

Molecular cloning of a member of a third class of Shaker-family K^+ channel genes in mammals

T. McCORMACK, E. C. VEGA-SAENZ DE MIERA, AND B. RUDY*

Department of Physiology and Biophysics and Department of Biochemistry, New York University Medical Center, 550 First Avenue, New York, NY 10016

Communicated by R. Llinás, April 5, 1990 (received for review February 5, 1990)

ABSTRACT We report the cloning of RKShIII A, a cDNA encoding a K^+ channel sequence expressed in rat brain. This cDNA encodes K^+ channel subunits that express in *Xenopus* oocytes a slow, 4-aminopyridine- and tetraethylammonium-sensitive, delayed rectifier-type K^+ channel activated by large membrane depolarizations. This gene belongs to the Shaker (Sh) family of K^+ channel genes, since the predicted protein has the same overall structure and shows significant homology to other members of this family. However, RKShIII A cannot be assigned to either of the two known classes of Sh-family genes in mammals based on sequence analysis. Notable features of the RKShIII A protein product include a probable cytoplasmic loop rich in prolines and a stretch very homologous to the *Drosophila* Shaw protein, both near the amino terminus.

Ion channels are ubiquitous membrane proteins with important and multiple functions in both excitable and nonexcitable cells (1). Potassium (K^+) channels are particularly diverse and are present in all eukaryotic cells (1, 2). In neurons specific combinations of various ion channels underlie the generation of many different signal waveforms and firing patterns and thus contribute to the complexity of neuronal information coding and integration (3).

Two unrelated sequences encoding voltage-dependent K^+ channels have been characterized, clearly representing two distinct families of genes. The first family, the Shaker (Sh) family, encodes proteins of several hundred amino acids that are similar to those encoded in the Shaker gene in *Drosophila*, a gene that generates several products by alternative splicing (4–6), and includes several members in mammals (7–14) and in *Drosophila* (15). A gene encoding a voltage-dependent K^+ channel with extremely slow kinetics cloned originally from kidney (16) defines the second K^+ channel gene family. The products of the Sh-family genes consist of a core region with six hydrophobic sequences of which probably five are membrane-spanning domains. Between the third and fourth hydrophobic segments there is a sequence, thought to be membrane-spanning (17, 18), consisting of an arginine (sometimes lysine) at every third position and hydrophobic amino acids in the other positions. This motif, believed to be responsible for voltage sensing, is known as S4 and is also present in voltage-dependent Na^+ and Ca^{2+} channels (17, 18). In contrast, the gene cloned from kidney encodes a protein with a single membrane-spanning domain and no real S4 sequence.

A comparison of the known sequences in the Sh family suggests the presence of several groups or classes (13). We now report on the cloning of a K^+ channel cDNA from rat brain (RKShIII A) that clearly belongs to the Sh family.[§] A comparison with other members of this family suggests that this cDNA is a member of a third class of K^+ channel genes in the Sh family in mammals.

MATERIALS AND METHODS

DNA Sequencing. Sequences were obtained by the dideoxynucleotide chain-termination method using Sequenase (United States Biochemical) and plasmid DNA as template.

RNA Expression. The recombinant pBluescript (Stratagene) plasmid containing the RKShIII A insert was linearized by digestion with *Apa* I or *Xho* I, and full-length capped RNA transcripts were synthesized with T3 polymerase as described (19). Standard methods were used to inject RNA into *Xenopus laevis* oocytes and to record ionic currents (19, 20), except that often the oocytes were not defolliculated until use. All electrophysiological recordings were carried out at 21–22°C in ND96 solution (96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$ /1 mM $MgCl_2$ /5 mM Hepes, pH 7.5).

Southern Blot Analysis. Digested rat genomic DNA was electrophoresed into 0.8% agarose in 1× TBE buffer (21). Southern blot transfers into Hybond (Amersham) were prepared essentially as described (21). The blots were hybridized with ³²P-labeled DNA probes synthesized by the random hexamer primer method (22).

RESULTS AND DISCUSSION

Primary Structure of RKShIII A. The nucleotide and deduced amino acid sequence of clone RKShIII A is shown in Fig. 1. The predicted product of the cDNA is a protein of 563 amino acids with a calculated relative molecular mass of 61,894. The conceptual translation of RKShIII A (Fig. 1), a hydrophathy analysis (not shown), and a comparison with the amino acid sequence of three classes of Sh-family genes (Fig. 2) indicate that our sequence is similar in overall structure to other Sh-family genes. Like all Sh-family gene products, RKShIII A contains six hydrophobic regions (H1–H6) and a well conserved S4 domain.

A special feature of RKShIII A is a 44-amino acid sequence inserted in the amino end region of the protein (enclosed in a box in Fig. 2). The sequence contains several stretches of consecutive prolines, a rare feature found in some other proteins such as the hinge region of some IgGs (25), myelin A1 basic protein (26), and synapsin (27). In addition, two serines in this sequence are surrounded by amino acids similar to those that surround serines or threonines that undergo O-glycosylation (26). According to the proposed topology of this channel (Fig. 1), these putative O-glycosylation sites are on the cytoplasmic side and thus might be involved in the regulation of phosphorylation and protein assembly and targeting (28).

We find that the fifth hydrophobic domain (H5) is consistently one of the most conserved regions among known Sh K^+ channel sequences. This sequence is probably not hydrophobic enough to constitute a membrane-spanning domain (6, 18). Furthermore, if one assumes that H5 is not membrane-spanning, the predicted topology of Sh K^+ chan-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.

[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34052).

-79		
GCACGGTG AACATCTGGC CCACGAGAGC TTAACTTGG TGCTGTGTC GCCTTCCTA	ATC CTC AGA ATC TTC AAG CTT ACC CGC CAT TTC GTA GGT CTG AGA GTG CTC GGA	1116
-20	<u>Leu Leu Arg Ile Phe Lys Leu Thr Arg His Phe Val Gly</u>	<u>Leu Arg Val Leu Gly</u> 372
GTCTGTCTG AGCCACAGAG ATG GGC AAG ATC GAG AAC AAC GAG AGG GTG ATC CTC	S4	
	Met Gly Lys Ile Glu Asn Asn Glu Arg Val Ile Leu	12
36	CAC ACT CTT CGT GCG AGC ACC AAT GAA TTT TTG TTG CTG ATC ATC TTT CTG GCT	1170
	His Thr Leu Arg Ala Ser Thr Asn Glu <u>Phe Leu Leu Leu Ile Ile Phe Leu Ala</u>	390
AAT GTC GGA GGC ACC AGG CAC GAA ACC TAC CGC AGC ACT CTC AAG ACC CTT CCT	90	
Asn Val Gly Gly Thr Arg His Glu Thr Tyr Arg Ser Thr Leu Lys Thr Leu Pro	30	
GGA ACT CGC CTG GCC CTT CTC GCC TCC TCT GAA CCT CAG GGC GAC TGC CTG ACT	144	
Gly Thr Arg Leu Ala Leu Leu Ala Ser Ser Glu Pro Gln Gly Asp Cys Leu Thr	48	
GCT GCG GGT GAC AAG CTG CAG CCG CTG CCC CCT CCG CTG TCT CCA CCG CCG CGA	198	
Ala Ala Gly Asp Lys Leu Gln Pro Leu Pro Pro Pro Leu Ser Pro Pro Pro Arg	66	
CGC CCT CCC TTG TCC CTT GTC CCC AGC GGC TGC TCT GAG GGC GGC GCA GGC AAC	252	
Pro Pro Pro Leu Ser Pro Val Pro Ser Gly Cys Phe Glu Gly Gly Ala Gly Asn	84	
TGC AGT TCG CAC GGT GGC AAT GGC AGC GAC CAC CCT GGG GGA GGC CGC GAA TTC	306	
Cys Ser Ser His Gly Gly Asn Gly Ser Asp His Pro Gly Gly Gly Arg Glu Phe	102	
TTT TTC GAT CGC CAC CCA GGA GTC TTC GCC TAT GTG CTC AAC TAC TAC CGC ACG	360	
Phe Phe Asp Arg His Pro Gly Val Phe Ala Tyr Val Leu Asn Tyr Tyr Arg Thr	120	
GGC AAG CTG CAC TGC CCC GCC GAC GTG TGT GGA CCG CTC TTC GAG GAA GAG CTG	414	
Gly Lys Leu His Cys Pro Ala Asp Val Cys Gly Pro Leu Phe Glu Glu Glu Leu	138	
GCA TTC TGG GGC ATC GAT GAG ACC GAC GTG GAG CCC TGC TGC TGG ATG ACC TAC	468	
Ala Phe Trp Gly Ile Asp Glu Thr Asp Val Glu Pro Cys Cys Trp Met Thr Tyr	156	
AGG CAG CAC CGG GAC GCG GAG GAG GCC CTG GAT ATC TTC GAG ACA CCC GAC CTC	522	
Arg Gln His Arg Asp Ala Glu Glu Ala Leu Asp Ile Phe Glu Thr Pro Asp Leu	174	
ATC GGA GGC GAC CCT GGT GAT GAT GAG GAC CTA GGG GGC AAG AGA CTG GGC ATT	576	
Ile Gly Gly Asp Pro Gly Asp Asp Glu Asp Leu Gly Gly Lys Arg Leu Gly Ile	192	
GAG GAT GCT GCG GGG CTG GGA GGA CCC GAT GGC AAG TCT GGC CGC TGG AGG AAG	630	
Glu Asp Ala Ala Gly Leu Gly Gly Pro Asp Gly Lys Ser Gly Arg Trp Arg Lys	210	
CTG CAG CCT CGC ATG TGG GCT CTC TTT GAG GAC CCC TAT TCA TCC AGA GCC GCT	684	
Leu Gln Pro Arg Met Trp Ala Leu Phe Glu Asp Pro Tyr Ser Ser Arg <u>Ala Ala</u>	228	
AGG TTT ATT GCT TTT GCT TCT CTG TTC TTC ATT TTG GTT TCC ATC ACA ACC TTT	738	
<u>Arg Phe Ile Ala Phe Ala Ser Leu Phe Phe Ile Leu Val Ser Ile Thr Thr Phe</u>	246	
H1		
TGC CTG GAG ACA CAC GAA GCT TTC AAT ATT GTT AAA AAC AAG ACA GAG CCA GTC	792	
<u>Cys Leu</u> Glu Thr His Glu Ala Phe Asn Ile Val Lys Asn Lys Thr Glu Pro Val	264	
ATC AAC GGC ACC AGC GCT GTT CTC CAG TAT GAA ATC GAA ACG GAT CCT GCC TTG	846	
Ile Asn Gly Thr Ser Ala Val Leu Gln Tyr Glu Ile Glu Thr Asp Pro Ala <u>Leu</u>	282	
ACA TAT GTG GAA GGA GTG TGT GTG GGG TTT ACT TTT GAA TTT TTA GTC CGT	900	
<u>Thr Tyr Val Glu Gly Val Cys Val Val Trp Phe Thr Phe Glu Phe Leu Val Arg</u>	300	
H2		
ATT GTT TTC TCG CCC AAT AAA CTT GAG TTC ATC AAA AAT CTA TTG AAC ATC ATT	954	
<u>Ile Val Phe</u> Ser Pro Asn Lys Leu Glu Phe Ile Lys Asn Leu <u>Leu Asn Ile Ile</u>	318	
GAC TTT GTG GCC ATC CTC CCC TTC TAC TTA GAG GTG GGA CTC AGC GGG CTG TCT	1008	
<u>Asp Phe Val Ala Ile Leu Pro Phe Tyr Leu Glu Val Gly Leu Ser Gly Leu Ser</u>	336	
H3		
TCC AAA GCG GCT AAA GAT GTG CTC GGC TTT CTC AGG GTG GTT AGG TTT GTG AGG	1062	
Ser Lys Ala Ala Lys Asp Val Leu Gly <u>Phe Leu Arg Val Val Arg Phe Val Arg</u>	354	
CTG GGA GTT TTG ATA TTC GCT ACG ATG ATC TAC TAC GCT GAG CGA GTA GGG GCT	1224	
<u>Leu Gly Val Leu Ile Phe Ala Thr Met Ile Tyr Tyr Ala</u> Glu Arg Val Gly Ala	408	
H4		
CAA CCT AAT GAT CCC TCA GCG AGT GAG CAC ACA CAG TTC AAA AAC ATC CCC ATT	1278	
Gln Pro Asn Asp Pro Ser Ala Ser Glu His Thr Gln Phe Lys Asn Ile Pro Ile	426	
GGT TTC TGG TGG GCT GTG GTG ACC ATG ACT ACC TTA GGC TAT GGG GAT ATG TAC	1332	
<u>Gly Phe Trp Trp Ala Val Val Thr Met Thr Thr Leu Gly Tyr Gly Asp Met Tyr</u>	444	
H5		
CCC CAA ACA TGG TCA GGG ATG TTG GTG GGG GCC TTG TGT GCT CTG GCT GGA GTG	1386	
<u>Pro Gln Thr Trp Ser Gly Met Leu Val Gly Ala Leu Cys Ala Leu Ala Gly Val</u>	462	
CTG ACC ATA GCT ATG CCT GTG CCC GTC ATT GTC AAC AAT TTT GGG ATG TAC TAC	1440	
<u>Leu Thr Ile Ala Met Pro Val Pro Val Ile Val Asn</u> Asn Phe Gly Met Tyr Tyr	480	
H6		
TCC TTG GCA ATG GCG AAG CAG AAA CTT CCA AGA AAA AGA AAG AAG CAC ATT CCT	1494	
Ser Leu Ala Met Ala Lys Gln Lys Leu Pro Arg Lys Arg Lys Lys His Ile Pro	498	
CCT GCC CCT CTG GCA AGC TCA CCT ACA TTT TGC AAG ACA GAA TTA AAC ATG GCT	1548	
Pro Ala Pro Leu Ala Ser Ser Pro Thr Phe Cys Lys Thr Glu Leu Asn Met Ala	516	
TGT AAC AGT ACC CAG AGT GAC ACA TGT CTG GGC AAA GAA AAC CGG CTT CTG GAA	1602	
Cys Asn Ser Thr Gln Ser Asp Thr Cys Leu Gly Lys Glu Asn Arg Leu Leu Glu	534	
CAT AAC AGA TCA GTG TTA TCA GGT GAC GAC AGT ACA GGA AGT GAG CCG CCA TTA	1656	
His Asn Arg Ser Val Leu Ser Gly Asp Asp Ser Thr Gly Ser Glu Pro Pro Leu	552	
TCA CCT TCC GGA AAG GCT CCC CAT CAG ACG CTC TAG TACCAGAG ACAAAAACAG	1710	
Ser Pro Ser Gly Lys Ala Pro His Gln Thr Leu *	563	
AAGAGGGGAA ACATGTITCC TGTTGACGAC AGGTGATTAC ACGTGGCCTT CTGATGGAGG	1770	
AATCAGGAAA GATAACTGCA AAGATGTGT CATTACTGGT TACACGCAAG CCGAGGCCAG	1830	
ATCTCTACT TAATGACTTG GGAAAGCCAC AAAACATGAA AGAAAGTGTT GTACAGAATT	1890	
TATCATGGAT TTTTGCTGCG TGAAAATGGG ACATTGAGAT TTAGECATTC AAGGATTGTA	1950	
CTGGAAACTT CTGCTACTGA ATGTGACCGG ATGTGACCGG TTTGTGTTCA GAAGAGTTCC	2010	
CGCATTTCT GAGGCATTTA AAGCTTTGTA AGAAATGGG GCTGGAACTG AAATGGGCTT	2070	
TGCCAAGGAA ATATTTCTGCT TGTCGACAGA CCAGAGTCCA CAAAAACAAT GTAAATACCA	2130	
ACGTGTGCAT GGGTCCACCC TTCTCACAGT CTATAGCAEC AGGGAGCCAC ATTCATTGTC	2190	
AGCATCTGTG AAGCTTCAAG AAATGCCAC AAGGCTCTGA ATGGCTCTCC TCGGCTCAT	2250	
GGTGGTTTTG CTGCAAAITA CAGAGGGCCAG TGAATCCAAT GTAGTATAGG TCTGTITTTCA	2310	
TTTCTTTTAT TTGTACATTA CGTGTGTAT CTACAAACAC CGATTGCCCTC ATTTCTCTGC	2370	
AAACCACTTA TATATCCGCT TGTTGAACTAT GTTTGAACT TTGTAGTCT CATAGAAAAT	2430	
CTAACCATTT TTATGTCATG TATTCAATTA GTTCTGGAAC GCTGTACTA TCTAGAAAGC	2490	
AGTCATCACC AGTATTCATC GTATAAGTCT TTTGTGCTG TTACTTCAAT TATAAATATT	2550	
AAAAATATAA ATTCGGCAA TGAGAAATATT TTTTATATA ATGATCAAGG AAAATGTCAG	2610	
TATATAGTAG AATATTATCA AATTATATCC TAAAGTGTCT AATTTGCAAT AAAGAGATAT	2670	
TCTTCAATCG ATTCCTTTTT TTCGTGAGCT TTTGTGGCTA TGAAGCGTGT GTTTGCTCTC	2730	
ATCATCTGCT TAGTTGAAC TGTTGAAGG TTTTTCATCT TCTTAATCA ATATTCCAG	2790	
AATCTCTTAG TCCCGCTGGG ATTCTGAATA TACTCTATAG TCCCTATATA AACCCCTGTA	2850	
TCTGTACTCT TTTGTGACCA TCTCAAGGTG CATGCCAAT CGTTGTGATA AGCCAATGGA	2910	
CATGTACTA ACTGAAATGA AGAATAAAG TCAAACGATC TGGGGATAAA CTTGAATCCT	2970	
ATCTGATTAA ATGAT	2985	

FIG. 1. Nucleotide and deduced amino acid sequence of RKShIIIa. The name given to this clone attempts to indicate its relationship to other K⁺ channel sequences. The R indicates the clone is from rat, K that it encodes a K⁺ channel, Sh that it belongs to the Sh family of genes, III that it is a member of the third known class of genes in this family, and A that it is the first known member of this class in rat. We have not indicated tissue of origin in the name, because other sequences have been found in more than one tissue (e.g., RCK4 and RHK1, found in brain cortex and heart, respectively, are the same sequence). The numbers indicate the nucleotide and amino acid positions with the chosen initiation codon as 1. The ATG triplet chosen as the initiation codon is the second found in the sequence, but it produces the longest possible open reading frame and predicts a protein that is highly homologous to other Sh K⁺ channel sequences. The first stop codon in frame is shown by an asterisk. Hydrophobic domains (H1–H6) and the S4 motif are underlined. Consensus sequences for N-glycosylation (23) are indicated with filled squares. Only two of these (Asn-259 and -266) are extracellular in the expected topology of the protein assuming that the amino terminus is intracellular (because of a lack of signal-peptide sequence) and that H1–H4, S4, and H6 are membrane-spanning domains (6, 18). The clone containing the RKShIIIa insert was obtained by screening (21) a rat brain cDNA library in λZAP (Stratagene) with DNA probes derived from the amino-terminal end of a partial clone obtained previously. Hybridization to nitrocellulose replicas of cultures was carried out in 50% (vol/vol) formamide/5× SSPE at 42°C (21). Another isolate was partially sequenced. It is identical to RKShIIIa except that in the 3' end instead of a T as the last nucleotide it contains a stretch of 10 A residues. The initial partial clone was obtained by screening, at low stringency [20% formamide/5× SSPE at 30°C], an oligo(dT)-primed rat brain λgt11 cDNA library with an oligonucleotide probe corresponding to the first 60 bases of the (H5) domain of the *Drosophila* Shaker H4 cDNA (4). The recombinant pBluescript plasmid containing the RKShIIIa insert was obtained from the purified λZAP clone by *in vivo* excision using Stratagene protocols.

nel subunits will be similar to that of the homologous repeats in voltage-dependent Na⁺ and Ca²⁺ channels (18). We propose that H5 enters the membrane partially and helps to form

the K⁺ selectivity filter. If a K⁺ channel is a tetramer of Sh-family gene products (18), the center of the H5 sequences could form a ring containing 12 hydroxyl groups. These

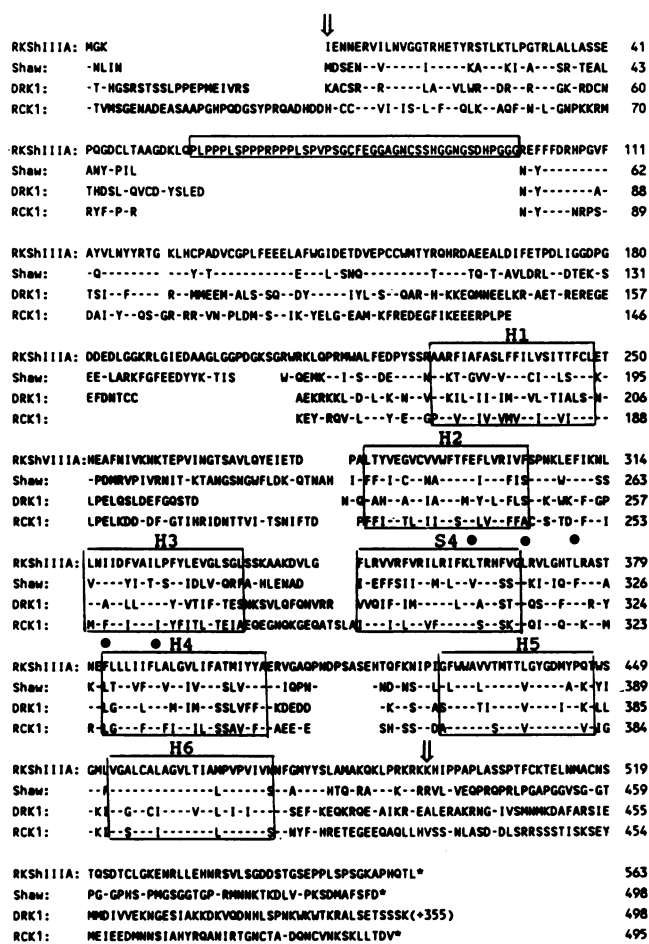


FIG. 2. Comparison of predicted amino acid sequences of RKShIIIA and other Sh K⁺ channel genes. The complete sequences of RKShIIIA, Shaw, and RCK1 are shown. DRK1 extends 355 amino acids further at the carboxyl end. Identical amino acids are shown with dashes. The sequences were aligned to maximize long stretches of homology. Gaps in the sequence are shown as blanks. The beginning and the end of the central core region used for calculations of identity are marked by arrows. The H1–H6 and S4 sequences and a 44-amino acid insert peculiar to RKShIIIA are boxed. The leucines in heptad repeat ("leucine zipper" motif) adjacent to the S4 domain are indicated with filled circles (24). In RKShIIIA the fourth leucine is replaced by phenylalanine.

hydroxyls could provide the water-like environment which dehydrates K⁺ ions selectively (1, 29).

Functional Properties of RKShIIIA Channels. *In vitro* transcribed RNA from RKShIIIA expresses K⁺ channels when injected into *Xenopus* oocytes. Large currents absent in uninjected oocytes are observed in oocytes injected with RKShIIIA transcripts (Fig. 3 A–C). The reversal potential of these currents, determined from tail-current analysis (19), depends on external K⁺ concentration as expected for a K⁺-selective channel. A reversal potential of –85 mV is obtained in 2 mM external K⁺ and –55 mV in 10 mM (data not shown). These values are close to the expected equilibrium potential for K⁺ (–95 mV and –55 mV, respectively), assuming an internal K⁺ concentration of 90 mM, and indicate a very high selectivity for K⁺ ions (1). The currents expressed by RKShIIIA activate slowly, starting at about –10 mV, and their rise time decreases with increasing depolarization (Fig. 3 A–C). These currents inactivate very slowly (Fig. 3B) and activate with a delay (Fig. 3C). All these are features of delayed rectifier-type K⁺ channels (1, 2).

A conductance–voltage curve obtained from five experiments is presented in Fig. 3D. The conductance begins to rise

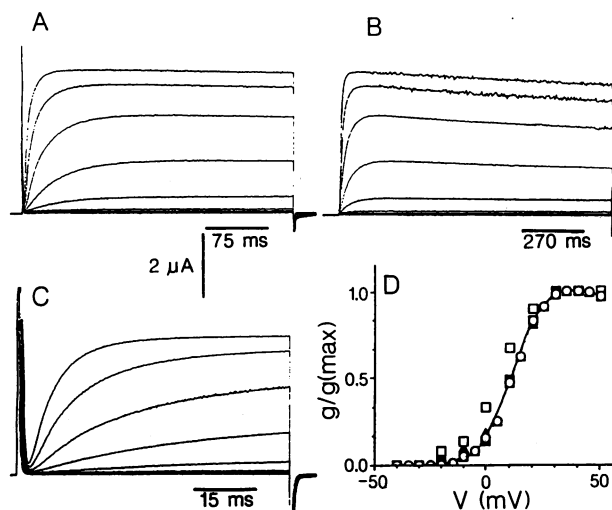


FIG. 3. Electrophysiological properties of ion currents expressed by RNA transcripts of RKShIIIA. (A–C) Currents recorded under voltage clamp. Depolarizing pulses, from –80 to +40 mV with 10-mV increments, were delivered at a rate of every 4 sec (A and C) or 8 sec (B) from a holding potential of –100 mV. The oocytes were bathed in ND96 solution and the electrodes were filled with 3 M KCl. (D) Plot of normalized conductance (conductance at the indicated potential divided by the maximum conductance) obtained from five oocytes and three different preparations of RNA. In one case (open circles) depolarizing pulses were given in 5-mV increments. The conductance was calculated (1) by dividing the steady-state current at the end of 500-msec depolarizing pulses by the driving force using a reversal potential of –85 mV.

between –15 and –10 mV and increases steeply to a maximum value. Sometimes a decline in conductance is observed with large depolarizations. Half-maximal conductance is obtained at around 13 mV and the limiting slope of the curve is estimated to be 7–7.5 mV for an *e*-fold change in conductance. The reduced steepness exhibited by RKShIIIA, compared to *Drosophila* Shaker channels (19), may be due to the smaller number of basic residues in the S4 domain (six vs. seven). However, amino acids in other regions of the protein may also influence the steepness of conductance–voltage relations (13).

The currents expressed by RKShIIIA are blocked 70–100% (depending on the membrane potential) by 1 mM tetraethylammonium, 5 mM Ba²⁺, and 1 mM 4-aminopyridine, three well known K⁺ channel blockers. The blockage of RKShIIIA channels by 4-aminopyridine seems to exhibit open-channel block behavior (1), contrary to what has been observed for 4-aminopyridine blockade of K⁺ channels in squid giant axon (30). Specifically, the degree of blockage of RKShIIIA currents by 4-aminopyridine increases with increasing depolarization, the rise time of the currents becomes faster, and at some voltages (e.g., at +20 mV) a time-dependent inactivation is induced (Fig. 4). Furthermore, repetitive depolarization produces further blockage (see *Inset* in Fig. 4B), as opposed to the gradual release of block observed for the delayed rectifier of the squid giant axon (30).

K⁺ Channel Classes. Based on sequence homology, and hence evolutionary relatedness, of previously known Sh-family sequences, two classes of genes (13) can be defined (classes I and II in Table 1). Members of a given class, in different species, are far more similar to each other than to members of different classes in the same species (Table 2). This suggests that these two classes originated from some ancestral sequence before the flies and mammals diverged (13–15). RKShIIIA is more similar to the *Drosophila* Shaw gene (15) than to any other known K⁺ channel sequence (Table 2) or any sequence in the GenBank data base (January

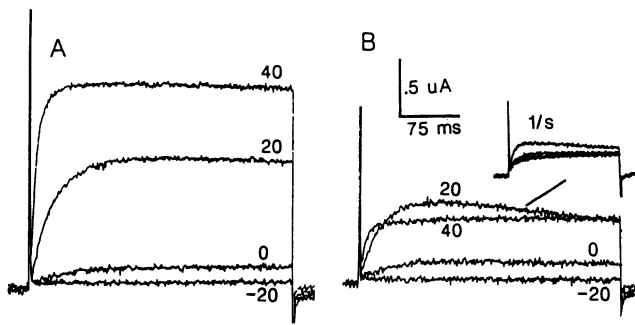


FIG. 4. 4-Aminopyridine block of RKShIIIA currents. Records of ion currents obtained as in Fig. 3 for depolarizing pulses to the indicated voltages are shown before (A) and after (B) the addition of 0.8 mM 4-aminopyridine. The *Inset* in B shows the currents during a depolarizing pulse to 20 mV applied at a frequency of 1/sec obtained after the experiment shown in B was finished.

1990), particularly in a long stretch near the amino terminus that is almost identical between these two sequences (Fig. 2). Furthermore, genomic DNA analysis suggests that several genes of the same class, with similar homologies to Shaw, are present in the rat genome (Fig. 5). However, as shown in Table 2, the overall homology between RKShIIIA and Shaw is significantly less than that among class I or class II genes (e.g., between RCK1 in rat and H4 in *Drosophila*, or between DRK1 in rat and Shab in *Drosophila*). Outside the stretch in the amino terminus, RKShIIIA is more similar to Shaw than to other Sh genes in the sixth hydrophobic domain (H6) and the sequence immediately following H6, but it is more similar to RCK1 or DRK1 in the region around the S4 domain (Fig. 2).

RKShIIIA is clearly different from class I or class II genes and thus we consider it a member of a third Sh gene class in mammals. Class I and II genes have vertebrate and invertebrate counterparts. RKShIIIA and Shaw are probably related genes; however, their evolutionary relatedness is different from that between class I and class II genes. Thus, their assignment to the same class of genes is tentative, and definite assignment must await further evolutionary analysis.

The physiological significance of these classes is not known. Inactivation is clearly not a defining parameter because, for example, class I includes channels that inactivate very fast and channels that inactivate very slowly and have delayed rectifier properties (Table 3). Differences in these properties apparently can be achieved without major changes in the core region. From the electrophysiological studies done thus far, we can distinguish two parameters of predicted physiological significance which appear to vary among classes (Table 3): class I members activate quickly and at relatively low (but not subthreshold) voltages, while

Table 1. Shaker family of K⁺ channel genes

Class	Rat	Human	<i>Drosophila</i>
I	RCK1 = RBK1 (10, 8)	HuKI (12, 13)	Shaker (4-6)
	RCK5 = RBK2 (10, 9)	HuKIV (12, 13)	
	RCK4 = RHK1 (10, 11)	HuKII (12, 13)	
	RCK3 (10)	HuKIII (12, 13)	
		HuKV (12, 13)	
II	DRK1 (14)		Shab (15)
III	RKShIIIA		Shaw? (15)
	(this paper)		
IV			Shal (15)
V			Shaw? (15)

This table was modified from ref. 13. A homologue of RCK1, RBK1, and HuKI is known in mouse (MBK1, ref. 7).

Table 2. Fraction of identical amino acids in analogous positions between pairs of Sh K⁺ channel proteins

	Shaw	DRK1	Shab	RCK1	H4
RKShIIIA	0.52	0.42	0.41	0.41	0.40
Shaw		0.41	0.40	0.41	0.41
DRK1			0.72	0.42	0.40
Shab				0.39	0.37
RCK1					0.76

The fractions of identical amino acids in overlapping sequences in the core region were calculated using an alignment like the one shown in Fig. 3 but which included also Shab and H4. H4 is one of the products of the *Drosophila* Shaker gene (4). Shab (15) and Shaw (15) are *Drosophila* genes, and DRK1 (14) and RCK1 (10) are rat genes.

class II and class III channels (DRK1 and RKShIIIA) activate relatively slowly and require large-depolarizations. RKShIIIA currents activate at particularly high voltages. These differences might explain why there seems to be much more expression of class I genes than of class II or III genes in brain, since most neuronal K⁺ channels activate in the voltage range of class I channels. The three gene classes also differ in the number of positively charged residues in the S4 domain (Table 3).

Other properties such as developmental regulation, cellular localization, posttranslational modification, or participation in heteromultimer formation (31, 32) might turn out to be important properties of Sh channel classes. For example, RKShIIIA and Shaw lack a consensus sequence for cAMP-dependent phosphorylation present in most Sh K⁺ channel sequences.

Functional Significance. One major task in the future of K⁺ channel gene research is to understand the physiological significance of the various K⁺ channel subunits that are being cloned. Because the functional properties of a given K⁺ channel will depend on whether it is a homo- or heteromultimer of Sh-family gene products (19, 31, 32), on the interactions of this aggregate with smaller subunits (20, 33), and on posttranslational modifications (2), the results of the expression of a particular Sh gene will depend on the cellular context. It is interesting in this regard that the properties

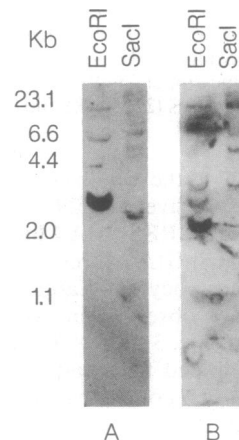


FIG. 5. Southern analysis of rat genomic DNA. DNA was digested with an excess of *EcoRI* or *SacI*. The Southern blots were hybridized (21) under conditions of relatively low stringency (30% formamide/5× SSPE at 37°C) with one of two probes. The first probe (A) contains the region that is most similar between Shaw and RKShIIIA (nucleotides 301-501); the second probe (B) contains the sequence encoding the H3 through H4 domains (nucleotides 964-1296), where Shaw and RKShIIIA are less similar. A single strong hybridization band and several faint but discrete hybridization bands are observed with both probes, suggesting the presence of several genes homologous to both regions of RKShIIIA. *HindIII*-digested λ DNA was used as size markers. Kb, kilobases.

Table 3. Electrophysiological properties of three classes of Sh K⁺ channels

Class	Channel (ref.)	S4 Z*	k _{in} , [†] msec	V _{on} , [‡] mV	t _{on} , [§] msec
I	H4 (19)	7	4	-40	5
	RCK1 (10)	7	—	-50	15
	RCK5 (10)	7	—	-55	6
	RHK1 (11)	7	30	-40	10
	RCK3 (10)	7	1000	-55	14
II	DRK1 (14)	5	—	-25	120
III	RKShIIIA	6	—	-10	80

Approximate values were determined from published data, at room temperature. All the experiments used a similar bathing solution except for those on DRK1, which used Co²⁺ instead of Ca²⁺.

*Number of positive charge residues in S4.

[†]Time constant of inactivation at 0 mV. A dash implies inactivation is very slow or very small.

[‡]Activation voltage, the approximate voltage at which the conductance starts to rise.

[§]Activation rate at 0 mV determined as time to peak for rapidly inactivating currents, and time to rise between 10% and 90% for slowly inactivating and noninactivating currents.

observed here are very similar to those of one of the K⁺ currents (*I_{Ks}*) observed in *Xenopus* oocytes injected with whole brain poly(A)⁺ RNA (34). RKShIIIA channels activate at about the highest voltages one may expect physiologically. If RKShIIIA is expressed in the plasma membrane, and if its properties resemble those in the oocyte, we expect this channel to be particularly important in the termination of prolonged depolarizations (above 0 mV) such as plateau potentials or in the regulation of the frequency and duration of spike bursts (3). A channel such as RKShIIIA would play little role in subthreshold phenomena or in shaping the waveform of fast Na⁺ spikes.

Note Added in Proof. After this paper was submitted we became aware of a sequence, obtained from NG108 cells, just published by Yokoyama *et al.* (35). Their sequence (NGK2) is similar (but not identical) to that of RKShIIIA and appears to be the same as that of a related cDNA (bn13) from PC12 pheochromocytoma cells (Vega *et al.*, ref. 36). Sequence analysis suggests that NGK2 (or bn13) and RKShIIIA are two different members of the same gene class as defined here. Interestingly, although the amino acid sequence differences between NGK2 and RKShIIIA are similar to those found among class I gene members, their electrophysiological properties are very similar.

The research presented here would not have been possible if B.R. had not been given a joint appointment at the Department of Biochemistry at New York University Medical Center. We thank N. Godson (Chairman of the Department of Biochemistry) and R. Llinás (Chairman of the Department of Physiology and Biophysics) for their support and J. W. Lin for his tremendous help in many aspects of this work. We thank R. Dunn (McGill University) and L. Buck (Columbia University) for their cDNA libraries. B.R. would especially like to thank S. Kamb, L. Iverson, K. McCormack, M. Ramaswami, M. Mathew, M. Gautam and Prof. N. Davidson for their trust and patience in teaching him many of the techniques used here and would like to dedicate this paper to them. This research was supported by U.S. Public Health Service Grant GM26976.

- Hille, B. (1984) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
- Rudy, B. (1988) *Neuroscience* 25, 729–750.

- Llinás, R. (1988) *Science* 242, 1654–1664.
- Kamb, A., Tseng-Crank, J. & Tanouye, M. A. (1988) *Neuron* 1, 421–430.
- Schwarz, T., Tempel, B., Papazian, D., Jan, Y. N. & Jan, L. Y. (1988) *Nature (London)* 331, 137–142.
- Pongs, O. N., Kecskemethy, N., Muller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H., Canal, I., Llamazares, S. & Ferrus, A. (1988) *EMBO J.* 7, 1087–1096.
- Tempel, B. L., Jan, Y. N. & Jan, L. Y. (1988) *Nature (London)* 332, 837–839.
- Christie, M. J., Adelman, J. P., Douglass, J. & North, A. J. (1989) *Science* 244, 221–224.
- McKinnon, D. (1989) *J. Biol. Chem.* 264, 8230–8236.
- Stuhmer, W., Ruppersberg, J., Schroter, K., Sakmann, B., Stocker, M., Giese, K., Perschke, A., Baumann, A. & Pongs, O. (1989) *EMBO J.* 8, 3235–3244.
- Tseng-Crank, J., Tseng, G.-N., Schwartz, A. & Tanouye, M. A. (1990) *FEBS Lett.*, in press.
- Kamb, A., Weir, M., Rudy, B., Varmus, H. & Kenyon, C. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4372–4376.
- Mathew, M. K., Ramaswami, M., Gautam, M., Kamb, A., Rudy, B. & Tanouye, M. A. (1989) *Neurosci. Abstr.* 15, 540.
- Frech, G. C., VanDongen, A. M. J., Schuster, G., Brown, A. M. & Joho, R. H. (1989) *Nature (London)* 340, 642–645.
- Butler, A., Wei, A., Baker, K. & Salkoff, L. (1989) *Science* 243, 943–947.
- Takumi, T., Ohkubo, H. & Nakanishi, S. (1988) *Science* 242, 1042–1045.
- Numa, S. (1987) *Biochem. Soc. Symp.* 52, 119–143.
- Catterall, W. A. (1988) *Science* 242, 50–60.
- Iverson, L., Tanouye, M., Lester, H., Davidson, N. & Rudy, B. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5723–5727.
- Rudy, B., Hoyer, J. H., Lester, H. A. & Davidson, N. (1988) *Neuron* 1, 649–658.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
- Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* 132, 6–13.
- Marshall, R. D. (1974) *Biochem. Soc. Symp.* 40, 17–26.
- McCormack, K., Campanelli, J. T., Ramaswami, M., Mathew, M. K., Tanouye, M. A., Iverson, L. E. & Rudy, B. (1989) *Nature (London)* 340, 103.
- Putnam, F. W. (1987) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, New York), Vol. 5, pp. 49–140.
- Eylar, E. H. (1972) *Adv. Exp. Med. Biol.* 32, 215–240.
- Sudhof, T. C., Czernik, A. J., Kao, H., Takei, K., Johnston, P. A., Horiuchi, A., Kanazir, S. D., Wagner, M. A., Perin, M. S., Camilli, P. D. & Greengard, P. (1989) *Science* 245, 1474–1480.
- Hart, G. W., Haltiwanger, R. S., Holt, G. D. & Kelly, W. G. (1989) *Ciba Found. Symp.* 145, 102–118.
- Bezanilla, F. & Armstrong, C. M. (1972) *J. Gen. Physiol.* 60, 588–608.
- Yeh, J. Z., Oxford, G. S., Wu, C. H. & Narahashi, T. (1976) *J. Gen. Physiol.* 68, 519–539.
- Haugland, F. N. & Wu, C. F. (1990) *J. Neurosci.* 10, 1357–1371.
- McCormack, K., Lin, J. W., Ramaswami, M., Tanouye, M. A., Iverson, L. E. & Rudy, B. (1990) *Biophys. J.* 57, 209 (abstr.).
- Rehm, H. & Lazdunski, M. (1988) *Proc. Natl. Acad. Sci. USA* 85, 4919–4923.
- Hoyer, J. H., Ahmed, I., Davidson, N., Lester, H. & Rudy, B. (1989) *Neurosci. Abstr.* 13, 177.
- Yokoyama, S., Imoto, K., Kawamura, T., Higashida, H., Iwabe, N., Miyata, T. & Numa, S. (1989) *FEBS Lett.* 259, 37–42.
- Vega-Saenz de Miera, E., Sen, K., Serodio, P., McCormack, T. & Rudy, B. (1990) *Neurosci. Abstr.* 16, in press.