

Genomic Organization, Chromosomal Localization, Tissue Distribution, and Biophysical Characterization of a Novel Mammalian *Shaker*-related Voltage-gated Potassium Channel, *Kv1.7**

(Received for publication, September 8, 1997, and in revised form, December 16, 1997)

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We report the isolation of a novel mouse voltage-gated *Shaker*-related K⁺ channel gene, *Kv1.7* (*Kcna7/KCNA7*). Unlike other known *Kv1* family genes that have intronless coding regions, the protein-coding region of *Kv1.7* is interrupted by a 1.9-kilobase pair intron. The *Kv1.7* gene and the related *Kv3.3* (*Kcnc3/KCNC3*) gene map to mouse chromosome 7 and human chromosome 19q13.3, a region that has been suggested to contain a diabetic susceptibility locus. The mouse *Kv1.7* channel is voltage-dependent and rapidly inactivating, exhibits cumulative inactivation, and has a single channel conductance of 21 pS. It is potently blocked by noxiustoxin and stichodactylotoxin, and is insensitive to tetraethylammonium, kalitoxin, and charybdotoxin. Northern blot analysis reveals ~3-kilobase pair *Kv1.7* transcripts in mouse heart and skeletal muscle. *In situ* hybridization demonstrates the presence of *Kv1.7* in mouse pancreatic islet cells. *Kv1.7* was also isolated from mouse brain and hamster insulinoma cells by polymerase chain reaction.

Ion channels that exhibit a variety of gating patterns and ion selectivity are critical elements that transduce signals in diverse cell types (1). Voltage-gated potassium-selective (*Kv*)¹ channels represent the largest family within this class of proteins (2), and perform many vital functions in both electrically excitable and nonexcitable cells. Following initiation of an ac-

tion potential in nerve and muscle cells, *Kv* channels play the important role of repolarizing the cell membrane (1). *Kv* channels can also modulate hormone secretion, for example insulin release from pancreatic islet cells (3–6), and regulate calcium signaling during mitogen-stimulated activation of lymphocytes (7).

Kv channels in mammalian cells are encoded by an extended family of at least nineteen genes (2). The largest subfamily, *Kv1*, is related to the fly *Shaker* gene and contains six members, *Kv1.1–Kv1.6* (2). The *Shaker* gene has 21 exons, which can be alternatively spliced to generate at least five functionally distinct transcripts (8, 9). In contrast, the protein-coding regions of each of the six known mammalian *Kv1* genes and the three known *Xenopus* homologues are contained in a single exon (2, 10), precluding alternative splicing as a means of generating functionally different proteins. The evolutionary significance of this pattern of organization remains a puzzle.

Here we report the identification of a novel mammalian gene, *Kv1.7* (*Kcna7/KCNA7*), that has a genomic organization distinct from the other members of the vertebrate *Kv1* subfamily. We have defined the chromosomal location of this gene in the mouse and human genome, determined its tissue distribution, and characterized the biophysical and pharmacological properties of the cloned channel.

EXPERIMENTAL PROCEDURES

Isolation and Characterization of *mKv1.7*, *hKv1.7*, *hKv3.3*, and *hKv3.4* DNA Clones—Three overlapping genomic clones (KC225, KC254, and KC256) were isolated from an AKR/J mouse genomic library screened with a mixture of *mKv1.1* and *rKv1.5* cDNA probes, as described previously (10), and mapped by multiple and partial restriction endonuclease digests, and by dideoxy sequencing. *Kv1.7* cDNAs were amplified by the polymerase chain reaction (PCR) from mouse brain and from the hamster insulinoma cell line, HIT-T1S, using intron-flanking primers (5'-TCTCCGTACTCGTCATCGG-3' within S1 and 5'-AAATGGGTGTCCACCCGGTC-3' on the 3' side of S5). The resulting 588-bp PCR fragments were sequenced.

Cosmid clones encoding *hKv1.7* and *hKv3.3* (11) were isolated from a human chromosome 19-enriched library, Library F (12), screened with *mKv1.7* and *mKv3.3* probes. A 1.9-kb cDNA fragment of the *Shaw* family gene, *hKv3.4*, was isolated from a human pancreatic library (13) screened with a mixture of *hKv3.1* (0.9-kb *XbaI/HindIII*), *hKv3.3* (1.4-kb *PstI/EcoRI*), and *mKv3.4* (0.9-kb *HindII/EcoRI*) probes at a final stringency of 0.5 × SSC and 0.1% SDS at 55 °C (8 × 10⁶ phage screened). The isolated clone spans the region from S1 through the 3' end of the coding region (0.6 kb), and 1.3 kb of the 3'-noncoding region.

Mice—Pancreatic tissue sections were obtained from 9–16-week-old diabetic-prone (*db/db*) and healthy (*db/+*) C57BL/KsJ mice. Mice ho-

* This work was supported in part by United States Public Health Service Grants AI-24783, Shannon Award GMD054872 (to K. G. C.), and NS14609 (to M. D. C.); by Glaxo Inc. (to K. G. C.); by United States Department of Energy Contract W-7405-Eng-48 (to H. W. and B. B.); and by the National Institutes of Health, NCI, DHHD, under Contract NO1-CO-74101 with ABL (C. M. H., N. G. C., and N. A. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF032099–AF032101.

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¹ The abbreviations used are: *Kv*, voltage-gated potassium selective; PCR, polymerase chain reaction; RBL, rat basophilic leukemic; bp, base pair(s); mb, millibase pair(s); kb, kilobase pair(s).

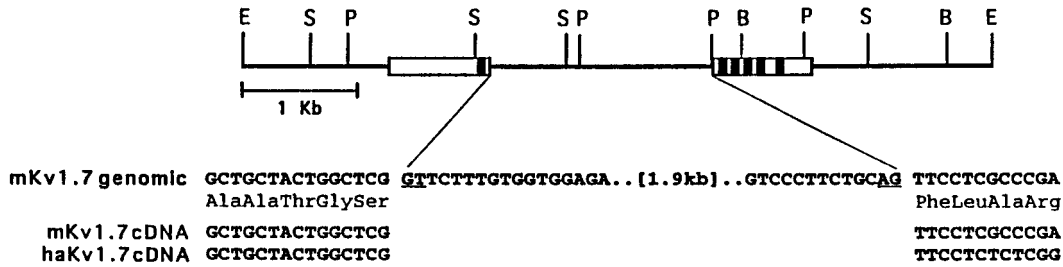


FIG. 1. Genomic organization of mouse *Kv1.7*. Top, the *mKv1.7* coding sequence is indicated by the two stippled boxes, and the six bars within these regions indicate the putative membrane-spanning domains S1 through S6. Restriction sites are indicated as follows: B, *Bgl*II; E, *Eco*RI; P, *Pst*I; S, *Sac*I. Bottom, comparison of the genomic sequence of *mKv1.7* with that of mouse and hamster (*haKv1.7*) cDNAs shows the splice donor and acceptor sites which form the boundaries of the single intervening sequence.

mozygous for the autosomal recessive diabetic susceptibility gene *db*, a mutated form of the leptin receptor (14, 15) on chromosome 4, develop diabetes beginning at about 6 weeks of age (16).

Mapping Mouse and Human Chromosomal Locations of *Kv1.7* and *Kv3.3*—Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F₁ females and (C57BL/6J) males, and a total of 205 N₂ mice were used to map the two mouse genes, *mKv1.7/Kcna7* and *mKv3.3/Kcnc3*, as described previously (11, 17–20). The probe for *mKv1.7* was the entire 6.4-kb *Eco*RI fragment shown in Fig. 1, and that for mouse *Kv3.3* was a 4-kb genomic *Hind*III fragment containing the entire 3'-exon (11). Although 155 mice were analyzed for all markers and are shown in this segregation analysis, up to 188 mice were typed for some pairs of markers. Recombination frequencies were calculated as described (11, 17–20) using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to account for the allele distribution patterns. Fluorescent *in situ* hybridization of cosmids to metaphase human chromosomes was carried out as described previously (21, 22).

Northern Blot Assays—A Northern blot of poly(A)⁺ RNA from mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (CLONTECH Inc., Palo Alto, CA) was probed with the mouse *Kv1.7*-specific 3'-noncoding region sequence. The *Pst*I/*Sac*I *Kv1.7* 3'-noncoding region was labeled by the random primer method (Boehringer Mannheim Random Primed DNA labeling kit). The RNA blot was hybridized at 60 °C for 18 h, washed at a final stringency of 0.2 × SSC and 0.1% SDS at 60 °C for 1 h, and exposed to x-ray film at –80 °C with an intensifying screen for 3 days.

In Situ Hybridization—cRNA probes labeled with α-³⁵S-labeled UTP (1300 Ci/mmol) were alkaline-denatured to an average size of 100 nucleotides and used for *in situ* hybridization on pancreatic frozen sections (6–10 μm thick) from *db/db* mice. Briefly, sections were hybridized overnight at 42 °C, RNase treated, washed five times in 0.5 × SSC at 65 °C, coated with Ilford K5 photographic emulsion, and exposed at 4 °C for varying times. After development, the sections were counterstained with hematoxylin and eosin Y and examined with a Leitz Aristoplan microscope equipped with reflected polarized light to visualize silver grains in dark field. The probes used for hybridization were as follows: *insulin*, 1.6-kb human insulin gene including the 5'- and 3'-flanking sequences (ATCC no. 57399); *hKv3.4*, 1.9-kb cDNA fragment spanning S1 through the 3' end of the coding region (0.6 kb), and 1.3 kb of the 3'-noncoding region; *mKv1.7*, 540-bp *Pst*I/*Sac*I fragment containing 29 bp of C-terminal coding sequence and 511 bp of 3'-noncoding sequence.

Electrophysiological Studies—To make a *mKv1.7* expression construct we amplified a 588-bp fragment from mouse brain cDNA spanning the region encoded by the two *Kv1.7* exons using reverse transcriptase PCR (5'-primer, 5'-TCTCCGTAAGTCATCTCGG-3'; 3'-primer, 5'-AAATGGGTGTCCACCCGGTC-3'). Exon 1 (850-bp *Bsp*HI/*Bin*I fragment), a 283-bp *Bin*I/*Bgl*II fragment of our 588-bp PCR product, and exon 2 (747-bp *Bgl*II/*Hind*III), were ligated together at *Bin*I and *Bgl*II sites to generate "fragment I" (1880 bp). Fragment I was blunt-ended at the 5' end and cloned into the pBluescript vector at *Sma*I/*Hind*III sites. To remove the 5'-NCR from fragment I, and for the purpose of cloning this fragment into the pBSTA expression vector, we introduced a unique *Bam*HI site just upstream of the initiator methionine using PCR: 5'-primer, 5'-ACAAAAGCTTCATATGACTACAAG-GAAAGCT-3'; and 3'-primer: 5'-AAGCGCAACCCGGCCACG-3'. The resulting PCR product (corresponding to the first 233 nucleotides of the coding region) was spliced to fragment I at the *Nco*I site, and the 1870-nucleotide fragment was ligated to the pBSTA vector.

mKv1.7 cRNA was transcribed *in vitro* (Ambion Kit, Austin, TX) and

diluted in a 0.1–0.5% fluorescein-dextran (*M_w* 10,000, Molecular Probes, Eugene, OR) in 100 mM KCl. Rat basophilic leukemic (RBL) cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and glutamine, and were plated onto glass coverslips one day prior to use for electrophysiological experiments. RBL cells were injected with cRNA using pre-pulled injection capillaries (Femtotip) in combination with an Eppendorf microinjection system (micromanipulator 5171 and transjector 5242; Madison, WI) as described previously (23). Four to six hours later, fluorescent cRNA-injected cells were evaluated electrophysiologically.

All membrane currents were recorded at room temperature (22–26 °C) with a LIST EPC-7 amplifier (Heka Elektronik, Germany). Series resistance compensation was employed if the current exceeded 2 nA, and the command input was controlled by a PDP 11/73 computer via a digital-to-analog converter. Capacitative and leak currents were subtracted using a P/8 procedure and the holding potential in all experiments was –80 mV. When membrane currents exceeded 2 nA 80% series resistance compensation was used.

RESULTS

The Protein-coding Region of *mKv1.7* Contains an Intron Unlike Its Vertebrate Homologues

A restriction map of a 6.4-kb *Eco*RI DNA fragment containing the entire mouse *Kv1.7* coding region is shown in Fig. 1. The coding region is contained in two exons separated by a 1.9-kb intron. The upstream exon encodes the entire N terminus, S1, and part of the S1-S2 loop. The downstream exon contains the region extending from the S1-S2 loop to the C-terminal end of the protein. The intron-exon splice sites were determined by comparing the genomic sequence with cDNA sequences obtained from the hamster insulinoma cell line, HIT-T1S, and from mouse brain (Fig. 1).

The complete coding sequence of the *mKv1.7* is shown in Fig. 2. The *mKv1.7* sequence is identical in the N terminus from bp 52 to 996 with the mouse EST sequence AA021711. Betsholtz *et al.* (24) amplified a short segment of *Kv1.7* cDNA spanning the S5/S6 region from mouse (MK-6), rat (RK-6), and hamster (HaK-6) insulin-producing cells using PCR. Our sequence is identical to their MK-6 sequence, except for four nucleotide changes.

The deduced *mKv1.7* protein consists of 532 amino acids and contains six putative membrane-spanning domains, S1–S6 (Fig. 2). The hydrophobic core of this protein shares considerable sequence similarity with other *Shaker* family channels, while the intracellular N and C termini and the external loops between S1/S2 and S3/S4 show little conservation. The protein contains conserved sites for post-translational modifications as indicated in Fig. 2. As do all other *Shaker*-related channels, *mKv1.7* has a potential tyrosine kinase phosphorylation site (RPSFDAVLY) in its N-terminal region (2); the proline-rich stretch within the N terminus (see residues 29–42) may be a binding site for SH3 domains of tyrosine kinases. Two protein kinase C consensus sites (Ser/Thr-X-Arg/Lys) are present in the cytoplasmic loop between S4 and S5 of *mKv1.7*; at least one

ACTGTCACCCGGGCAATCCGAAGATGAGGCGAGAGGATGCCACAAGTTCGCGGTACAGCTTAAATGACAGCGCTGAGGAGAGCTGTCTAAATAC -141
AAACAACAATAAATAGCCATGATGCCATACGCCCTGCAATCCAGCATTGGGAGGTGGAGGAGGATCATAAATTCAGGTCAGTTCAGCTACATAAGACCTCGGGTCATGAATCCAAAAGCCAAACAATAAATAG -1
ATG ACT ACA AGG AAA GCT CAA GAG ATC CAC GGA AAA GCG CCG GGT GGC AGT GPT TCC ACA GGT GTG GGA AGC GCA GAG GGC GOC CCT AGC CCC GGG GGA ACA CCG 108
Met Thr Thr Arg Lys Ala Gln Glu Ile His Gly Lys Ala Pro Gly Lys Val Ser Thr Gly Val Gly Thr Ala Glu Thr Ala Glu Val Thr Pro 36
CCC CCT CCC CCG CGC CTT GCG CCG ACT TTC CAT GCT ATT TTT ACC GCG CGA CAC CGG ACA CCC GAC TGG GGT GGC TGC GGC GTC GGG GCC ACA CGT CCG TTC ACC GGT 216
Pro Pro Pro Pro Arg Pro Gly Arg Thr Phe His Ala Ile Phe Thr Arg Arg His Arg Thr Pro Asp Trp Gly Gly Cys Gly Val Gly Ala Thr Arg Pro Phe Thr Gly 72
CGC CCG GGC TGT GCG CGC CAT GGA GCC ACG GTG CCC GGC GGC CTG CGC TGC TGS GAG CGG CTG GTG CTC AAC GTG GCC GGG TTG CGC TTC GAG ACC CGC GCG CGC ACG 324
Arg Pro Gly Cys Ala Thr Arg His Gly Arg Thr Ala Ala Leu Arg Cys Gly Thr Arg Leu Val Leu Asn Val Ala Gly Leu Arg Phe Glu Thr Glu Thr Ala Thr Arg Thr 108
CTC GGC CGC TTC CCG GAC ACG CTG CTG GGG GAC CCG GTG CGC CGC AGC CGC TTC TAC GAC GGC GCG CGC GCC GAG TAT TTC TTC GAC CGA CAC TY-K CGG CCC AGC TTC GAT 432
Leu Gly Arg Phe Pro Asp Thr Leu Leu Gly Asp Pro Val Arg Arg Ser Arg Phe Tyr Asp Gly Ala Arg Ala Glu Tyr Phe Phe Asp Arg His Arg Pro Ser Phe Asp 144
GCG GTG CTC TAC TAC TAC CAG TCG GGC GGC CCG CTG AGA CCG CCG GCG CAC GTG CCC CTC GAC GTC TTC CTG GAG GAG GTG TCC TTC TAC GGG CTG GGG CCG CCG CTG 540
Ala Val Leu Tyr Tyr Tyr Gln Ser Gly Gly Arg Leu Arg Arg Pro Ala His Val Pro Leu Asp Val Phe Leu Glu Val Ser Phe Thr Gly Leu Gly Arg Leu 180
GCG CCG CTG CCG GAG GAC GAG GGC TGC GCG GTC GCC GAG CCG CCG CTG CCC CCG CCC TTT GCG CGT GAC CTC TGG CTG CTC TTC GAA TTT CCT GAG AGC TCG GAG GCT 648
Ala Arg Leu Arg Glu Asp Glu Gly Cys Ala Val Ala Glu Arg Pro Leu Pro Pro Phe Ala Arg Gln Leu Trp Leu Leu Phe Glu Phe Pro Glu Ser Ser Gln Ala 216
GCG CGC GTG CTC GCC GTG GTC TCC GTA CTC GTC ATC CTG GTC TCC ATC GTG GTC TTT TGC CTC GAG ACA CTG CCA GAC TTC CGC GAC GAC CGC GAT GAC CCG GGG CTC 756
Ala Arg Val Leu Ala Val Val Ser Val Leu Val Ile Leu Val Ser Ile Val Val Phe Cys Leu Glu Thr Leu Pro Asp Phe Arg Asp Asp Arg Asp Asp Pro Gly Leu 252
GCG CCG GTA GCG GCT GCT ACT GGC TCG TTC CTC GCT CCG CTC AAT GGC TCC AGT CCC AFG CCA GGA GCC CCT CCC CGA CAG CCC TTC AAC GAT CCA TTC TTT GTG GTG 864
Ala Pro Val Ala Ala Ala Thr Gly Ser Phe Leu Arg Leu Asn Gly Ser Ser Pro Arg Ser Pro Gly Ala Ser Pro Arg Gln Pro Phe Asn Arg Pro Phe Phe Val Val 288
GAG ACC CTG TGT ATC TGC TGG TTC TCC TTT GAG CTG CTG GTG CAT CTG GTG GCC TGC CCT AGC AAA GCT GTG TTC TTC AAG AAT GTG ATG AAC CTA ATT GAC TTC GTG 972
Glu Thr Leu Cys Ile Cys Trp Phe Ser Phe Glu Leu Leu Val His Leu Val Ala Cys Pro Ser Lys Ala Val Phe Phe Lys Asn Val Met Asn Leu Ile Asp Phe Val 324
GCC ATC CTG CCT TAC TTC GTG GCC CTG GGC ACG GAG TTA GCC CCG CAG CCG GGT GTG GGC CAG CCG GCT ATG TCC CTG GCC ATC CTA AGG GTC ATC CGA TTG GTG CGT 1080
Ala Ile Leu Pro Tyr Phe Val Ala Leu Gly Thr Glu Leu Ala Arg Gln Arg Gly Val Gly Gln Pro Ala Met Ser Leu Ala Ile Leu Arg Ile Arg Leu Val Arg 360
GTC TTC CGC ATC TTC AAG CTC TCC AGG CAT TCG AAG GGT CTA CAG ATC TTG GGT GAG ACA CTG CCG GCT TCC ATG CGT GAG CTA GGT CTC CTC ATC TTC CTC TTC 1188
Val Phe Arg Ile Phe Lys Leu Ser Arg His Ser Lys Gly Leu Gln Ile Leu Gly Gln Thr Leu Arg Ala Ser Met Arg Glu Leu Gly Leu Leu Ile Phe Phe Leu Phe 396
ATT GGC GTG GTC CTC TTT TCC AGC GCA GTC TAC TTT GCT GAA GTG GAC CCG GTG GAC ACC CAT TTC ACC AGC P ATC CCG GAG TCC TTT TGG TGG GCA GTG GTC ACC ATG 1299
Ile Gly Val Val Leu Phe Ser Ser Ala Val Tyr Phe Ala Glu Val Asp Arg Val Asp Thr His Phe Thr Ser Ile Pro Glu Ser Phe Trp Trp Ala Val Val Thr Met 432
ACC ACG GTT GGC TAT GGG GAC ATG GCA CCC GTC ACC GTG GGT GGC AAG ATC GTG GGC TCT CTG TGT GCC ATT GCA GGT GTG CTC ACC ATC TCT CTG CCT GTG CCT GTC 1404
Thr Thr Val Gly Tyr Gly Asp Met Ala Pro Val Thr Val Gly Gly Lys Ile Val Gly Ser Leu Cys Ala Ile Ala Gly Val Leu Thr Ile Ser Leu Pro Val Pro Val 468
ATT GTC TCT AAC TTT AGC TAC TTT TAC CAC CCG GAG ACA GAG GGC GAA GAG GCA GGG ATG TAC AGC CAT GTG GAC ACA CAG CCC TGC GGT ACC CTG GAG GGC AAG GCT 1512
Ile Val Ser Asn Phe Ser Tyr Phe Tyr His Arg Glu Thr Glu Gly Glu Ala Gly Met Tyr Ser His Val Asp Thr Gln Pro Cys Gly Tyr Leu Glu Gly Lys Ala 504
AAT GGG GGG CTG GTG GAC TCT GAG GTG CTT GAA CTC CTC CCA CCA CTC TGG CCC CCT GCA GGG AAA CAC ATG GTG ACT GAG GTG GAG GGG TCA ACT GGG GTC TCC AGG 1620
Asn Gly Gly Thr Arg Asp Ser Leu Pro Leu Glu Leu Pro Leu Ala Gly Thr Pro Ala Gly His Met Val Thr Glu Val * 532
GAGCAGTGGGGTGGGAGGGAGGGAAGGCGAGGTGCTGGTAAAGGACTAAGATGGTAAACAATCTTAATCTGAAGGCTGTGACATGTTGGCGGGTACAGATTCTAGGGGGACCTTATGTGACAGGAATTGCCA 1763
GGATTTGGGTTGTGTCAGGGCTCCCGCCATTGATTCGCAATGTGGTAGAGCTGTGCAATTCAGCCGGGGCTCTATGGGTAGCAGTGTAAAGAGACTTGGCCATAGATTGTGAGTTTCTTAGGTTCTCGGGGTCCTCCATGG 1906
GGGCAAGTGTGGTCCCGTAACTGATAGGACTTTGGGCTCTAGGGCAAGTCGGCTCTAGGTTCTTGAGGACTGTTAAAGAGTGTGAGCTTGTGGTACATGCTCACACACAGAGTTATGTGG 2049
TGATACATAGCAGATTGTGTGGATTCATGTTCTTCTAGGACTCAAGTTATTTAGATCTGTGAGCTCTGGAGTCTGAGACAGGATGTGATCACTCTGGTCTCTGATGGAAAAGGGTTAGGGTATAAAGATTAGACAGA 2192
GCACAGACTCAGATAAAGGACAGAGACCCCGCCACACACACACACAATCTCTGTGTTGTGGAGGGTTATAGGACTGGAAATCATTTTAGGGCCATGCAAAAGCAGATGGTGTGACCCCAATGATGTCCTCCGAACAG 2335
TTGAGTCTGATCACTTCTACGGTCTCATGGGACTTGTGTACTGTGAGTCCCTGGAACTTTGGTTGAAATTTGACTGTATGTGAGGGCCATTTAAACAGTAGTACAGGCTCACAGCAGATAAAAATAACTACAGGGAG 2478
CAAGCATGGGCACGTGGATGTGTGGCCCGTTGAACAAGTTTACTCAGGGTTATGCAATGTGAGAGCTGGTGTAGTGTCTGTATCTCTGGGTCCCGCTGCCCGAAGCACCTAGAAAAGCAGATGGAACTAGTAACTGCCTGTCT 2621
TGCTCATGTGGACCCATTTCTATGTTGTCCTATGCTAGTACAAAGATTGAGAGGCTGGTGGAGCCACAAGGAAAGAAAACAGTAGGGCAAGAGTAGTACTTGGGAAAAGATGTGGGAGGGGACAGAGACTTAGA 2764
AGAARAAGATGTGAGAGAAGGAGCAGGAGGAGCAGGGAGGGGGAAGAGGGGAGGGGAGAGACAGACAGCAGGTTAAAGAGGAAGAGATGTGGGGGGGGGGGAGAGACCTGGAGCCCAAGGGTCACTCTCTCT 2907
ATTCTCTGAGTCATAACAGACTCTAAGCTATGGCTCAGCATCCCTTAGAGCCAAAGGAGTCCACCACACTCTCGAGGTGCTCTGTGTTCTTAGGACAGAAAACCAAGGCTGTAGTGTGCTGTGCTTCTCAAGTTACCA 3050
CGGGTCATAGACCAAGCTGGTCAGGTGGCTGCTGCACACCCCAACAATCTCTGGTGTGACAGGATTTGCTGAGGCCACTGGCTGTCTCTTTTATCCCTTAAACAGGGTCCCTCTTTGTAGTGTGGCTGTC 3193
CTGGAACTCACTACGTAGA

FIG. 2. Nucleotide sequence and deduced amino acid sequence of mouse Kv1.7. The six putative membrane-spanning domains (S1 through S6) and pore-forming region (P) are indicated. The potential sites for phosphorylation by tyrosine kinase (TY-K) and protein kinase C (PKC) are shown. The position of the single intron between S1 and S2 is indicated by an arrow, and the critical residue for tetraethylammonium block (Ala-441, within the P region) is shown in bold and underlined.

of these sites is present in all members of the Kv1 family (2). mKv1.7, like Kv1.6, lacks an N-glycosylation site in the extracellular loop linking the S1 and S2 transmembrane segments; this consensus sequence is conserved in all other Kv1 family genes.

Fig. 3 shows a phylogenetic tree of the entire Shaker family of genes based on parsimony analysis of a nucleotide sequence alignment (generated from the amino acid sequence alignment) using the program PAUP (25). Our analysis places mKv1.7 within the Shaker family of genes. The mKv1.7 gene does not cluster with any of the known genes, and its position within the tree is not firmly established.

Kv1.7 Is Located on Mouse Chromosome 7 and Human Chromosome 19q13.3

The mKv1.7/Kcna7 gene resides on mouse chromosome 7 (Fig. 4A), ~0.5 centimorgan telomeric to the Shaw-related K⁺ channel gene, mKv3.3/Kcnc3, and ~2.4 centimorgans centromeric of MyoD1 (myoblast differentiation factor). The most centromeric marker in this study was Gpi1 (glucose phosphate isomerase 1), which mapped ~6.1 centimorgans centromeric to mKv3.3/Kcnc3.

The interval on mouse chromosome 7 containing mKv1.7/Kcna7 and mKv3.3/Kcnc3 shares a region of homology with human chromosomes 19q13 and 11p15, and the human homologues of these K⁺ channel genes may therefore be expected to reside on one of these chromosomes. Confirming this predic-

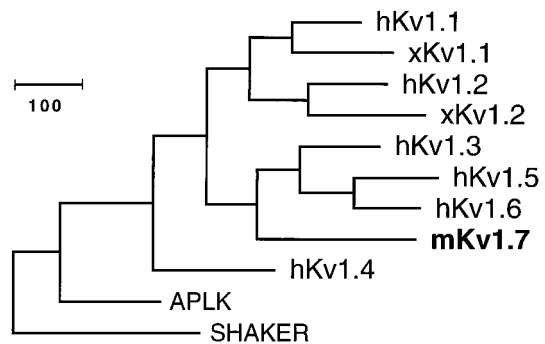


FIG. 3. Proposed phylogenetic relationship of mKv1.7 and other Shaker-related Kv channel genes. This tree is based on parsimony analysis of nucleotide sequence alignments using the program PAUP (25). Horizontal distance represents the number of nucleotide substitutions in each lineage, with the scale bar at the upper left representing 100 substitutions. m, mouse; h, human; x, Xenopus; APLK, Aplysia; SHAKER, Drosophila Shaker.

tion, we mapped both genes to human 19q13.3-13.4 using fluorescent *in situ* hybridization. The idiogram of human chromosome 19 shown in Fig. 4B, and a more detailed map shown in Fig. 4C, indicate that hKv1.7/KCNA7 is located ~1.3 mb centromeric of hKv3.3/KCNC3. The genes for both muscle glycogen synthase (GYS1) and the histidine-rich calcium protein (HRC) also map to this region; Kv1.7/KCNA7 lies ~25 kb telomeric to GYS1 and ~200 kb centromeric to HRC (Fig. 4C).

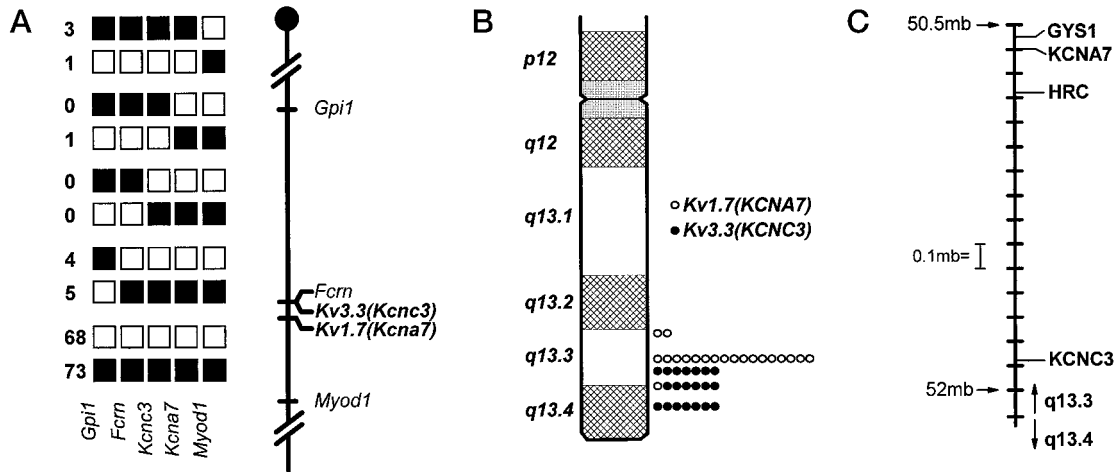


FIG. 4. **Chromosomal localization of the mouse and human *Kv1.7* genes.** *A*, mouse chromosome 7. (*Left*) results of segregation analysis in a (C57BL/6J \times *M. spretus*) F_1 \times C57BL/6J interspecific backcross. The genes indicated are as follows: *Gpi1*, glucose phosphate isomerase-1; *Fcfn*, Fc-receptor, neonatal form; *Myod1*, myoblast differentiation factor-1. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci are *Gpi1* - 11/181 - *Fcfn* - 0/184 - *mKv3.3/Kcnc3* - 1/188 - *mKv1.7/Kcna7* - 4/167 - *Myod1*. The recombination frequencies expressed as genetic distances in centimorgans \pm the S.E. are: *Gpi1* - 6.1 ± 1.8 - [*Fcfn*, *mKv3.3*] - 0.5 ± 0.5 - *mKv1.7* - 2.4 ± 1.2 - *Myod1*. Filled boxes indicate the presence of the C57BL/6J allele, and open boxes the presence of the *M. spretus* allele. (*Right*) diagram showing the deduced order of genes on mouse chromosome 7, with the centromere shown at the top. *B*, human chromosome 19. Diagram shows the deduced order of genes on human chromosome 19. A single *hKv1.7/KCNA7* cosmid was mapped to 19q13.3 by fluorescent *in situ* hybridization (FISH). Ten cells were scored for each cosmid used, and for each of two *hKv3.3/KCNC3* cosmids, signal was present on both chromatids in a position corresponding to 19q13.3-q13.4. The positions where signal was observed for the two probes are indicated as open circles (*hKv1.7/KCNA7*) and solid circles (*hKv3.3/KCNC3*). *C*, detailed map of the relevant region of human chromosome 19. The positions of *KCNA7*, *KCNC3*, *GYS1*, and *HRC* are shown, with each cross-bar indicating a distance of 100 kb. The positions corresponding to 50.5 and 52 mb of chromosome 19 (37) are indicated, as is the point of demarcation between chromosome bands q13.3 and q13.4.

Interestingly, a putative diabetes susceptibility gene has been suggested to be present at 19q13.3 (26, 27), especially in Finnish families with associated hypertension and difficulties in insulin-stimulated glucose storage. This region has also been suggested to contain a modifier locus for cystic fibrosis (28).

Biophysical and Pharmacological Characterization of *Kv1.7* Channels

We carried out a detailed characterization of mKv1.7 channels expressed in RBL cells which express no native Kv currents (29, 30). The *mKv1.7* gene expressed in *Xenopus* oocytes produced currents (data not shown) similar to those obtained in RBL cells (Fig. 5).

Voltage Dependence—Patch clamp studies were performed in the whole-cell mode. Fig. 5A shows a family of outward currents elicited by a 200 ms depolarizing pulse from a holding potential of -80 mV in RBL cells injected with *mKv1.7* cRNA; no outward currents were detected in control cells (data not shown). The currents activate rapidly, and from the conductance-voltage curve shown in Fig. 5B we determined that the $1/2$ activation potential ($V_{1/2}$) is -20 mV.

Inactivation and Deactivation—Inactivation of mKv1.7 channels was rapid following a 200 ms depolarizing pulse from -80 to 40 mV (Fig. 5A). The rate of inactivation, measured by fitting the data to a single exponential function, yielded a time constant (τ_h) of 14 ± 2.1 ms (S.E., $n = 7$). As shown in Fig. 5C, the current became progressively smaller following repeated depolarizing pulses at 1-s intervals. This phenomenon, termed “cumulative inactivation,” is due to the accumulation of channels in the inactivated state which are then unavailable for opening. The related channels, Kv1.3 (7) and Kv1.4 (31), also display this behavior.

The kinetics of channel closing (deactivation) was determined by first opening the channels with a 15 ms conditioning depolarizing pulse, and then forcing the channels to close by repolarizing to different potentials (Fig. 5D). The time constant, τ_{tail} , of the resulting “tail” current was 5.1 and 5.3 ms at -60 mV in two cells.

Single-channel Conductance—We measured single-channel currents in an outside-out patch by applying 450-ms voltage ramps from -90 to 80 mV every second (Fig. 5E). Single channel events were seen at potentials more positive than ~ -15 mV. The single-channel conductance of 21 pS was estimated from the slope of the current recorded during an opening (Fig. 5E).

Pharmacology—We determined the pharmacological sensitivity of the mKv1.7 channel using methods described previously (30, 32), IC_{50} values in each case being determined when block reached steady-state. The channel was blocked by several non-peptide small molecule antagonists, 4-aminopyridine ($IC_{50} = 245 \mu M$), capsaicin ($25 \mu M$), cromakalim ($450 \mu M$), tedisamil ($18 \mu M$), nifedipine ($13 \mu M$), diltiazem ($65 \mu M$), and resiniferatoxin ($20 \mu M$). Surprisingly, the dihydroquinoline compound, CP-339,818, that blocks rapidly inactivating Kv1 channels in the nanomolar range (30), had little effect on mKv1.7 ($IC_{50} = 10 \mu M$). The channel was insensitive to externally applied tetraethylammonium ($C_{50} = 86$ mM), probably because the residue at the tetraethylammonium-binding site, Ala-441 (Fig. 2), is hydrophobic.

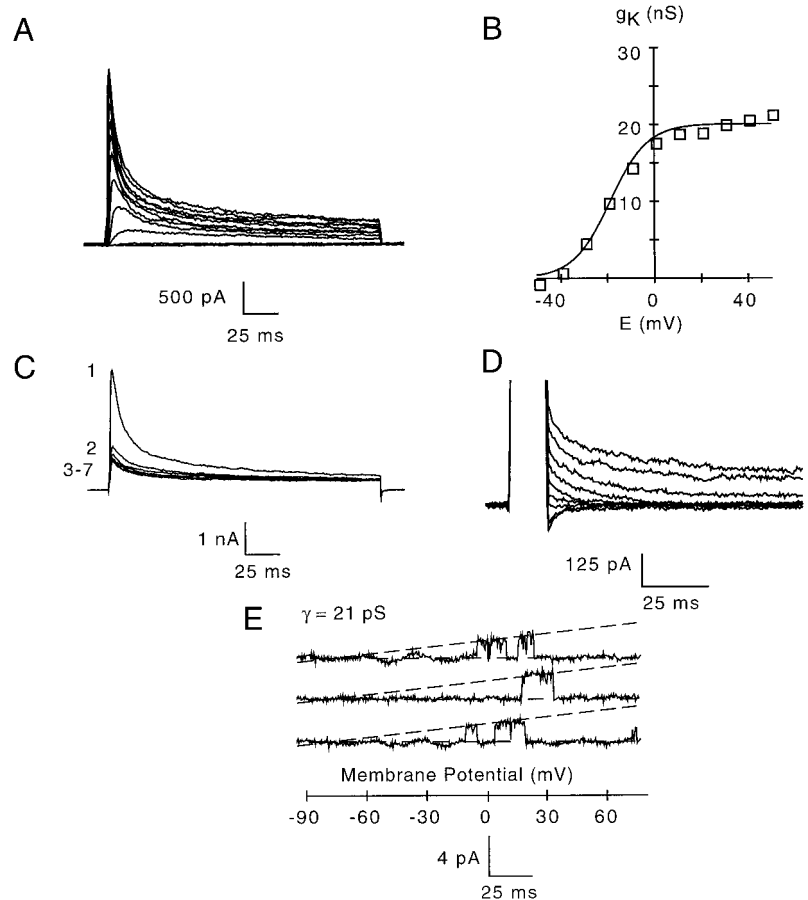
The mKv1.7 channel is also potently blocked by a peptide (ShK toxin) obtained from sea anemone *Stichodactyla helianthus* ($IC_{50} = 13$ nM), and by the scorpion toxins, noxiustoxin ($IC_{50} = 18$ nM) and margatoxin ($IC_{50} = 116$ nM). The channel was resistant to charybdotoxin ($IC_{50} > 1000$ nM) and kaliotoxin ($IC_{50} > 1000$ nM).

Expression of *mKv1.7* Transcripts in Different Tissues

Northern blot assays using a mKv1.7-specific probe revealed strongly hybridizing 3-kb bands in heart and skeletal muscle; faint bands of similar size were visible in liver and lung (together with larger 7–8-kb bands), but none were seen in spleen, kidney, testis, or brain (Fig. 6) We were able to isolate *mKv1.7* transcripts from mouse brain by PCR (see Fig. 1). mKv1.7 is also expressed in placenta, since the mouse EST AA021711 was derived from this tissue.

PCR analysis demonstrated the presence of haKv1.7 mRNAs

FIG. 5. **Kv1.7 currents.** A, family of mKv1.7 currents. The holding potential was -80 mV and depolarizing pulses were applied every 30 s. The test potential was changed from -50 to 50 mV in 10 -mV increments. B, peak K^+ conductance-voltage relation for currents shown in A. The line through the points was fitted with the Boltzmann equation: $g_k(E) = g_{k(max)} / (1 + \exp[(E_n - E)/k])$, with parameter values $g_{k(max)} = 20$ nS and $k = -8$ mV. C, cumulative inactivation of Kv1.7 currents. Currents were elicited by a train of six depolarizing voltage steps (200-ms duration) to 40 mV once every second from a holding potential of -80 mV. The current amplitude decreases significantly during this train of pulses from the largest (first trace) to the smallest (last). D, kinetics of deactivation of Kv1.7 currents. Tail currents were elicited by voltage steps from -100 to -40 mV after a 15-ms depolarizing prepulse to 40 mV. The tail current-decay time constants, τ_t , were measured by fitting single-exponential functions to the decay of the K^+ current during repolarization. E, single-channel currents of Kv1.7 in an outside-out patch. The broken line shows the slope conductance.



in hamster insulinoma cells (Fig. 1). We verified the presence of *mKv1.7* mRNA in pancreatic islet cells obtained from 9–16-week-old diabetic *db/db* mice by *in situ* hybridization (Fig. 7C) using a mKv1.7-specific antisense probe (12–14); *mKv1.7* mRNA was also present in islets from normal *db/+* mice (data not shown). Scattered acinar cells outside the islets also showed mKv1.7 hybridization (Fig. 7C). In contrast, *mKv3.4* mRNA was found in acinar cells surrounding islets, but not in islets, of both *db/db* (Fig. 7B) and *db/+* mice (data not shown). As a control, insulin mRNA was detected in both normal and diabetic islets, but not in acinar cells (Fig. 7A). A *Kv1.5*-specific probe did not show appreciable hybridization to islets (data not shown), despite a report of *Kv1.5* cDNA having been cloned from human insulinoma cells (33).

DISCUSSION

Unlike all other known mammalian *Shaker*-related genes (*Kv1.1–Kv1.6*) that have intronless coding regions (2, 9), the protein-coding region of *mKv1.7* is interrupted by a single 1.9-kb intron. The fly *Shaker* gene also contains an intron in the S1-S2 loop, raising the possibility that the intron in *Kv1.7* may be ancient, predating the divergence of flies and mammals. Both the mouse *Kv1.7* and the fly *Shaker* intron are placed between codons, *i.e.* they are “phase 0” introns. While this is consistent with their having a common origin it may also be fortuitous, since there are only three possible “phases.” Although we favor the idea that Kv introns were lost in the vertebrate lineage before their expansion by gene duplication (in which case the *Kv1.7* intron would represent a more recent insertion), the evolutionary history of this complex gene family remains to be elucidated.

Since *Kv1.7* mRNA is expressed in the mouse heart, we searched the literature for native cardiac A-type Kv currents

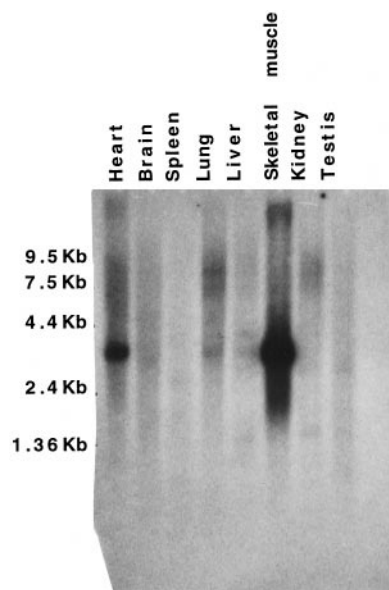


FIG. 6. **Expression of *Kv1.7* mRNA in tissues.** Northern blot assay.

with properties resembling those of Kv1.7. The Kv1.7 homotetramer shares many properties with the rapidly inactivating transient outward (I_{to}) current in cardiac Purkinje fibers, but not the I_{to} current in atrial and ventricular myocytes. Kv1.7 and the Purkinje I_{to} currents activate at negative potentials (~ -30 to -20 mV), inactivate rapidly ($\tau_h < 25$ ms), exhibit cumulative inactivation, are blocked by micromolar concentrations of 4-aminopyridine, and are resistant to >20 mM tetra-

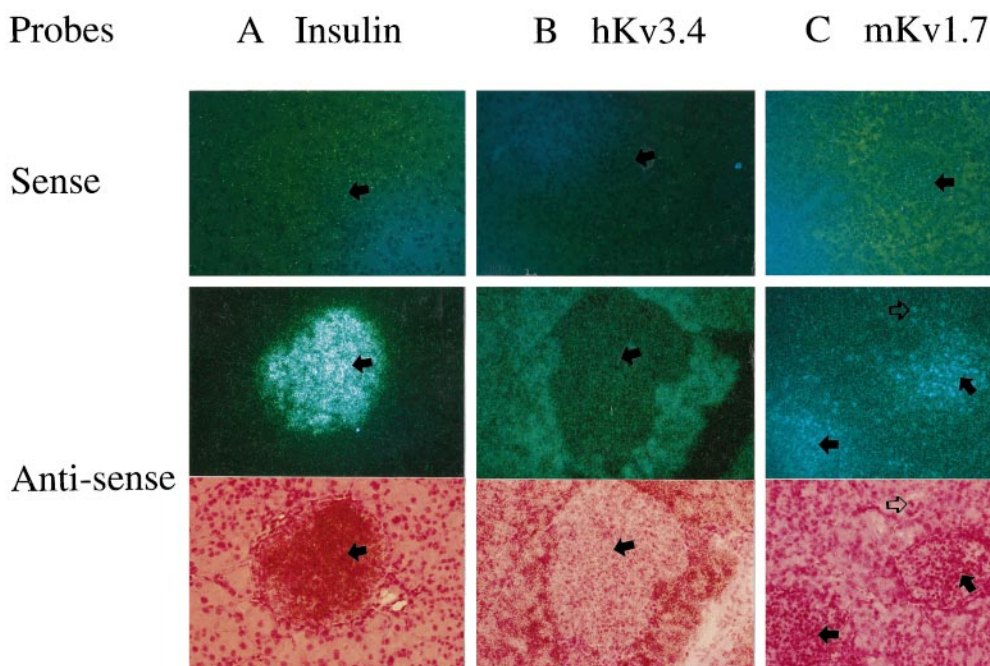


FIG. 7. *In situ* hybridization of mouse pancreas from diabetic *db/db* mice showing expression of *Kv1.7*, *Kv3.4*, and *insulin*. A–C, pancreatic sections from a *db/db* mouse hybridized with probes specific for insulin (A), *Kv3.4* (B), or *Kv1.7* (C). Top, sense probe, dark field; middle, antisense probe, dark field; bottom, antisense probe, bright field, showing the same field as the middle row. Filled arrow, pancreatic islet; open arrow, acinar cells that hybridized with *Kv1.7* antisense probe. A, sense and antisense probes, 0.1 ng/ml, 10 days of exposure; B, sense probe, 0.1 ng/ μ l, 10 days of exposure, and antisense probe, 0.5 ng/ μ l, 7 days of exposure; C, sense and antisense probes, 0.5 ng/ μ l, 1 month of exposure. Magnification: A and B, \times 425; C, \times 312.

ethylammonium (34–36) (this study). In contrast, the I_{to} current in atrial and ventricular muscle, a product of the *Kv4.3* gene, does not exhibit cumulative inactivation (36). These studies suggest that at least part of the Purkinje fiber I_{to} might be encoded by the *Kv1.7* gene, although more extensive biophysical and pharmacological studies are required to confirm the link, and the presence of *Kv1.7* mRNA and/or protein has yet to be demonstrated in these fibers. The abundant expression of *Kv1.7* mRNA in mouse heart suggests that this channel is also likely to be present in ventricular and/or atrial muscle where it may co-assemble with other *Kv1* family channels to form heterotetramers.

Recent studies suggest an important role for *Kv* channels in regulating islet cell function, specifically in repolarizing the membrane potential following each action potential during the glucose-induced bursting phase associated with insulin secretion (3–6). Despite these interesting findings, the genes encoding *Kv* genes in β -cells have not been identified. Although the *Kv1.5* gene was isolated from human insulinoma cells (33), we did not detect *Kv1.5* mRNA in normal or diseased islets. We have, however, demonstrated the presence of *Kv1.7* mRNA in these cells. Unlike the noninactivating *Kv* channels in pancreatic β -cells (3, 4), the *Kv1.7* homotetramer exhibits rapid C-type inactivation. Since *Kv1.7* mRNA is expressed in pancreatic islets, it is possible that heteromultimers composed of *Kv1.7* and other *Kv1* subunits constitute the native *Kv* channels in β -cells. Enhanced levels of such *Kv* channels would excessively hyperpolarize the membrane of β -cells and impair insulin secretion (5). The mapping of the *Kv1.7* gene to human chromosome 19q13.3, a region thought to contain a diabetic susceptibility gene (26, 27), also suggests that *Kv1.7* might contribute to the pathogenesis of type II diabetes mellitus in some humans.

In conclusion, we have described a novel *Kv1* family gene with a genomic organization distinct from all the other members of the family. The *Kv1.7* channel produces a typical A-type

current, and transcripts are expressed in the heart, skeletal muscle, brain, placenta, and pancreatic β -cells. This channel is biophysically and pharmacologically similar to the Purkinje fiber I_{to} current, and the gene may contribute at least one subunit to heteromultimeric *Kv* channels in pancreatic β -cells.

Acknowledgments—The assistance of F. Glaser, S. Plummer, B. Dethlefs, S. Hoffman, M. Christensen, T. Wymore, C. Chandy, and D. J. Gilbert is gratefully acknowledged. We are obliged to Dr. J. Aiyar for reading and improving our manuscript.

REFERENCES

- Hille, B. (1993) *Ionic Channels of Excitable Membranes*, 2nd Ed., Sinauer, Sunderland, MA
- Chandy, K. G., and Gutman, G. A. (1995) in *Handbook of Receptors and Channels: Ligand and Voltage-gated Ion Channels* (North, A., ed) pp. 1–72, CRC Press, Boca Raton, FL
- Smith, P. A., Bokvist, K., Arkhammar, P., Berggren, P. O., and Rorsman, P. (1990) *J. Gen. Physiol.* **95**, 1041–1059
- Smith, P. A., Ashcroft, F. M., and Rorsman, P. (1990) *FEBS Lett.* **261**, 187–190
- Philipson, L. H., Rosenberg, M., Kuznetsov, A., Lancaster, M. E., Worley, J. F., III, Roe, M. W., and Dukes I. D. (1994) *J. Biol. Chem.* **269**, 27787–27790
- Roe, M. W., Worley, J. F., 3rd, Mittal, A. A., Kuznetsov, A., DasGupta, S., Mertz, R. J., Witherspoon, S. M., 3rd, Blair, N., Lancaster, M. E., McIntyre, M. S., Shehee W. R., Dukes, I. D., and Philipson, L. H. (1996) *J. Biol. Chem.* **271**, 32241–32246
- Lewis, R. S., and Cahalan, M. D. (1995) *Annu. Rev. Immunol.* **13**, 623–653
- Pongs, O., Kecskemethy, N., Muller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H. H., Canal, I., Llamazares, S., and Ferrus, A. (1988) *EMBO J.* **7**, 1087–1096
- Schwarz, T. L., Papazian, D. M., Caretto, R. C., Jan, Y. N., and Jan, L. Y. (1988) *Nature* **331**, 137–142
- Chandy, K. G., Williams, C. B., Spencer, R. H., Aguilar, B. A., Ghanshani, S., Tempel, B. L., and Gutman, G. A. (1990) *Science* **247**, 973–975
- Ghanshani, S., Pak, M., McPherson, J. D., Strong, M., Dethlefs, B., Wasmuth, J. J., Salkoff, L., Gutman, G. A., and Chandy, K. G. (1992) *Genomics* **12**, 190–196
- deJong, P. J., Yokabata, K., Chen, C., Lohman, F., Pederson, L., McNinch, J., and van Dilla, M. (1990) *Cytogenet. Cell Genet.* **51**, 985
- Permutt, M. A., Koranyi, L., Keller, K., Lacy, P. E., Scharp, D. W., and Mueckler, M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 8688–8692
- Chen, H., Charlat, O., Tartaglia, L. A., Wolf, E. A., Weng, X., Ellis, S. J., Lakey, N. D., Culpepper, J., Moore, K. J., Breitbart, R. E., Duyk, G. M., Tepper, R., and Morgenstern, J. P. (1996) *Cell* **84**, 491–495
- Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I., and Friedman, J. M. (1996) *Nature* **379**, 632–635
- Shafir, E. (1992) *Diabetes Metab. Rev.* **8**, 179–208

17. Green, E. L. (1981) in *Genetics and Probability in Animal Breeding Experiments*, pp 77–113, Oxford University Press, New York
18. Jenkins, N. A., Copeland, N. G., Taylor, B. A., and Lee, B. K. (1982) *J. Virol.* **43**, 26–36
19. Saunders, A. M., and Seldin, M. F. (1990) *Genomics* **8**, 525–535
20. Copeland, N. G., and Jenkins, N. A. (1991) *Trends Genet.* **7**, 113–118
21. Brandriff, B. F., Gordon, L. A., Tynan, K. T., Olsen, A. S., Mohrenweiser, H. W., Fertitta, A., Carrano, A. V., and Trask, B. J. (1992) *Genomics* **12**, 773–779
22. Trask, B., Fertitta, A., Christensen, M., Youngblom, J., Bergmann, A., Copeland, A., de Jong, P., Mohrenweiser, H., Olsen, A., Carrano, A., and Tynan, K. (1993) *Genomics* **15**, 133–145
23. Ikeda, S. R., Soler, F., Zuhlke, R. D., Lewis, D. L. (1992) *Pflueg. Arch. Eur. J. Physiol.* **422**, 201–203
24. Betsholtz, C., Baumann, A., Kenna, S., Ashcroft, F. M., Ashcroft, S. J., Berggren, P. O., Grupe, A., Pongs, O., Rorsman, P., Sandblom, J., and Welsh, M. (1990) *FEBS Lett.* **263**, 121–126
25. Swofford, D. L. (1993) PAUP: Phylogenetic analysis using parsimony. Version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, IL
26. Groop, L. C., Kankuri, M., Schalin-Jantti, C., Ekstrand, A., Nikula-Ijas, P., Widen, E., Kuismanen, E., Eriksson, J., Franssila-Kallunki, A., Saloranta, C., and Koskimies, S. (1993) *N. Engl. J. Med.* **328**, 10–14
27. Elbein, S. C., Hoffman, M., Ridinger, D., Otterud, B., and Leppert, M. (1994) *Diabetes* **43**, 1061–1065
28. Estivill, X. (1996) *Nat. Genet.* **12**, 348–350
29. McCloskey, M., and Cahalan, M. D. (1990) *J. Gen. Physiol.* **95**, 208–222
30. Nguyen, Q.A., Kath, J., Hanson, D. C., Biggers, M. S., Canniff, P. C., Donovan, C. B., Mather, R. J., Bruns, M. J., Rauer, H., Aiyar, J., Lepple-Wienhues, A., Gutman, G. A., Grissmer, S., Cahalan, M. D., and Chandy, K. G. *Mol. Pharmacol.* **50**, 1672–1679, 1996
31. Wymore, R., Korenberg, J. R., Coyne, C. Chen, X-N, Hustad, C., Copeland, N. G., Gutman, G. A., Jenkins, N. A., Chandy, K. G. (1994) *Genomics* **20**, 191–202
32. Grissmer, S., Nguyen, A. N., Aiyar, J., Hanson, D. C., Mather, R. J., Gutman, G. A., Karmilowicz, M. J., Auperin, D. D., and Chandy, K. G. (1994) *Mol. Pharmacol.* **45**, 1227–1234
33. Philipson, L. H., Hice, R. E., Schaefer, K., LaMendola, J., Bell, G. I., Nelson, D. J., and Steiner, D. F. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 53–57
34. Reder, R. F., Miura, D. S., Danilo, P., Jr., and Rosen, M. R. (1981) *Circ. Res.* **48**, 658–668
35. Gintant, G. A., Cohen, I. S., Datyner, N. B., and Kline, R. P. (1992) in *The Heart and Cardiovascular System* (Fozzard, H., ed) 2nd Ed., pp. 1122–1166, Raven Press, New York
36. Dixon, J. E., Shi, W., Wang, H. S., McDonald, C., Yu, H., Wymore, R. S., Cohen, I. S., and McKinnon, D. (1996) *Circ. Res.* **79**, 659–668
37. Ashworth, L. K., Batzer, M. A., Brandriff, B., Branscomb, E., de Jong, P., Garcia, E., Garnes, J. A., Gordon, L. A., Lamerdin, J. E., Lennon G., Mohrenweiser, H., Olsen, A. S., Slezak, T., and Carrano, A. V. (1995) *Nat. Genet.* **11**, 422–427