

Potassium channels from NG108-15 neuroblastoma-glioma hybrid cells

Primary structure and functional expression from cDNAs

Shigeru Yokoyama*[†], Keiji Imoto*, Tetsuro Kawamura*[†], Haruhiro Higashida[†], Naoyuki Iwabe[°],
Takashi Miyata[°] and Shosaku Numa*

*Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Kyoto 606, [†]Department of Biophysics, Neuroinformation Research Institute, Kanazawa University School of Medicine, Kanazawa 920 and [°]Department of Biology, Kyushu University Faculty of Science, Fukuoka 812, Japan

Received 26 October 1989

The complete amino acid sequences of two potassium channel proteins from NG108-15 neuroblastoma-glioma hybrid cells have been deduced by cloning and sequencing the cDNAs. One of these proteins (NGK2) is structurally more closely related to the *Drosophila Shaw* gene product than to the *Shaker* and *Shab* gene products, whereas the other (NGK1) is identical with a rat brain potassium channel protein (BK2) which is more closely related to the *Drosophila Shaker* gene product. mRNAs derived from both the cloned cDNAs, when injected into *Xenopus* oocytes, direct the formation of functional potassium channels with properties of delayed rectifiers.

Potassium channel; cDNA cloning; cDNA expression; Delayed rectifier; Phylogeny; (NG108-15 cell)

1. INTRODUCTION

Voltage-gated potassium channels in excitable membranes play important roles in the control of cellular excitability [1]. Recently, the primary structures of some potassium channel proteins from *Drosophila* [2–6] and from mammalian brain [7–11] have been elucidated by cloning and sequencing the cDNAs. mRNAs derived from *Drosophila Shaker* cDNAs [12–14] and from rat brain cDNAs [10,11,15,16] direct the formation of functional potassium channels in *Xenopus* oocytes. The present investigation deals with cloning and sequence analysis of two cDNAs encoding distinct potassium channel proteins from mouse neuroblastoma × rat glioma hybrid cells (NG108-15) [17] and with functional expression of the cloned cDNAs. The evolutionary relationships among voltage-gated potassium channel proteins are discussed.

2. MATERIALS AND METHODS

2.1. Cloning and sequencing of cDNAs

NG108-15 cells were cultured and differentiated in the presence of 1 mM theophylline and 10 μ M prostaglandin E₁ as in [17]. Total

RNA was extracted [18], and poly(A)⁺ RNA isolated [19]. Randomly primed cDNA libraries were constructed in phage λ ZAPII (Stratagene) using poly(A)⁺ RNA as template. Double-stranded cDNA, prepared with the cDNA synthesis system (Bethesda Research Laboratories), was methylated by *Eco*RI methylase, blunted by T₄ DNA polymerase and ligated with the *Eco*RI linker dGGAATTCC. After *Eco*RI digestion, the cDNA was passed through a Sepharose CL-4B column. Fractions longer than ~0.4 kilobase pairs (kb) were pooled and ligated into the vector. The initial screening of a cDNA library was effected by hybridization at 50°C with a synthetic DNA probe prepared as follows. Two overlapping 105-mers (I, II) which comprise portions of the MBK1 cDNA [7] encoding a mouse brain potassium channel protein were prepared with an automatic DNA synthesizer (Applied Biosystems); I represents nucleotides 862–966 of the message strand, and II the sequence complementary to nucleotides 937–1041. After annealing the single strands, the complementary sequences were labeled by a fill-in reaction using a Klenow fragment and [α -³²P]dCTP as in [20]. For the screening of a second cDNA library, cDNA clones isolated initially served as probes for hybridization at 50°C. cDNA inserts from isolated clones were subcloned into pBluescript SK(–) by in vivo excision according to the Stratagene product protocol. Nested deletions were made [21] and DNA sequencing was carried out on both strands by the dideoxy chain termination method [22]. Blot hybridization analysis of poly(A)⁺ RNA from NG108-15 cells was performed as in [23], using the *Eco*RI(815)/*Bam*HI(1612) fragment from λ NGK2·19A4 or the *Pst*I(vector)/*Bam*HI(1612) fragment from pSPNGK2 as probe; restriction endonuclease sites are identified by numbers (in parentheses) indicating the 5'-terminal nucleotide generated by cleavage.

2.2. Synthesis of specific mRNAs

The recombinant plasmids pSPNGK1 and pSPNGK2, used for the synthesis of mRNAs specific for the NGK1 and the NGK2 protein (see section 3), respectively, were constructed as follows. pSP64(polyA) (Promega) was digested with *Eco*RI, blunted by T₄ DNA polymerase and ligated with the *Xho*I linker dCCTCGAGG. The resulting plasmid was cleaved with *Xho*I and ligated again to yield pSP64AX. The 1.8-kb *Pvu*II(–40)/*Bam*HI(vector) fragment from λ NGK1·23C2

Correspondence address: S. Numa, Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Yoshida, Sakyo-ku, Kyoto 606, Japan

The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no. Y07521

and the 3.0-kb *Bam*HI/*Hinc*II fragment from pSP64AX were ligated to yield pSPNGK1. The *Nar*I(−7)/*Nar*I(508) fragment from λ NGK2·18A1 was blunted by T₄ DNA polymerase and cleaved by *Hind*III. The resulting 0.23-kb fragment, the *Hind*III(224)/*Eco*RI(815) fragment from λ NGK2·18A1, the *Eco*RI(815)/*Bam*HI(1612) fragment from λ NGK2·19A4 and the 3.0-kb *Bam*HI/*Hinc*II fragment from pSP64AX were ligated to yield pSPNGK2. NGK1-specific and NGK2-specific mRNAs were synthesized in vitro, using *Xho*I-cleaved pSPNGK1 and pSPNGK2, respectively, as templates [24]. Transcription was primed with the cap dinucleotide G(5')ppp(5')G (0.5 mM) [25].

2.3. Electrophysiological measurements

Each of the specific mRNAs was injected into *Xenopus laevis* oocytes (mRNA concentration, 25 ng/ μ l; average volume injected per oocyte, ~50 nl). The injected oocytes were incubated at 19°C for 2–3 days in modified Barth's medium [26] containing cefmenoxime (0.1 mg/ml). Whole-cell currents were measured using a conventional two-microelectrode voltage clamp. The chamber was continuously perfused with normal frog Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, pH adjusted to 7.2 with NaOH). For ion substitution experiments, the concentration of KCl was raised at the expense of NaCl. The final KCl concentration ranged from 5 mM to 100 mM. Effects of pharmacological agents were assessed by measuring the current amplitudes at the end of 200-ms voltage steps to 0 mV. Whole-cell current records were low-pass filtered at 3 kHz and sampled at 2.5 kHz. For single-channel recording, oocytes were immersed in a nominally Ca²⁺-free K⁺ solution (100 mM KCl, 10 mM EGTA, 10 mM HEPES, pH adjusted to 7.2 with KOH) and inside-out patches were excised into the same solution. Patch pipettes were filled with normal frog Ringer solution. All electrophysiological measurements were carried out at 22 ± 1°C essentially following the procedures described in [15].

3. RESULTS AND DISCUSSION

A randomly primed cDNA library derived from NG108-15 cell poly(A)⁺ RNA was screened by hybridization with a synthetic DNA probe comprising a partial cDNA sequence for a mouse brain potassium channel protein (see section 2.1). From ~5 × 10⁵ plaques, two clones (λ NGK1·5C1 and λ 2NGK2·5B1) were isolated. A second randomly primed cDNA library (~3 × 10⁶ plaques) was screened by hybridization with the cDNA inserts of the initial clones, yielding two clones (including λ NGK1·23C2) that hybridized with λ NGK1·5C1 and six clones (including λ NGK2·18A1 and λ NGK2·19A4) that hybridized with λ NGK2·5B1. Nucleotide sequence analysis of clones λ NGK1·23C2 and λ NGK1·5C1 revealed that they encode a protein composed of 499 amino residues (NGK1) whose sequence is identical with that of the rat brain BK2 protein reported previously [9].

Fig.1 shows the cDNA sequence encoding the NGK2 protein, determined with clones λ NGK2·18A1, λ NGK2·19A4 and λ NGK2·5B1, together with the deduced amino acid sequence. The translational initiation site was assigned to the first ATG triplet that appears downstream of a nonsense codon, TAA (nucleotide residues −15 to −13). The nucleotide sequence surrounding the initiating codon agrees reasonably well with the consensus sequence [27]. A

translational termination codon, TGA, occurs in the frame after codon 511 specifying isoleucine. Thus the NGK2 protein is composed of 511 amino acid residues (including the initiating methionine) and has a calculated *M_r* of 57 925. The size of the NGK2 mRNA was estimated to be ~7000 nucleotides by blot hybridization analysis of poly(A)⁺ RNA from NG108-15 cells.

The NGK2 protein, like other potassium channel proteins [2–11], has a basic structure corresponding to a single internal repeat of the sodium channel and the calcium channel [28,29]. It contains five hydrophobic segments (S1, S2, S3, S5 and S6) and one positively charged segment (S4). The conserved charged residues in segment S2 and in segment S3 are also retained. The sequence similarity observed suggests that the NGK2 protein has the same transmembrane topology as proposed for the single internal repeats of the other voltage-gated ionic channels and that it forms a homo- or heteromultimer to surround a central pore as a channel. This model is consistent with both the two potential *N*-glycosylation sites (asparagine residues 220 and 229) being located on the extracellular side of the membrane.

Fig.2 shows an alignment of the amino acid sequence of the NGK2 protein with those of the *Drosophila* *Shaw*, *Shaker* and *Shab* proteins and of the mammalian RCK1, BK2 and drk1 proteins. The amino acid sequence of the NGK2 protein is more closely related to that of the *Shaw* protein (56% amino acid identities) than to those of the other potassium channel proteins (43%–45% amino acid identities). On the basis of the sequence alignment, a phylogenetic tree representing the evolutionary relationships among the seven potassium channel proteins was inferred by the neighbor-joining method [30], as shown in fig.3. According to the phylogenetic tree, known members of the potassium channel family are classified into three subfamilies: NGK2/*Shaw* subfamily, RCK1·BK2/*Shaker* subfamily and drk1/*Shab* subfamily. From the phylogenetic positions of the divergence of mammalian and *Drosophila* genes, it is evident that the three subfamilies diverged before the separation of vertebrates and insects. After this separation, mammalian genes belonging to the RCK1·BK2/*Shaker* subfamily further diverged during vertebrate evolution. The relationship between NGK2 and *Shaw* (56% amino acid identities) is less close than that between drk1 and *Shab* (72%), between RCK1 and *Shaker* (82%) or between BK2 and *Shaker* (80%). Thus it remains possible that NGK2 is not a mammalian counterpart of *Shaw*. Of interest would be the evolutionary relationships among *Shal*, another *Drosophila* potassium channel gene [6], and NGK2 and the other members of the potassium channel family.

mRNAs specific for the NGK1 and for the NGK2 protein were synthesized by transcription in vitro of the cloned cDNAs and were injected into *Xenopus* oocytes.

										5'-----CCGCGGTCTCTGTGC	-121
CCCCGACGGCTGGGGGGAGGGGGGAAGAGGCCCTGCGCCCCCTCCCGTCGCCAACTCCCTGGCGGAGCTCCCATGGGTGTGCTGGGCGCGCCATGCCTAAGGGGCGCCGCG											-1
1	10	20	30	40	50	60	70	80	90	100	110
Met Gly Gln Gly Asp Glu Ser Glu Arg	Ile Val Ile Asn Val Gly Gly Thr Arg His	Gln Thr Tyr Arg Ser Thr Leu Arg Thr Leu	Pro								
ATG GGC CAA GGG GAC GAG AGC GAG CGC	ATC GTG ATC AAC GTG GGC GGC ACG CGC	CAC CAG ACG TAC CGC TCG ACG CTG CGC	ACG CTG CCC								
120	130	140	150	160	170	180	190	200	210	220	230
Gly Thr Arg Leu Ala Trp Leu Ala Glu	Pro Asp Ala His Ser His Phe Asp Tyr Asp	Pro Arg Ala Asp Glu Phe Phe Phe Asp Arg	His								
GGC ACG CGG CTT GCC TGG CTG GCA GAG	CCG GAC GCC CAC AGC CAC TTC GAC TAT GAC	CCG CGT GCC GAC GAG TTC TTC TTC GAC	CGC CAC								
240	250	260	270	280	290	300	310	320	330	340	350
Pro Gly Val Phe Ala His Ile Leu Asn	Tyr Tyr Arg Thr Gly Lys Leu His Cys Pro	Ala Asp Val Cys Gly Pro Leu Tyr Glu Glu	Glu								
CCG GGC GTC TTC GCT CAC ATC CTG AAC	TAT TAC CGC ACC GGC AAG CTT CAC TGC	CCG GGC GAC GTG TGC GGC CGG CTC TAC	GAG GAG								
360	370	380	390	400	410	420	430	440	450	460	470
Leu Ala Phe Trp Gly Ile Asp Glu Thr	Asp Val Glu Pro Cys Cys Trp Met Thr Thr	Arg Gln His Arg Asp Ala Glu Glu Ala Leu	Asp								
CTG GCC TTC TGG GGC ATC GAC GAG ACG	GAC GTG GAG CCC TGC TGC TGG ATG ACC	TAT CGC CAG CAC CGA GAC GCT GAG GAG	CGC CTG GAC								
480	490	500	510	520	530	540	550	560	570	580	590
Ser Phe Gly Gly Ala Pro Leu Asp Asn	Ser Ala Asp Asp Ala Asp Ala Asp Gly Pro	Gly Asp Ser Gly Asp Gly Glu Asp Glu Glu	Glu								
AGC TTT GGC GGT GCG CCC TTG GAC AAC	AGC GCC GAC GAC GCG GAC GCC GAC GGC	CCC GGC GAC TCG GGC GAC GGC GGC GAC	CTG GAG								
600	610	620	630	640	650	660	670	680	690	700	710
Met Thr Lys Arg Leu Ala Leu Ser Asp	Ser Pro Asp Gly Arg Pro Gly Gly Phe Trp	Arg Arg Trp Gln Pro Arg Ile Trp Ala Leu	Phe								
ATG ACC AAG AGA TTG GCA CTC AGT GAC	TCC CCA GAT GGC CGG CCT GGC GGC TTC	TGG CGC TGG CAA CCG CGC ATC TGG	CGC CTG TTC								
720	730	740	750	760	770	780	790	800	810	820	830
Glu Asp Pro Tyr Ser Ser Arg Tyr Ala	Arg Tyr Val Ala Phe Ala Ser Leu Phe Phe	Ile Leu Val Ser Ile Thr Thr Phe Cys Leu	Glu								
GAG GAC CCC TAC TCA TCC CGC TAC GCG	CGG TAT GTG GCC TTT GCC TCC CTA TTC	ATC CTG GTC TCC ATC ACA ACC TTC TGT	CTG GAG								
840	850	860	870	880	890	900	910	920	930	940	950
Thr His Glu Arg Phe Asn Pro Ile Val	Asn Lys Thr Glu Ile Glu Asn Val Arg	Asn Gly Thr Gln Val Arg Tyr Arg Glu Ala	Glu								
ACT CAC GAG CGC TTC AAC CCC ATC GTG	AAC AAG ACC GAA ATC GAG AAC GTT CGA	AAC GGC ACC CAA GTG CGG TAC TAC	CGG GAA GCA GAG								
960	970	980	990	1000	1010	1020	1030	1040	1050	1060	1070
Thr Glu Ala Phe Leu Thr Tyr Ile Glu	Gly Val Cys Val Val Trp Phe Thr Phe Glu	Phe Leu Met Arg Val Val Phe Cys Pro Asn	Lys								
ACG GAG GCC TTC CTC ACC TAC ATC GAG	GGC GTC TGC GTG GTC TGC ACC TTC GAG	TTC CTC ATG CGT GTC GTC TTC TGC	CCC AAC AAG								
1080	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190
Val Glu Phe Ile Lys Asn Ser Leu Asn	Ile Ile Asp Phe Val Ala Ile Leu Pro Phe	Tyr Leu Glu Val Gly Leu Ser Gly Leu Ser	Ser								
GTG GAA TTC ATC AAG AAC TCC CTC AAT	ATC ATT GAC TTT GTG GCC ATT CTC CCC	TTC TAC CTG GAG GTG GGC CTA AGC	GGC CTG TCC								
1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310
Lys Ala Ala Lys Asp Val Leu Gly Phe	Leu Arg Val Val Arg Phe Val Arg Ile	Leu Arg Ile Phe Lys Leu Thr Arg His Phe	Val								
AAA GCC GCC AAG GAC GTT CTG GGC TTC	CTG CGC GTC GTC CGC TTC GTG CGC ATC	CTG CGC ATC TTC AAG CTG ACC CGC	CAC TTC GTG GGC								
1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
Leu Arg Val Leu Gly His Thr Leu Arg	Ala Ser Thr Asn Glu Phe Leu Leu Leu Ile	Ile Phe Leu Ala Leu Gly Val Leu Ile Phe	Ala								
CTG AGG GTC CTG GGC CAC ACG CTC CGT	GCC AGC ACC AAC GAG TTC CTG CTG CTT	ATC ATC TTC CTG GCC CTG GGA GTG	CTC ATC TTT GCC								
1440	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550
Thr Met Ile Tyr Tyr Ala Glu Arg Ile	Gly Ala Gln Pro Asn Asp Pro Ser Ala Ser	Glu His Thr His Phe Lys Asn Ile Pro Ile	Gly								
ACC ATG ATC TAC TAC GCC GAG AGG ATA	GGG GCA CAG CCC AAT GAC CCC AGC GCC	AGC GAA CAC ACA CAC TTT AAA AAC	ATC CCC ATC GGC								
1560	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670
Phe Trp Trp Ala Val Val Thr Met Thr	Thr Leu Gly Tyr Gly Asp Met Tyr Pro Gln	Thr Trp Ser Gly Met Leu Val Gly Ala Leu	Cys								
TTC TGG TGG GCT GTG GTC ACC ATG ACG	ACA CTG GGC TAT GGA GAC ATG TAT CCC	CAG ACG TGG TCT GGA ATG CTG GTG	GGA GCC TTG TGT								
1680	1690	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790
Ala Leu Ala Gly Val Leu Thr Ile Ala	Met Pro Val Pro Val Ile Val Asn Asn	Phe Gly Met Tyr Tyr Ser Leu Ala Met	Ala Lys Gln								
GCT CTG GCT GGT GTG CTG ACC ATT GCC	ATG CCG GTG CCT GTC ATC GTG AAC	AAT TTT GGG ATG TAC TAC TCT TTA	GCC ATG GCT AAG CAG								
1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910
Lys Leu Pro Lys Lys Lys Lys Lys His	Ile Pro Arg Pro Pro Gln Leu Gly Ser Pro	Asn Tyr Cys Lys Ser Val Val Asn Ser Pro	His								
AAA CTA CCA AAG AAA AAA AAG AAG CAT	ATT CCG CGG CCA CCA CAG CTG GGA	TCT CCC AAT TAT TGT AAA TCT GTC	GTA AAC TCT CCA CAG								
1920	1930	1940	1950	1960	1970	1980	1990	2000	2010	2020	2030
His Ser Thr Gln Ser Asp Thr Cys Pro	Leu Ala Gln Glu Glu Ile Leu Glu Ile	Asn Arg Ala Gly Arg Lys Pro Leu Arg	Gly Met Ser								
CAC AGT ACT CAG AGT GAC ACA TGC CCG	CTG GCC CAG GAA GAA ATT TTA GAA	ATT AAC AGA GCA GGT AGG AAA CCT	CTC AGA GGC ATG TCG								
144	145	146	147	148	149	150	151	152	153	154	155
ATC TGA CCTTTCACCTCCGCCCTGTAGCAATGATTCCAGATCCAGTCAGACTGCTTCTTAGTTCCACGGGCGACCCAGGATCCTGTGCCAACTTTGAGTTGTGGAGCCTGGGAC	1648										
CCCAGGGAGATGCTGGGCGGC-----3'											

Fig.1. Nucleotide sequence of the cDNA encoding the NGK2 protein and the deduced amino acid sequence. The nucleotide sequence was determined using clones λ NGK2-18A1 (carrying nucleotides -136 to 1014), λ NGK2-19A4 (665-1669) and λ NGK2-5B1 (925-1362). Nucleotide residues are numbered in the 5' to 3' direction from the first residue of the ATG initiation triplet and the preceding residues are indicated by negative numbers. Numbers of the nucleotide residues at the right-hand end of the individual lines are given. Amino acid residues are numbered from the initiating methionine. The putative transmembrane segments S1-S6 are overlined; the termini of each segment have been tentatively assigned.

Large outward currents were elicited in injected oocytes upon depolarization from a holding potential of -60 mV under voltage clamp (fig.4A,B). The size of the current at 0 mV was $18.0 \pm 10.7 \mu\text{A}$ (mean \pm SD, $n = 29$) for oocytes injected with the NGK1-specific mRNA and $25.4 \pm 10.9 \mu\text{A}$ ($n = 26$) for oocytes injected with the NGK2-specific mRNA. Under the same experimental conditions, non-injected oocytes showed small outward currents, but the size of the current was at most $0.15 \mu\text{A}$

($0.07 \pm 0.05 \mu\text{A}$, $n = 30$). When the test potential was varied, the outward current began to appear at -30 mV in oocytes injected with the NGK1-specific mRNA, whereas the outward current was elicited only at more positive potentials in oocytes injected with the NGK2-specific mRNA (fig.4A-C). The outward currents in oocytes injected with the NGK1-specific and with the NGK2-specific mRNA showed fast activation, attaining maximal amplitudes in tens of milliseconds,

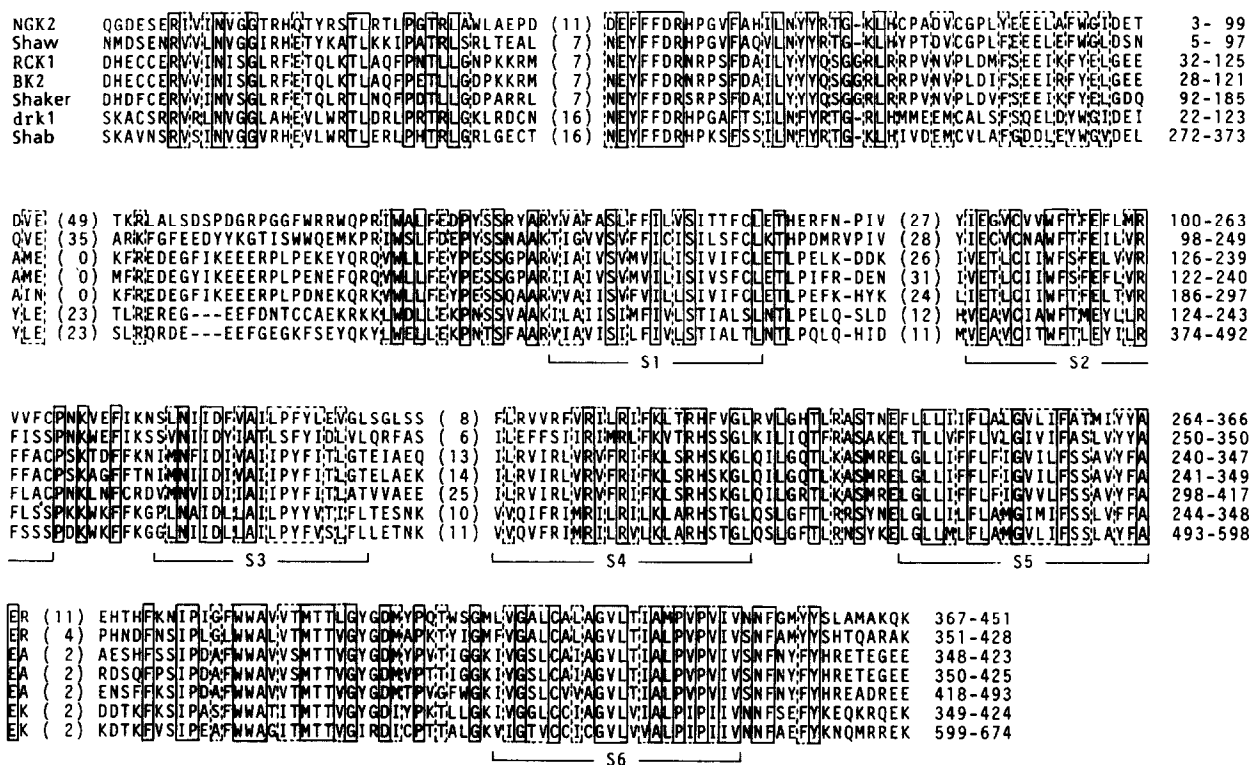


Fig.2. Alignment of the amino acid sequences of different potassium channel proteins. The one-letter amino acid notation is used. The seven sequences compared (from top to bottom) are as follows: NGK2; Shaw2 [6]; RCK1 [8]; BK2 [9] (NGK1); SHA1 [2]; drk1 [10]; Shab11 [6]. Amino acid residues are numbered from the initiating methionine, and numbers of the residues on each line are given on the right-hand side. Sets of seven identical residues at one aligned position are enclosed with solid lines, and sets of seven identical or conservative residues [31] at one aligned position with broken lines. Highly divergent segments are represented by the sum of residues in each segment given in parentheses. For evaluating sequence similarity, a continuous stretch of gaps (-) was counted as one substitution regardless of its length. The positions of segments S1-S6 are indicated (see the legend to fig.1).

but were inactivated very slowly during 1-s voltage steps to 0 mV, the decrease in current size being $9 \pm 3\%$ ($n=9$) and $13 \pm 7\%$ ($n=9$) of the peak value, respectively.

Ion substitution experiments showed that the reversal potential of the tail currents shifted by $+55.6 \pm 1.8$ mV ($n=6$) and by $+53.1 \pm 2.0$ mV ($n=6$) per 10-fold increase of external K^+ concentration, respectively, in

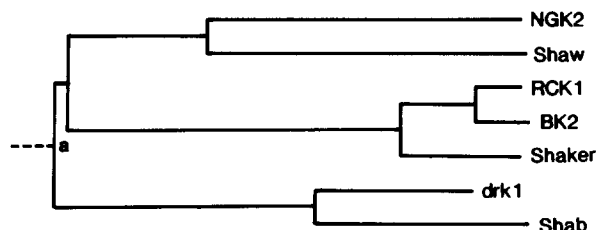


Fig.3. Phylogenetic tree of the potassium channel family. The neighbor-joining method [30] was used; the calculation procedure for the phylogenetic tree inference has been described previously [32]. The deepest root 'a' was determined by comparing the amino acid sequences of the potassium channel proteins with those of sodium channels and calcium channels (data not shown). Lengths of horizontal lines of the tree are proportional to estimated numbers of amino acid substitutions.

oocytes injected with the NGK1-specific and with the NGK2-specific mRNA. These values are close to the theoretical value of $+58.6$ mV, calculated from the Nernst equation, and indicate that the outward currents observed in injected oocytes are carried by potassium ions.

The outward currents were examined for sensitivity to pharmacological agents added to the bathing solution. The concentrations required for inhibiting the currents by 50% (IC_{50}) were as follows (the former and latter values refer to oocytes injected with the NGK1-specific and with the NGK2-specific mRNA, respectively): 0.2 mM and 0.6 mM 4-aminopyridine; >10 mM and 0.1 mM tetraethylammonium; and 0.8 mM and 1 mM quinine. Apamin (1 μ M) and tolbutamide (100 μ M) were ineffective.

Single-channel currents were recorded from inside-out membrane patches isolated from injected oocytes using a nominally Ca^{2+} -free K^+ solution on the cytoplasmic side (fig.4D,E). The channel in patches of oocytes injected with the NGK1-specific mRNA showed a slope conductance of 17.5 ± 0.4 pS (5 patches), but smaller conductance levels were frequently observed

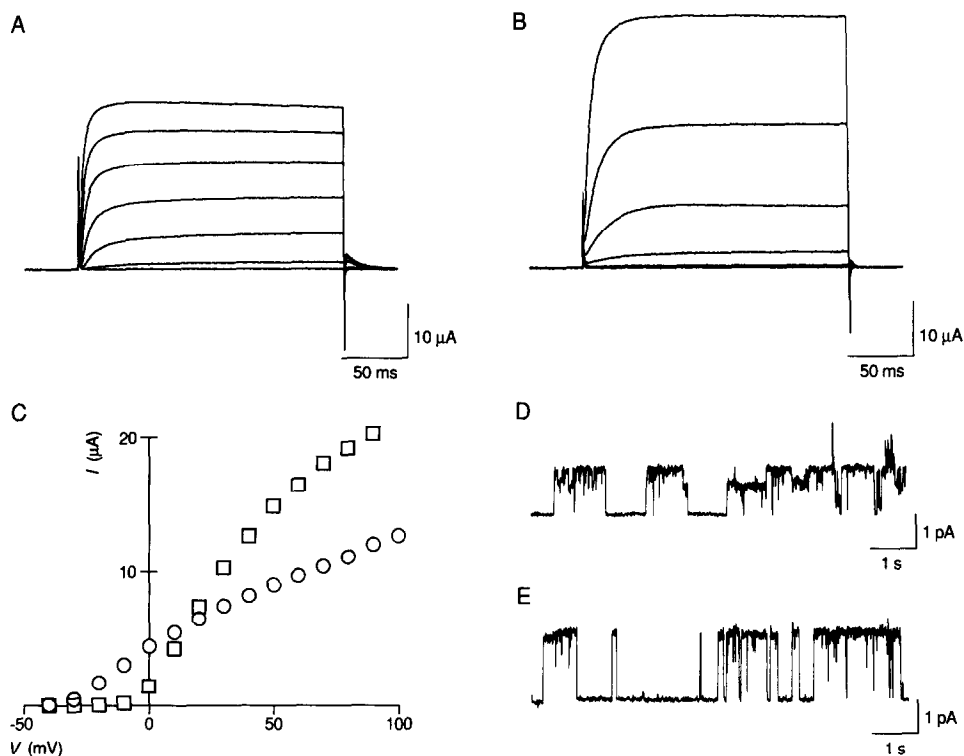


Fig.4. Properties of outward currents in *Xenopus* oocytes injected with the NGK1-specific and with the NGK2-specific mRNA. (A,B) Outward currents in response to depolarizing voltage steps in an oocyte injected with the NGK-1 specific mRNA (A) or with the NGK2-specific mRNA (B). The membrane potential was held at -60 mV and stepped to test potentials ranging from -40 mV to $+20$ mV in 10 -mV increments. In B, overlapping records were obtained upon depolarization to -40 mV, -30 mV and -20 mV. (C) The whole-cell current-voltage relation obtained from an oocyte injected with the NGK1-specific mRNA (circles) or with the NGK2-specific mRNA (squares). The oocytes used for C were different from those used for A and B. (D,E) Single-channel currents from an oocyte injected with the NGK1-specific mRNA (D) or with the NGK2-specific mRNA (E). Inside-out patch. Outward current upwards. Membrane potential, 0 mV. Records were low-pass filtered at 0.1 kHz and sampled at 0.25 kHz.

(fig.4D). The channel in patches of oocytes injected with the NGK2-specific mRNA showed a larger slope conductance (26.4 ± 1.7 pS; 6 patches), and subconductance levels rarely occurred (fig.4E).

The cDNA expression studies indicate that both the NGK2 protein, which is closely related to the *Drosophila Shaw* gene product, and the NGK1 protein, which is a member of the RCK1 · BK2/*Shaker* subfamily, form potassium channels with properties of delayed rectifiers, although the NGK2 and NGK1 potassium channels exhibit some kinetic and pharmacological differences. It has been reported that *Drosophila Shaker* cDNAs are expressed to produce A-type potassium channels [12–14], while expression of mammalian RCK1 and related cDNAs of the same subfamily yields potassium channels with properties of delayed rectifiers and possibly A-type channels [11,15,16]. The drk1 protein, which is a mammalian counterpart of the *Drosophila Shab* gene product, is expressed as a delayed rectifier class channel [10]. The molecular basis for the electrophysiological differences between these voltage-gated potassium channels remains to be elucidated.

Acknowledgements: This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation, the Japanese Foundation of Metabolism and Diseases, and the Japan Heart Foundation.

REFERENCES

- [1] Hille, B. (1984) *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland, MA.
- [2] Tempel, B.L., Papazian, D.M., Schwarz, T.L., Jan, Y.N. and Jan, L.Y. (1987) *Science* 237, 770–775.
- [3] Schwarz, T.L., Tempel, B.L., Papazian, D.M., Jan, Y.N. and Jan, L.Y. (1988) *Nature* 331, 137–142.
- [4] Pongs, O., Kecskemethy, N., Müller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H.H., Canal, I., Llamazares, S. and Ferrus, A. (1988) *EMBO J.* 7, 1087–1096.
- [5] Kamb, A., Tseng-Crank, J. and Tanouye, M.A. (1988) *Neuron* 1, 421–430.
- [6] Butler, A., Wei, A., Baker, K. and Salkoff, L. (1989) *Science* 243, 943–947.
- [7] Tempel, B.L., Jan, Y.N. and Jan, L.Y. (1988) *Nature* 332, 837–839.
- [8] Baumann, A., Grupe, A., Ackermann, A. and Pongs, O. (1988) *EMBO J.* 7, 2457–2463.
- [9] McKinnon, D. (1989) *J. Biol. Chem.* 264, 8230–8236.
- [10] Frech, G.C., Van Dongen, A.M.J., Schuster, G., Brown, A.M. and Joho, R.H. (1989) *Nature* 340, 642–645.

- [11] Stühmer, W., Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Giese, K.P., Perschke, A., Baumann, A. and Pongs, O. (1989) *EMBO J.* 8, 3235-3244.
- [12] Timpe, L.C., Schwarz, T.L., Tempel, B.L., Papazian, D.M., Jan, Y.N. and Jan, L.Y. (1988) *Nature* 331, 143-145.
- [13] Iverson, L.E., Tanouye, M.A., Lester, H.A., Davidson, N. and Rudy, B. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5723-5727.
- [14] Timpe, L.C., Jan, Y.N. and Jan, L.Y. (1988) *Neuron* 1, 659-667.
- [15] Stühmer, W., Stocker, M., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A. and Pongs, O. (1988) *FEBS Lett.* 242, 199-206.
- [16] Christie, M.J., Adelman, J.P., Douglass, J. and North, R.A. (1989) *Science* 244, 221-224.
- [17] Nirenberg, M., Wilson, S., Higashida, H., Rotter, A., Krueger, K., Busis, N., Ray, R., Kenimer, J.G. and Adler, M. (1983) *Science* 222, 794-799.
- [18] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) *Biochemistry* 18, 5294-5299.
- [19] Aviv, H. and Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* 69, 1408-1412.
- [20] Lauffer, L., Garcia, P.D., Harkins, R.N., Coussens, L., Ullrich, A. and Walter, P. (1985) *Nature* 318, 334-338.
- [21] Henikoff, S. (1987) *Methods Enzymol.* 155, 156-165.
- [22] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.
- [23] Thomas, P.S. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5201-5205.
- [24] Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K. and Green, M.R. (1984) *Nucleic Acids Res.* 12, 7035-7056.
- [25] Konarska, M.M., Padgett, R.A. and Sharp, P.A. (1984) *Cell* 38, 731-736.
- [26] Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S. and Sakmann, B. (1986) *Pflügers Arch. Gesamte Physiol.* 407, 577-588.
- [27] Kozak, M. (1984) *Nucleic Acids Res.* 12, 857-872.
- [28] Numa, S. and Noda, M. (1986) *Ann. N.Y. Acad. Sci.* 479, 338-355.
- [29] Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. and Numa, S. (1987) *Nature* 328, 313-318.
- [30] Saitou, N. and Nei, M. (1987) *Mol. Biol. Evol.* 4, 406-425.
- [31] Dayhoff, M.O., Schwartz, R.M. and Orcutt, B.C. (1978) in: *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3 (Dayhoff, M.O. ed.) pp. 345-352, National Biomedical Research Foundation, Silver Springs, MD.
- [32] Iwabe, N., Kuma, K., Hasegawa, M., Osawa, S. and Miyata, T. (1989) *Proc. Natl. Acad. Sci. USA*, in press.