional mutations in mice. *Genome Res* 1997;7:293-8.
15. Sonner JM, Gong D, Li J, et al. Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. *Anesth Analg* 1999;89:1030-4.
16. Eisele JH, Eger EI II, Muallem M. Narcotic properties of carbon dioxide in the dog. *Anesthesiology* 1967;28:856-65.
17. Medhurst AD, Rennie G, Chapman CG, et al. Distribution analysis of human two pore domain potassium channels in tissues of the central nervous system and periphery. *Mol Brain Res* 2001;86:101-14.
18. Patel AJ, Honore E. Properties and modulation of mammalian 2P domain K⁺ channels. *Trends Neurosci* 2001;24:339-46.
19. Crawley JN, Belknap JK, Collins A, et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* 1997;132:107-24.
20. Simpson EM, Linder CC, Sargent EE, et al. Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. *Nat Genet* 1997;16:19-27.

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