been reported, it will be interesting to see if this pattern of modulation of rapid gating kinetics holds true for other neurotransmitters and other cells[24,26]. Changes in the kinetics of opening and closing might be consistent with allowed voltage-dependence of gating reported by Bean[19].

The pharmacological properties of NA inhibition of whole-cell N-type channel current and transmitter release are very similar. This points to a functional relationship between these phenomena and reinforces earlier evidence that dihydropyridine-insensitive N-type $Ca^{2+}$ channels are the main $Ca^{2+}$ entry mechanism controlling transmitter release from sympathetic neurons[6]. Interestingly, the triggering of transmitter release may be dominated by L-type channels in certain other neuronal systems[30-32]. Stimulation of α-adrenergic receptors is coupled to inhibition of N-type $Ca^{2+}$-channels and reduction of transmitter release by means of a G protein but not by a readily diffusible second messenger such as cAMP (as in current hypotheses[2-4]), nor by protein kinase C (as in sensory neurons[33]). A relatively direct coupling mechanism would be appropriate for rapid feedback control of transmitter release. The feedback may also be localized, because (1) the concentration of NA falls off steeply with increasing distance from the release sites, (2) the α-adrenergic modulation of N-type $Ca^{2+}$ channels works only at short range, and (3) the attenuation of $Ca^{2+}$ entry may strongly affect only nearby release sites. Our results do not exclude additional effects of NA on potassium channels[34,35], possibly mediated by clonidine-sensitive $\alpha_2$-receptors[34] and lowered cAMP[35], that would result in less localized decreases in transmitter release through reduction of action-potential duration and global $Ca^{2+}$ entry. □

Received 4 May; accepted 14 July 1989.


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ACKNOWLEDGEMENTS. We thank David Friel, Keith Bley, Rick Aldrich and Dan Madison for suggestions, Dr Friel for the use of single channel analysis programs and Dr Aldrich for use of computers. This work was supported by the NIH. D.L. is a postdoctoral fellow of the American Heart Association, California Affiliate.

# A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning

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**VOLTAGE-activated potassium channels play an important part in the control of excitability in nerve and muscle. Different $K^+$ channels are involved in establishing the resting potential, determining the duration of action potentials, modulation of transmitter release, and in rhythmic firing patterns and delayed excitation[1]. Using *in vitro* transcripts made from a directional complementary DNA library we have isolated, by expression cloning in *Xenopus* oocytes, a novel $K^+$-channel gene (*drk1*). Functionally, *drk1* encodes channels that are $K^+$ selective and belong to the delayed rectifier class of channels, rather than the A-type class encoded by the *Shaker* gene of *Drosophila*. The channels show sigmoidal voltage-dependent activation and do not inactivate within 500 ms. Structurally, *drk1* encodes an amino-acid sequence which is more closely related to the *Drosophila Shab* gene than to the *Shaker* gene.**

Several cDNA clones encoding $K^+$ channels have been isolated from *Drosophila*[2-8]. Microinjection of *Xenopus* oocytes, with RNA transcribed *in vitro* from some of the clones produces voltage-dependent, fast transient outward $K^+$ currents, characteristic of A-type channels[9-11]. The different cDNA clones that have been isolated contain an identical core region, yet differ in the regions encoding the amino and carboxyl termini of the proteins. Using *Shaker* sequence information, two mammalian $K^+$-channel cDNA clones encoding the same gene were recently isolated by cross-hybridization[12,13]. Expression of rat cDNAs in *Xenopus* oocytes yielded $K^+$ currents with properties of delayed rectifier channels[14] and possibly A-type channels[15]. We designed a sequence-independent approach to isolate cDNA clones encoding channel and receptor genes expressed in the brain. Size-fractionated rat-brain messenger RNA, enriched for mRNA between 3.3 and 4.2 kilobases (kb), was used to generate a directional cDNA library in the transcription-competent vector λZAP[16]. Pools of 100,000 recombinant phages (independent cloning events) were amplified and used to prepare DNA templates for RNA synthesis. Following microinjection of *in vitro* synthesized RNA, *Xenopus* oocytes were tested for outward currents ($I_K$) produced by depolarizing voltage steps. Transcripts from pools of 100,000 recombinants yielded small $I_K$-like currents. One pool was divided into smaller 'cocktails', each containing 10,000 recombinants. Three of seven such cocktails elicited sustained outward currents of several hundred nA in amplitude upon depolarization of the oocyte membrane. The currents activated relatively slowly (>100 ms for full activation) and did not inactivate during the test pulse (500 ms). One cocktail was chosen and, by reducing the pool size to 1,000, then 100 and finally 12 recombinants, we eventually isolated a single 'positive' clone (*drk1*) with a 3.4-kb insert.

The cDNA clone we isolated encoded a $K^+$ channel with the properties of a delayed rectifier[1]. The channels opened at test potentials more positive than −20 mV and showed sigmoidal voltage-dependent activation (Fig. 1*a*, *b*). The time to half-maximal activation ranged from 20 to 100 ms. Injection of as little as 20 pg *in vitro*-synthesized transcripts produced $I_K$-current amplitudes of up to 1 μA at +40 mV. At higher current



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densities, resulting from injection of 2 ng RNA, it was possible to record from macropatches containing several hundred channels, where the changes in potential are complete in much less than 1 ms (manuscript in preparation). Voltage dependence and kinetics were similar for both macropatch and whole-cell recording. To characterize the ionic selectivity of these channels we determined the reversal potential from tail-current measurements (Fig. 1*c*). Substituting external $Na^+$ with *N*-methyl-D-glucamine$^+$ (NMDG) had a negligible effect, whereas varying the external $K^+$ concentration ($[K^+]$) shifted the reversal potential with a slope of 48 mV per 10-fold charge in external $[K^+]$ (Fig. 1*d*), which is characteristic of channels selective for $K^+$ ions over $Na^+$ ions. These $K^+$ currents were blocked by 4-aminopyridine with a 50% inhibitory concentration ($IC_{50}$) of 0.5 mM and by tetraethylammonium with an $IC_{50}$ of 10 mM (data not shown). The outward current at +20 mV was independent of the presence of $Ca^{2+}$ in the bath and insensitive to $Co^{2+}$ (2 mM) or $Cd^{2+}$ (0.2 mM), eliminating the possibility of the presence of $Ca^{2+}$-activated $K^+$ channels. Neither apamin nor charybdotoxin at concentrations up to 1 μM had any effect on the channels.

The DNA sequence of *drk1* was determined and shows an open reading frame of 2,559 nucleotides (Fig. 2*a*) encoding a protein of 853 amino acids (with a calculated relative molecular mass of 95,294). The 5′-untranslated region contains 13 nucleotides (the first 8 nucleotides shown in Fig. 2*a* are linker-derived) and no upstream ATG preceding the assigned initiation codon (that is, the first ATG found). The TGA stop codon is followed by a 3′-untranslated region of ~800 nucleotides ending in a poly(A) tail of ~50 residues. The beginning of the derived protein sequence contains hydrophilic and charged amino acids and presumably does not represent a signal peptide. A hydropathy plot of the deduced amino-acid sequence shows features reminiscent of all voltage-gated ion channels cloned so far (Fig. 2*b*). In the N-terminal half of the molecule, there are six hydrophobic regions that have been attributed to membrane spanning segments (boxed regions Fig. 2*b*). The other half of the protein encoded by *drk1* does not show main hydrophobic regions and probably represents a cytoplasmic tail of >400 amino-acid residues. It is longer than the corresponding C-termini of all other $K^+$ channels known and it does not show similarity to any known sequence in the protein-sequence data bank. This part of the *drk1* product contains two consensus sequences for cyclic AMP-dependent phosphorylation[17]. The tail may be important in modulation of channel activity and/or targeting of the channel to specific subcellular compartments.

The N-terminal half of the amino-acid sequence derived from the *drk1* clone is similar to the core regions of $K^+$ channels of

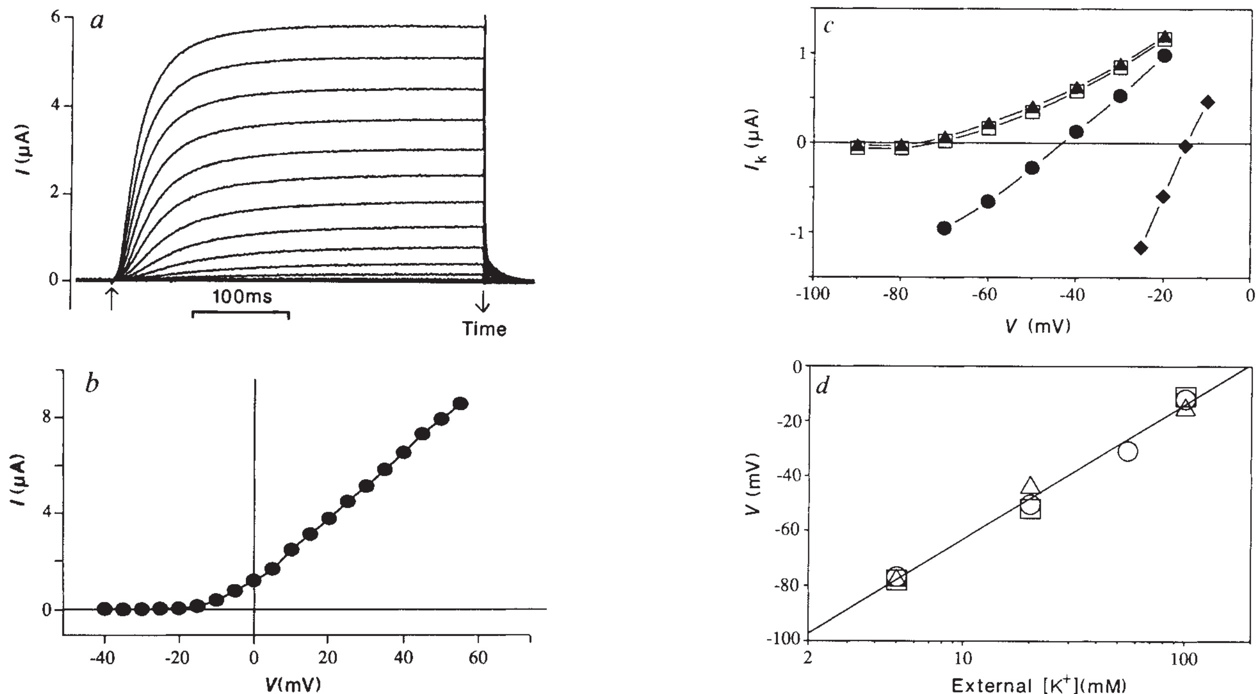


FIG. 1 Delayed rectifying kinetics and $K^+$ selectivity of *drk1* mRNA expressed in *Xenopus* oocytes. *a*, Family of $K^+$ currents elicited by 400 ms voltage steps from a holding potential of −50 mV to test potentials ranging from −40 mV to +30 mV in 5-mV increments. The beginning and the end of the pulse are indicated by arrows. Leakage and capacity currents are corrected for by adding the response to a voltage step of equal size but opposite sign. *b*, Steady-state current–voltage relationship for the experiment shown in *a*. The current at the end of the 400-ms test pulse is plotted against membrane potential. Threshold for activation was −15 mV. *c*, The effect of varying external $K^+$ and $Na^+$ concentrations on the instantaneous current–voltage relationship. The $K^+$ conductance was activated by a 200-ms voltage step to 0 mV and repolarized to potentials between −10 mV and −100 mV in 10-mV increments. The instantaneous current following the repolarization is estimated by fitting a double-exponential function to the tail-currents which is extrapolated back to the time of the step. The resulting instantaneous currents are corrected for a linear leakage component estimated from the steady-state currents between −50 and −100 mV and plotted against voltage. The external solutions contained $MgCl_2$ (2 mM), $CoCl_2$ (2 mM), HEPES (10 mM, pH 7.4) to which was added (mM): NaCl (100), KCl (5) (□), NMDG (100), KCl (5) (▲), NMDG (85), KCl (20) (●), NMDG (5), KCl (100) (◆). NMDG was used to replace $Na^+$. *d*, Reversal potential as a function of external $K^+$ concentration. Reversal potentials were measured from instantaneous current–voltage relationships as shown in *c*. Results from three oocytes are combined. A linear least-squares fit is shown with a slope of 48 mV per 10-fold change in external $[K^+]$.

METHODS. A directional rat-brain cDNA library enriched for full-length inserts between 3.3–4.2 kb[16] was used for *in vitro* transcription of sense-strand RNA using T7 RNA polymerase and the 5′-cap analogue $m^7G(5')ppp(5')G$ (ref. 19). After microinjection of RNA (5 ng), *Xenopus* oocytes were screened 3–4 days later for the expression of delayed rectifying $K^+$ channels using a two-electrode voltage-clamp technique (ref. 20, and R.H.J. *et al.*, manuscript in preparation). A 'positive' pool of 100,000 recombinants was subdivided into pools of progressively smaller sizes and eventually led to the isolation of the clone *drk1*. Oocytes were isolated from *Xenopus* frogs as described elsewhere (ref. 20, and R.H.J. *et al.*, manuscript in preparation) and kept in modified Barth's solution at 19 °C. For screening they were transferred to a recording chamber and kept in a solution containing (mM): NaCl (140), KCl (2), $CaCl_2$ (2), $MgCl_2$ (2), HEPES (10, pH 7.4). Electrodes were filled with KCl (2 M), HEPES (10 mM, pH 7.2). Their resistance in the bath ranged from 1 to 3 MΩ. Oocytes were voltage-clamped using an Axoclamp 2A (Axon Instruments).



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*a*

```
   1                                      MetThrLysHisGlySerArgSerThrSerSerLeuPro
 -21  AATTCCGGTCGCTGGTCTGGCATGACGAAGCATGGCTCGCGCTCCACCAGCTCGCTGCCG

  14  ProGluProMetGluIleValArgSerLysAlaCysSerArgArgValArgLeuAsnVal
  40  CCCGAGCCCATGGAGATCGTGCGCAGCAAGGCGTGCTCGCGCCGGGTGCGCCTCAACGTC

  34  GlyGlyLeuAlaHisGluValLeuTrpArgThrLeuAspArgLeuProArgThrArgLeu
 100  GGGGGCCTGGCGCACGAGGTGCTGTGGCGCACTCTGGACCGCCTGCCCCGCACGCGGCTG

  54  GlyLysLeuArgAspCysAsnThrHisAspSerLeuLeuGlnValCysAspAspTyrSer
 160  GGCAAGCTTCGGGACTGCAACACGCACGACTCCCTGCTCCAGGTGTGCGACGACTACAGC

  74  LeuGluAspAsnGluTyrPhePheAspArgHisProGlyAlaPheThrSerIleLeuAsn
 220  CTTGAGGACAACGAGTACTTCTTCGACCGCCACCCTGGCGCCTTTACCTCCATTCTCAAT

  94  PheTyrArgThrGlyArgLeuHisMetMetGluGluMetCysAlaLeuSerPheSerGln
 280  TTCTACCGCACCGGCCGGCTGCACATGATGGAGGAGATGTGCGCGCTGAGCTTCAGCCAG

 114  GluLeuAspTyrTrpGlyIleAspGluIleTyrLeuGluSerCysCysGlnAlaArgTyr
 340  GAGCTGGACTACTGGGGCATCGATGAGATCTACCTGGAGTCCTGCTGCCAGGCCCGCTAT

 134  HisGlnLysLysGluGlnMetAsnGluGluLeuLysArgGluAlaGluThrLeuArgGlu
 400  CACCAAAAGAAGGAGCAGATGAACGAGGAGCTGAAGCGCGAGGCCGAGACGCTGCGGGAG

 154  ArgGluGlyGluGluPheAspAsnThrCysCysAlaGluLysArgLysLysLeuTrpAsp
 460  CGGGAGGGCGAGGAGTTTGACAACACGTGCTGTGCAGAGAAGAGGAAGAAACTCTGGGAT
                                                         S1
                         ***
 174  LeuLeuGluLysProAsnSerSerValAlaAlaLysIleLeuAlaIleIleSerIleMet
 520  CTGCTGGAGAAGCCCAACTCGTCGGTGGCCGCCAAGATCCTGGCCATCATCTCGATCATG

 194  PheIleValLeuSerThrIleAlaLeuSerLeuAsnThrLeuProGluLeuGlnSerLeu
 580  TTCATCGTCCTGTCCACCATCGCTCTGTCACTCAACACGCTGCCTGAGCTGCAGAGCCTA

 214  AspGluPheGlyGlnSerThrAspAsnProGlnLeuAlaHisValGluAlaValCysIle
 640  GACGAGTTCGGCCAGAGCACGGATAACCCGCAGCTGGCGCACGTGGAGGCGGTGTGCATC
           S2
 234  AlaTrpPheThrMetGluTyrLeuLeuArgPheLeuSerSerProLysLysTrpLysPhe
 700  GCATGGTTCACCATGGAGTACTTGCTGAGGTTCCTGTCCTCCCCCAAGAAATGGAAGTTC
                                                   S3
 254  PheLysGlyProLeuAsnAlaIleAspLeuLeuAlaIleLeuProTyrTyrValThrIle
 760  TTCAAGGGCCCCCTCAACGCCATTGACCTACTGGCCATCCTGCCCTACTACGTCACCATC
                     ***
 274  PheLeuThrGluSerAsnLysSerValLeuGlnPheGlnAsnValArgArgValValGln
 820  TTCCTCACAGAATCCAACAAGAGCGTGCTGCAGTTCCAGAACGTGCGCCGGGTGGTCCAG
                                 S4
 294  IlePheArgIleMetArgIleLeuArgIleLeuLysLeuAlaArgHisSerThrGlyLeu
 880  ATCTTTCGCATCATGCGCATCCTTCGGATCCTGAAGCTGGCCCGTCACTCCACTGGCCTG

 314  GlnSerLeuGlyPheThrLeuArgArgSerTyrAsnGluLeuGlyLeuLeuIleLeuPhe
 940  CAGTCCTTGGGCTTCACCCTGCGCAGAAGCTACAACGAGCTGGGCCTGCTCATCCTCTTC
                 S5
 334  LeuAlaMetGlyIleMetIlePheSerSerLeuValPhePheAlaGluLysAspGluAsp
1000  CTCGCGATGGGCATCATGATCTTCTCCAGCCTGGTCTTCTTCGCGGAGAAGGATGAGGAC

 354  AspThrLysPheLysSerIleProAlaSerPheTrpTrpAlaThrIleThrMetThrThr
1060  GACACCAAGTTCAAAAGCATCCCCGCCTCTTTCTGGTGGGCTACCATCACCATGACAACT

 374  ValGlyTyrGlyAspIleTyrProLysThrLeuLeuGlyLysIleValGlyGlyLeuCys
1120  GTTGGCTATGGAGACATCTACCCGAAGACTCTCCTGGGGAAAATCGTGGGGGGCTCTGT
                              S6
                                                           ***
 394  CysIleAlaGlyValLeuValIleAlaLeuProIleProIleIleValAsnAsnPheSer
1180  TGCATTGCCGGGGTCCTGGTGATTGCTCTCCCCATCCCCATCATCGTCAATAACTTCTCC

 414  GluPheTyrLysGluGlnLysArgGlnGluLysAlaIleLysArgArgGluAlaLeuGlu
1240  GAGTTCTACAAGGAGCAGAAGCGCCAGGAGAAAGCCATCAAGCGGAGGGAGGCTCTGGAG

                     ***   ●
 434  ArgAlaLysArgAsnGlySerIleValSerMetAsnMetLysAspAlaPheAlaArgSer
1300  AGAGCCAAGAGGAATGGCAGCATCGTGTCCATGAACATGAAGGACGCCTTCGCCCGGAGC

 454  IleGluMetMetAspIleValValGluLysAsnGlyGluSerIleAlaLysLysAspLys
1360  ATCGAGATGATGGACATCGTGGTGGAGAAAAATGGCGAGAGCATAGCTAAGAAGGATAAA
                                                              ●
 474  ValGlnAspAsnHisLeuSerProAsnLysTrpLysTrpThrLysArgAlaLeuSerGlu
1420  GTGCAAGATAACCACCTGTCCCCCAACAAGTGGAAATGGACCAAGAGGGCACTCTCCGAG

 494  ThrSerSerSerLysSerPheGluThrLysGluGlnGlySerProGluLysAlaArgSer
1480  ACCAGCTCCAGTAAGTCCTTTGAAACCAAGGAGCAGGGATCCCCCGAAAAGGCCAGGTCC

 514  SerSerSerProGlnHisLeuAsnValGlnGlnLeuGluAspMetTyrSerLysMetAla
1540  TCGTCTAGTCCACAGCACTTGAACGTCCAGCAGCTGGAAGACATGTACAGTAAGATGGCC

 534  LysThrGlnSerGlnProIleLeuAsnThrLysGluMetAlaProGlnSerLysProPro
1600  AAGACACAGTCCCAGCCCATCCTCAACACCAAGGAGATGGCCCCGCAGAGCAAGCCTCCA

 554  GluGluLeuGluMetSerSerMetProSerProValAlaProLeuProAlaArgThrGlu
1660  GAGGAGCTGGAGATGAGCAGCATGCCCAGCCCCGTGGCCCCTCTGCCCGCACGCACGGAG

 574  GlyValIleAspMetArgSerMetSerSerIleAspSerPheIleSerCysAlaThrAsp
1720  GGCGTCATCGACATGCGGAGCATGTCCAGCATTGACAGCTTCATCAGCTGTGCCACGGAC

 594  PheProGluAlaThrArgPheSerHisSerProLeuAlaSerLeuSerSerLysAlaGly
1780  TTCCCTGAAGCCACCAGATTCTCCCACAGTCCTCTGGCATCCCTCTCCAGCAAGGCTGGG

 614  SerSerThrAlaProGluValGlyTrpArgGlyAlaLeuGlyAlaSerGlyGlyArgLeu
1840  AGCAGCACAGCCCCGGAGGTGGGCTGGCGGGGGGCTCTGGGTGCCAGCGGCGGGAGGCTC

 634  ThrGluThrAsnProIleProGluThrSerArgSerGlyPhePheValGluSerProArg
1900  ACGGAGACCAACCCCATTCCTGAGACCAGCCGCTCTGGTTTCTTCGTGGAGAGTCCCAGG

 654  SerSerMetLysThrAsnAsnProLeuLysLeuArgAlaLeuLysValAsnPheValGlu
1960  AGTTCCATGAAGACCAACAACCCCTTGAAGCTGCGAGCGCTCAAGGTCAACTTCGTGGAG

 674  GlyAspProThrProLeuLeuProSerLeuGlyLeuTyrHisAspProLeuArgAsnArg
2020  GGCGACCCCACCCCGCTGCTGCCCTCCCTGGGCTTGTATCACGATCCTCTTAGGAACAGA

 694  GlyGlyAlaAlaAlaAlaValAlaGlyLeuGluCysAlaSerLeuLeuAspLysProVal
2080  GGAGGGGCAGCGGCTGCAGTTGCGGGACTGGAGTGTGCCTCCCTCTTAGACAAGCCCGTG

 714  LeuSerProGluSerSerIleTyrThrThrAlaSerAlaArgThrProProArgSerPro
2140  CTGAGCCCAGAGTCCTCCATCTACACCACAGCAAGTGCCAGGACGCCCCCTCGCTCCCCT

 734  GluLysHisThrAlaIleAlaPheAsnPheGluAlaGlyValHisHisTyrIleAspThr
2200  GAGAAACACACAGCAATAGCATTCAACTTCGAGGCGGGAGTCCACCATTACATAGACACC

 754  AspThrAspAspGluGlyGlnLeuLeuTyrSerValAspSerSerProProLysSerLeu
2260  GACACAGATGACGAGGGTCAGCTGCTCTACAGCGTGGACTCCAGCCCTCCCAAAAGTCTC

 774  HisGlySerThrSerProLysPheSerThrGlyAlaArgThrGluLysAsnHisPheGlu
2320  CACGGGAGTACCAGTCCCAAGTTCAGCACTGGAGCTAGAACGGAGAAGAACCACTTCGAG

 794  SerSerProLeuProThrSerProLysPheLeuArgProAsnCysValTyrSerSerGlu
2380  AGCTCCCCCTTGCCCACCTCCCCTAAGTTCTTAAGGCCGAACTGTGTCTACTCCTCAGAA
                                                          ***
 814  GlyLeuThrGlyLysGlyProGlyAlaGlnGluLysCysLysLeuGluAsnHisThrPro
2440  GGGTTGACTGGAAAAGGCCCTGGGGCTCAAGAGAAATGCAAGCTGGAGAACCATACCCCC

 834  ProAspValHisMetLeuProGlyGlyGlyAlaHisGlySerThrArgAspGlnSerIle
2500  CCGGATGTGCACATGCTGCCTGGGGGAGGAGCACACGGGAGCACTAGGGATCAGAGTATC

2560  TGAGCTCCCTACCACAGAGGGCATCTGCA-----(approx 800 nucleotides)-----3'end
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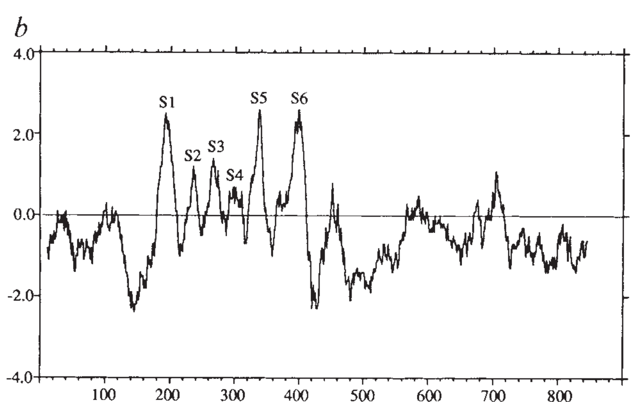


FIG. 2 *a*, Nucleotide- and deduced amino-acid sequence of *drkl*. The numbers indicate amino-acid and nucleotide positions starting at the designated initiation codon. To obtain the longest possible reading frame, the first ATG triplet was chosen as the initiation codon. The sequence flanking the second in-frame ATG (position 49–51), however, is in better agreement with the consensus sequence for translation initiation[21]. Amino acids 77–83 (heavy line on top) are conserved in all $K^+$ channels cloned so far. The boxes outline potential transmembrane segements S1–S6. Consensus sequences for *N*-glycosylation[22] are shown by asterisks. Only one site (position 279) faces the extracellular side in the assumed topology of the channel. Two consensus sequences for cAMP-dependent phosphorylation (●) are found in the cytoplasmic tail[17]. *b*, Hydropathy plot of the amino-acid sequence of *drk1*. The deduced amino-acid sequence of *drk1* was subjected to a hydropathy analysis according to Kyte and Doolittle, with a window size of 22 amino acids[25]. Segments S1–S6 correspond to the boxed regions in Fig. 2. Amino-acid positions are shown on the abscissa. The relative hydrophopicity index is shown on the ordinate.

METHODS. The DNA sequence of *drk1* was determined for both strands of the insert. Several restriction fragments were subcloned into M13mp19 vectors using standard recombinant DNA technology and the sequences were determined using an ABI automated DNA sequenator[23,24].

*Drosophila* (those encoded by *Shaker*)[6–8], rat and mouse[12–15], and to the core regions of two different putative $K^+$ channels encoded by the genes *Shab* and *Shaw*[18]. Figure 3 shows an alignment of the *drk1* sequence (corresponding to residues 18–430) with sequences of the *Drosophila* genes *Shaker*, *Shab*, and *Shaw* and with one of rat brain (*rck1*). In this core region, the percentage of identical amino acids in analogous positions between channels is given in Table 1. The six putative membrane-spanning segments (S1–S6) and a stretch of seven amino acids (Asn-Glu-Tyr-Phe-Phe-Asp-Arg) preceding S1 show the highest conservation within the core region of the five channels analysed. The segment S4 is probably the voltage-sensor and is characterized by a series of positively charged amino acids at every third position. The S4s of the *Shab* and *drk1* products have five positive charges; those of the *rck1* and *Shaker* products contain two additional positive flanking charges. Only four such charges



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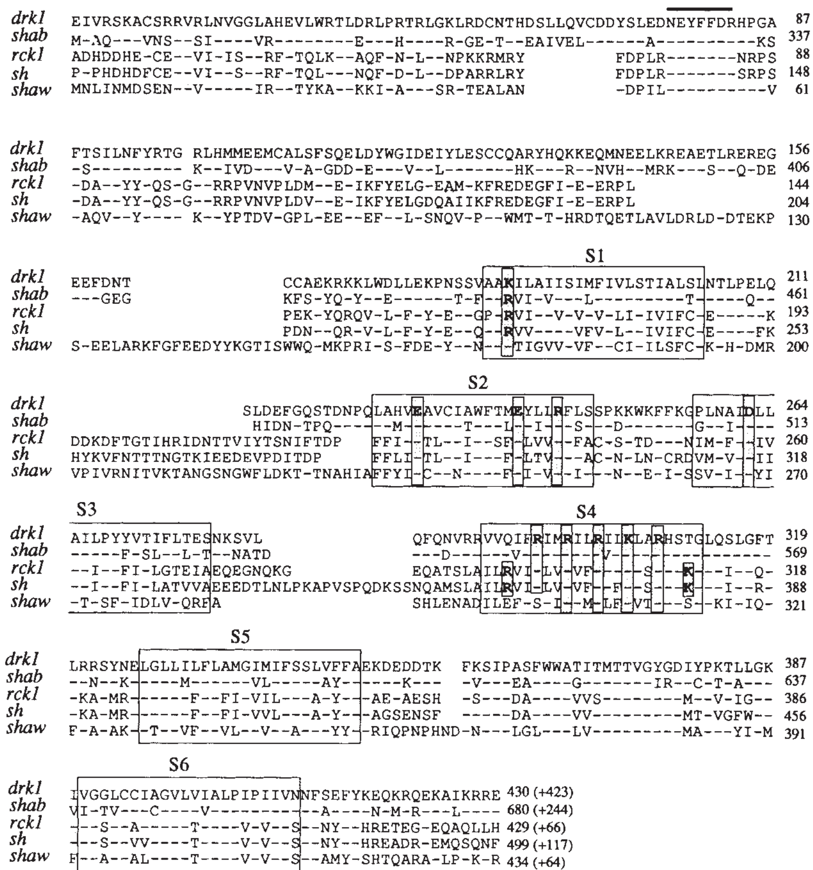

FIG. 3 Comparison of amino-acid sequences of different K$^+$ channels. The sequences of five different K$^+$ channels were aligned to reveal similarities over the areas indicated. Amino-acid positions are shown by numbers. The lengths of the missing C-termini are shown in parentheses. Identical amino acids are indicated by dashes; gaps in the sequences, by empty spaces. The *Shaker* gene encodes an A-type K$^+$ channel and is derived from the *Shaker*-region of *Drosophila*[4]. Two *Drosophila* cDNA clones, *Shab* and *Shaw*, whose functions are not known, are distinct from the various *Shaker* clones[18]. The gene *rck1* was isolated from a rat-brain cDNA library using a Shaker sequence as a screening probe[13]. Two families, each containing two members, are apparent. The gene *drk1* is most similar to *Shab*, whereas *rck1* is most similar to *Shaker*. The putative transmembrane regions (S1–S6) are boxed. The positive charges in the postulated voltage-sensor (S4) are shaded, as well as the conserved charged residues in other transmembrane regions. The longest stretch of identical residues present in all the channels is outlined by a heavy line. We used the EuGene software by Molecular Biology Information Resources at the Department of Cell Biology, Baylor College of Medicine, for the sequence alignment.

are present in S4 of the protein encoded by *Shaw*; this is the only (putative) K$^+$ channel known to have a negatively charged amino acid (glutamate) at a position where other K$^+$ channels have a positively charged arginine residue. It will be interesting to see whether these charge differences are responsible for some of the different kinetic properties of A-type and delayed rectifier channels. From the comparative alignment shown in Fig. 3 and Table 1 it is clear, on the basis of the conserved core sequences, that *drk1* and *Shab* are two members of the same family of genes, and *rck1* and *Shaker* are members of another family. The *drk1/Shab*-family and *rck1/Shaker* family are related and belong to a superfamily which includes a third family with *Shaw* as its only member so far. Of the *drk1/Shab* family only *drk1* has been expressed (this report), and we have shown that it encodes a K$^+$ channel with properties of a delayed rectifier. Of the *rck1/Shaker* family several members have been expressed. The *Shaker* clones encode A-type channels[9-11], whereas expression of *rck1* yields K$^+$ currents with properties of delayed rectifier-type channels[14] and possibly A-type channels[15]. The function of *Shaw* is unknown. The fact that *rck1* and *Shaker* genes are more closely related to one another than to *drk1* encoding the new K$^+$ channel indicates that the *rck1/Shaker* and *drk1/Shab* families are evolutionarily older than flies and mammals. The most conserved region in all K$^+$ channels sequenced so far is the stretch of seven amino acids near the beginning of the core. Because it is present in all types, this stretch may be a general hallmark of K$^+$ channels. This region seems well-suited to initiate a specific search by means of an oligonucleotide-directed screening approach to identify unknown members of the K$^+$ channel superfamily. □

TABLE 1 Percentage of identical amino acids in analogous positions between pairs of different K$^+$-channel types

| | *Shab* | *Shaker* | *Shaw* | *rck1* |
|---|---|---|---|---|
| *drk1* 70 | 70 | 40 | 41 | 40 |
| *rck1* | 39 | 74 | 40 | |
| *Shaw* | 40 | 42 | | |
| *Shaker* | 36 | | | |

Percentages were calculated using the alignment in Fig. 3. The results show that *drk1* is most closely related to *Shab*, whereas *rck1* is most similar to *Shaker*.

Received 10 May; accepted 19 July 1989.


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ACKNOWLEDGEMENTS We thank Drs Tom Caskey and Richard Gibbs, and Donna Muzny (Howard Hughes Medical Institute) for their help with automated DNA sequencing. Charybdotoxin was a gift from Drs G. J. Kaczorowski and M. L. Garcia. This work was supported in part by an Advanced Technology Program Award from the State of Texas (R.H.J.) and by the National Institutes of Health (A.M.B.).



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