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ISOLATION OF THE PARTIAL GENOMIC SEQUENCE FOR RHK7(KV1.7), A NOVEL SHAKER-LIKE VOLTAGE SENSITIVE POTASSIUM CHANNEL EXPRESSED IN RAT HEART

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ABSTRACT

We have used the polymerase chain reaction to isolate partial sequences of voltage-sensitive potassium channels expressed in the rat heart. One of these PCR fragments represented a novel sequence and was used to screen a rat genomic library. We isolated a genomic clone, RHK7(Kv1.7), that contains a 987 bp open reading frame representing a putative novel potassium channel. An analysis of the deduced amino acid sequence revealed that this channel is a member of the Shaker-related subclass of vertebrate potassium channels. Northern blot analysis confirmed that RHK7(Kv1.7) is expressed in rat heart tissue. A unique feature of this RHK7(Kv1.7) genomic clone is that it does not appear to code for the amino terminal end of the potassium channel. It does, however, contain a 3' splice acceptor consensus sequence. We postulate that, unlike other Shaker-related vertebrate potassium channel genes, the complete RHK7(Kv1.7) gene contains an intron.

INTRODUCTION

Potassium channels play an essential role in governing the excitability of cardiac cells. These channels are largely responsible for governing the membrane potential of resting cardiac cells; in addition, they help determine action potential shape and duration[1]. Recently, cardiac potassium channels have gained attention as a major target for therapeutic intervention. Potassium channel blocking drugs tend to delay cell repolarization and prolong the action potential[2]. They are well known to have antiarrhythmic effects[3] and are also effective in suppressing ventricular fibrillation[4]. A second class of drugs, the potassium channel openers, also have therapeutic potential. These agents have antihypertensive effects and may protect the myocardium during ischemia[5,6]. The clinical use of drugs affecting potassium channels, however, has been limited by a lack of specificity of drug action[7]. One obstacle to achieving such specificity may be the large number of potassium channel subtypes in different tissues and even within single cells. Clearly, a better understanding of cardiac potassium channels, particularly at the molecular level, may not only enhance our understanding of cardiac physiology but may also facilitate the design and testing of more specific therapeutic agents.

At least ten different potassium currents, each of which may be due to one or more distinct potassium channel subtypes, have so far been identified in the heart. These currents can be roughly divided into two groups: ligand-gated currents and voltage-gated currents. Of the ligand-gated currents, four appear to be mediated by relatively large-conductance channels that are closed under normal physiologic conditions. One of these, Ik(ATP)[8,9] is activated when levels of intracellular ATP are low. This channel may open during hypoxic conditions to decrease heart rate and oxygen demand. Two other currents, Ik(AA) and Ik(PC) have been identified in neonatal rat atrial cells[10] and are activated by arachidonic acid and phosphatidylcholine, respectively. Like Ik (ATP), these currents may activate under conditions of physiologic stress to help preserve cell function. A fourth ligand-gated current, Ik(Na)[11] is activated by increased intracellular sodium. Its function is not yet clear; it may be important in

the event of Na/K pump failure or during disturbances of Na+/Ca++ exchange.

Two other ligand gated currents, Ik(Ach) and Ik(Ca++) are thought to play crucial roles in the heart under normal, physiologic conditions. Ik(Ach)[12] is activated by acetylcholine and is likely to play an important role in mediating the heart's response to parasympathetic stimulation. This current may be particularly important in the SA node, where it would result in a slowing of the heart rate. Less is known about the calcium-activated current, Ik(Ca++)[13], which appears to contribute to repolarization during the initial part of the action potential.

In addition to the ligand-gated currents, at least four distinct voltage-gated potassium currents have been identified in cardiac tissues. One of these, Ik(i)[14], is an inwardly rectifying current that is active at hyperpolarized potentials and may help anchor the resting potential of cardiac cells. Ik[15,16], a delayed rectifier, opens in response to depolarization. This current is responsible for the late repolarization phase of the action potential. A third voltage-gated current, Ik(p)[17], is also activated by depolarization but remains open for the entire duration of the action potential. It may prevent the action potential plateau from becoming too positive, an event which could result in decreased calcium influx and decreased contractility. The fourth voltage-gated current, Ik(to) is a transient outward or rapidly inactivating current. This current has been described in nodal tissue[18], ventricle[19,20,21], and crista terminalis[22], and is likely to be due to several different channels. Ik(to) is active during phase two of the action potential and is thought to help regulate action potential duration.

In recent years, it has become possible to move from the study of potassium currents to the study of potassium channels at a molecular level. The cloning of the Shaker locus in Drosophila provided the starting point for such investigation[23,24,25]. The Shaker gene codes for at least five different voltage-sensitive potassium channel proteins by alternative splicing.[26] These channel proteins all share a common core region but have divergent carboxyl and amino termini. These divergent ends play an

important role in determining the different elecrophysiologic properties of the channels[27].

The precise structure of these voltage-sensitive potassium channels has not yet been determined. The conserved core region of the Shaker sequence contains six hydrophobic domains, which have been referred to as H1-H6[24]. This region also contains an S4 domain, a sequence containing a series of positively charged amino acids. The S4 domain is also found in voltage-sensitive sodium[28] and calcium[29] channels, and is postulated to serve as the voltage sensor. A widely held model states that six of these domains, H1, H2, H3, S4, H4, and H6 are membrane-spanning[30 31]. The H5 domain is postulated to be on the exterior side of the membrane, where it may form part of the channel pore[32], whereas the amino and carboxyl termini are thought to be cytoplasmic. A comparison with the structures of voltage-sensitive sodium and calcium channels suggests that four potassium channel proteins may come together as multimers to form a complete channel. Several recent studies in which heteromultimeric channels have been expressed and characterizied support this hypothesis[33 34].

Since the cloning of Shaker, three distinct but homologous potassium channels, Shab, Shaw, and Shal, have been cloned in Drosophila.[35] Many vertebrate genes encoding voltage sensitive potassium channels have also been identified, primarily in nervous tissue. Like the original Shaker channels, these gene products vary widely in their terminal regions but share highly conserved hydrophobic and S4 domains. The vertebrate channels can generally be classified into four subfamilies based on the homology of their core regions to those of the Drosophila channels[36]. These subfamilies so far include a Shaker-related subfamily (Kv1.1-Kv1.6[37 38 39 40 41 42 43 44]), a Shab-related subfamily (Kv2.1[45]), a Shaw-related subfamily (Kv3.1-Kv3.4 [46 47 48 49]), and and a Shal-related subfamily (Kv4.1-Kv4.2[50 51]). One potassium channel that does not fit into these categories is IsK, a channel orginally cloned in rat kidney[52]. This channel, which was found mainly in epithelial cells, contains only one transmembrane domain and has little similarity to other cloned potassium channels. The electophysiologic properties of most of these channels have been determined

by expressing them individually in Xenopus oocytes. The currents they produce are diverse, and include rapidly inactivating, non-inactivating, and delayed rectifier currents.

The search for potassium channel genes has only recently been extended to the heart. Several channels have now been cloned from cardiac libraries, including RHK1(Kv1.4),[53] and RK1-RK5 (Kv1.1, Kv1.2, Kv1.4, Kv1.5, and Kv4.2)[54] from rat heart, mIsK from mouse[55], and HK1 (Kv1.5) and HK2 (Kv1.4) from humans[56]. Expression of drk1 (Kv2.1) and Kv3.3 in cardiac tissue has also been reported[54, 49]. The heart channels so far identified are identical to channels found in the brain and in other tissues.

To further characterize cardiac voltage-sensitive potassium channels at the molecular level, we have used the polymerase chain reaction to screen rat heart cDNA for novel channels. We then screened a rat genomic library with one of the PCR products to isolate a genomic clone. This report describes the partial genomic sequence of RHK7 (Kv1.7), a novel, Shaker-like voltage-sensitive potassium channel that is expressed in rat heart. This channel is unique among Shaker-related homologs in that it appears to have an intron between the H1 and H2 domains. Moreover, unlike other Shaker-like channels and channels homologous to Shaw, Shab and Shal, RHK7 (Kv1.7) does not appear to be expressed in brain.

MATERIALS AND METHODS

PCR of rat heart mRNA:

Rat heart poly-A selected mRNA was copied into first-strand cDNA using oligo(dT) priming and reverse transcriptase. Degenerate oligonucleotides were constructed to correspond to the coding regions for amino acids MTTVGYG and PVPVIVS. The amino acids correspond to residues 440-447 and 473-480 of the Shaker A1 gene.[57] The nucleotide sequences were 5'ATGACNACNGTNGGNTANGG3' (Sense primer) and 5'TNACNATNACTGGNACNGG3' (antisense primer), where N is equal to A, T, G, or C. PCR conditions were the following: 94 degrees C for 30 seconds, 50 degrees C for 30 seconds, and 72 degrees C for 90 seconds for 40 cycles, followed by 72 degrees C for 10 min. The PCR products were separated on an agarose gel and fragments of the predicted 120bp length were isolated and subcloned into the Sma1 site of the BlueScript SK+ vector.

DNA sequence analysis of PCR products:

The PCR products were sequenced using single stranded DNA template. Recombinant plasmids were isolated from BSJ-72 host cells and digested with EcoR1 and Not1 to confirm presence of 120bp inserts. Single-stranded plasmid DNA was then synthesized from recombinants containing appropriate inserts using the following protocol. A single colony of BSJ-72 cells containing recombinant plasmid was inoculated into 2 ml 2XYT broth, 100ug/ml carbenicillin and M13K07 phage (Stratagene). Cells were incubated at 37 degrees for 60 min., and Kanamycin was added to a final concentration of 70ug/ml. Cells were then incubated at 37 degrees overnight. Cells were spun in microfuge, 10 min., and 1 ml supernatant was transferred to a fresh tube. 200 ul 20% PEG, 2.5M NaCl was added and the phagemids were allowed to precipitate 15 min. at room temperature. The tubes were spun in a microfuge for 5 min, and the supernatant removed. The pellet was resuspended in 200ul TE and 20ul NaAcetate, and extracted with phenol, phenol:CHCl3, and CHCl3. DNA was precipitated with 2.5 vol. EtOH at -80 degrees. The single stranded DNA was recovered by spinning 20 min. in a microfuge, washed with 70% EtOH and resuspended in 20ul TE buffer. Single stranded DNA template was sequenced by a variation of the chain termination technique, using the Sequenase version 2.0 sequencing kit (United States Biochemical Corp).

Preparation of 32-P labelled PCR fragment probe:

Probes were constructed from one of the novel PCR fragments (PCR10) using a random priming technique. To isolate the PCR10 fragment, BSJ-72 cells containing the recombinant BlueScript Sk+ plasmid

containing the PCR10 fragment were grown overnight in LB broth and 50 ug/ml carbenicillin. 1.5 ml of culture was spun for 3 min. in a microfuge; the pellet was resuspended in 200 ul 50 mM glucose, 25 mM Tris pH 8.0, 10 mM EDTA and allowed to incubate 5 min at room temperature. 400 ul 0.2 N NaOH, 1% SDS was added; the mixture inverted 15 times; and iced for 5 min. 300 ul 3M KAcetate pH 4.8 was added; the mixture inverted 10 times; and iced for 10 min. The mixture was then microfuged 10 min; the supernatant transferred to a fresh tube; and microfuged for an additional 10 min. The supernatant was extracted twice with phenol:CHCl3 alcohol and once with CHCl3/Isoamyl alcohol. DNA was precipitated with an equal volume of isopropanol for 10 min. at room temperature. The pellet was spun out 10 min in a microfuge, washed with 70% EtOH, dried, and then resuspended in 100 ul TE, 20 ug/ml RNase and incubated 30 min at 37 degrees.

The isolated plasmid DNA was digested with BamH1 and Pst1 in a total volume of 400 ul for two hours at 37 degrees (the Sma1 site is not recreated after subcloning; BamH1 and Pst1 are the nearest flanking sites in the polylinker). The solution was then extracted with an equal volume of phenol: CHCl3 and with CHCl3. DNA was precipitated by adding 1/10 volume NaAcetate pH 5.2 and 2.5 volumes EtOH, and freezing at -80 degrees. DNA was then pelleted by spinning 20 min. in a microfuge; washed with 70% EtOH, and resuspended in 25 ul ddH2O. The cut plasmid DNA was then run on a 2% low gelling temperature TAE agarose gel, and the 120 bp PCR10 fragments were excised. The agarose fragments containing the DNA were heated 15 min. at 65 degrees C. The melted agarose was then extracted twice with phenol and once with phenol: CHCl3. DNA was precipitated with 1/10 volume NaAcetate, 2.5 volumes EtOH at -80 degrees C, pelleted by spinning 20 min. in a microfuge, washed with 70 % EtOH and resuspended in 25 ul ddH2O. Approximately 1.25 ug of DNA was recovered.

To make the radiolabelled probe, 0.1 ug of the 120-bp PCR fragment DNA (PCR10) was labelled using a random priming labelling kit from Boehringer Mannheim Biochemicals. The probe was labelled to a specific activity of 3.4 X 10^8 CPM/ug with 32-P labelled dCTP. The probe DNA was purified on a Biogel P-60 column and boiled for 10 min immediately prior to adding to the hybridization buffer (see below).

Screening of the rat genomic DNA library:

Approximately 500,000 plaques from a rat genomic lambda-DASH library (Stratagene) were screened with the PCR10 fragment. To plate out the library, LE 392 e.coli were grown overnight in NZY broth supplemented with 0.2% Maltose. Cells were spun at 7K for 10 min. in a Sorvall centrifuge and resuspended in 20 ml. of 10mM MgSO4. 50,000 phage in a total volume of 100ul SM Buffer were then added to 600ul resuspended LE392 cells and incubated for 20 min. at 37 degrees C. This mixture was then added to 6.5

ml NZY top agarose and 2.5 ml. NZY broth at 55 degrees C, and plated on to dried NZY plates. The plates were incubated approximately 7 hours, until plaques had formed, and then refrigerated at 4 degrees for two hours. Filter lifts were performed with S+S NC nitrocellulose filters (Schleicher and Schuell). Filters were denatured for 1 min. in 0.5N NaOH, 1.5M NaCl; neutralized for 5 min. in 0.5M Tris pH 7.4, 1.5M NaCl; and rinsed 30 sec. in 2X SSC. Filters were dried on Whatman 3mm paper and baked 2 hours at 80 degrees C. Filters were then washed in 5X SSC, 0.5% SDS, 1mM EDTA for one hour at 42 degrees C.

Prehybridization was performed for 4 hours at 42 degrees C in 50% formamide, 5X SSC, 1X Denhardt's solution, 1% SDS, 20mM NaPhosphate pH 7.0, 0.1 mg/ml denatured salmon sperm, using a total volume of 50 ml buffer per ten filters. Hybridization was performed overnight at 42 degrees C in fresh buffer with the addition of 0.1ug of 32-P labelled PCR10 probe (see above). Filters were washed twice for 30 min. in 2X SSC, 0.1% SDS at room temperature and twice for 30 min. in 0.1X SSC, 0.1% SDS at 65 degrees C. Filters were then autoradiographed overnight at -80 degrees using Kodak XAR film.

Isolation of RHK 7 genomic clones:

Nine independently hybridizing phage from the genomic library screen were isolated and stored in 0.5 ml SM buffer over 20 ul CHCl3. The phage were then replated and screened in the manner described above. This process was repeated until a pure phage stock was obtained. The titer of the resulting phage stocks was too low for use in isolating phage DNA. High titer phage stocks were prepared by isolating 10 plaques from a single clone, storing overnight in 0.5 ml SM buffer, and then plating 10 ul of this stock on to NZY plates as previously described. The plates were grown until confluent lysis occurred. 5 ml of SM buffer and 20 ul of CHCl3 was then added to each plate, and the plates were rocked gently for 5 hours at 4 degrees C. The SM buffer was drained, the cell debris spun out at 10K for 15 min., and the resulting pure phage stock stored over 40 ul CHCl3 at 4 degrees C. The resulting titer was approximately 10^5 pfu/ul.

DNA was isolated from four of the hybridizing phage. To isolate phage DNA, LE 392 cells were first grown overnight in 50 ml of NZY broth, 0.2% Maltose and then resuspended in 20 ml of iced 10 mM MgSO4. Approximately 10^7 phage from the high titer stocks were added to 200 ul of the resuspended cells, incubated for 20 min. at 37 degrees C., inoculated into 25 ml of prewarmed NZY broth and incubated for approximately 5 hours at 37 degrees C. Upon cell lysis, 0.4 ml CHCl3 was added and the mixture incubated for an additional 20 minutes. 2ug/ml RNase and 2ug/ml DNase were then added and the mixtures incubated for one hour at 37 degrees C. Phage were precipitated by adding an equal volume of 20% PEG, 2.5M NaCl in SM buffer (without gelatin) and incubating on ice 90 min. The phage pellets were spun out for 20 min at 7K in a Sorvall centrifuge and the

supernatant decanted off. The pellets were resuspended in 2 ml SM-gelatain with 5mM EDTA, 0.1% SDS, 50 ug/ml proteinase K and incubated 30 min. at 56 degrees C. The solution was extracted with phenol, phenol:CHCl3 and CHCl3. The isolated phage DNA was precipitated with an equal volume of isopropanol at -80 degrees; spun 20 min. in a microfuge, washed with 70% EtOH and resuspended in 100 ul TE buffer.

Southern blots of phage DNA:

Phage DNA fragments hybridizing to the PCR10 probe were detected by Southern blotting. Phage DNA from the four independent clones was cut with BamH1, and the resulting DNA fragments were separated on a 0.7% TAE agarose gel. The gel was denatured in 0.2N NaOH, 0.6 M NaCl for 30 min. and neutralized in 0.5M Tris pH 7.5, 1.5M NaCl for 30 min. DNA was transferred to NYTRAN (Schleicher and Schuell) filter paper in 20X SSC for 16 hours. The filter was washed twice for 15 minutes in 2X SSC, blotted dry on Whatman 3mm paper, and baked at 80 degrees for two hours. The filter was then washed for one hour in 0.1X SSC, 0.5% SDS at 42 degrees C. Prehybridization was in 50% formamide, 5X SSC, 20mM Naphosphate, 1X Denhardts solution, 1% SDS, 100ug/ml salmon sperm for 4 hours at 42 degrees. Hybridization was for 16 hours at 42 degrees in the same buffer, with the addition of radiolabelled probe. The probe was constructed from 0.1ug of the PCR10 fragment DNA , and was prepared and radiolabelled as described above. The filter was washed twice in 2X SSC, 0.1% SDS for 30 min. at room temperature and twice in 0.1X SSC, 0.1% SDS for 30 min. at 65 degrees. Autoradiography was done using Kodak XAR paper.

Subcloning and sequencing of the RHK7 clone:

A common 2.3 Kb BamH1 fragment noted to hybridize to the PCR10 probe in all four phage DNA samples was subcloned and sequenced. Phage DNA from a single clone was digested with Bam H1 and the DNA fragments were separated on a 1% TAE gel. The gel was stained with EtBr, and the 2.3 Kb fragment was located. Na45 DEAE membrane (Schleicher and Schuell) was then inserted directly above this fragment, and the fragment was run on to the paper. The DNA was eluted by incubating the filter paper in 200 ul 1.0M NaCl, 0.1mM EDTA, 20mM Tris pH8.0 at 65 degrees for one hour. The eluant was extracted with isoamyl alcohol and the DNA precipitated at -80 degrees with 2.0 vol EtOH and glycogen carrier. The DNA was pelleted by spinning for 20 minutes in the microfuge, washed with 70% EtOH, and resuspended in 100 ul TE. To remove any remaining salts, the DNA was reprecipitated with 1/10 vol NaAcetate and 2.5 vol EtOH at -80 degrees C, pelleted, washed with 70% EtOH, and resuspended in 10 ul ddH2O.

BlueScript SK+ plasmid DNA was digested with BamH1, and immediately prior to gel loading was dephosphorylated with calf intestinal phosphatase in the presence of 10mM ZnCl2 and 100 mM Tris pH 9. The cut

plasmid was purified on a 1% TAE agarose gel. DNA was isolated using Na45 DEAE membrane in the manner described above.

Ligations were performed in a total volume of 10 ul using T4 DNA ligase (BRL) and incubating at 14 degrees C overnight. 5 ul of ligation mixture was then used to transform DH5 alpha competent cells. Transformants were selected on LB plates containing carbenicillin and topped with IPTG and X-gal. Plasmid DNA was isolated from DH5 alpha cells and checked for the appropriate 2.3 Kb insert by digesting with BamH1 and separating the fragments on a 1% TAE agarose gel. Plasmids containing the appropriate insert were then used to transform BSJ-72 cells and were sequenced from single stranded template using the protocol described above.

Northern Blots of Rat Heart and Rat Brain RNA:

Northern blots of rat heart and rat brain RNA were probed with the 2.3 Kb BamH1 fragment containing the RHK7 clone. The blots used were identical to those described by Baldwin et. al.[48] Poly(A)+ RNA for these blots was isolated from rat brain tissues and heart using the Fast-Track mRNA isolation procedure (Invitrogen). The RNA was fractionated on 0.7% agarose gels containing formaldehyde and transferred to Nytran (Schleicher and Schuell) membranes.

The Nytran membrane was prehybridized for four hours in 50% formamide, 5X SSC, 20mM NaPhosphate, 0.1%SDS, 100ug/ml salmon sperm DNA at 42 degrees, and hybridized for 16 hours at 42 degrees in the same buffer with the addition of the probe. The probe was constructed from 0.1ug of the 2.3 Kb BamH1 DNA fragment containing the RHK7 clone. This fragment was isolated as described above and labelled with 32-P dCTP using a random priming labelling kit from Boehringer Mannheim Biochemicals to a specific activity of $6.8X10^8$ CPM/ug. After hybridization, the filter was washed twice in 2X SSC, 0.1% SDS for 30 min. at room temperature and twice in 0.1X SSC, 0.1% SDS for 30 min. at 65 degrees. Autoradiography was performed using Kodak XRP paper at -80 degrees for two weeks.

The size of the hybridizing transcripts was determined by comparison to a lambda RNA ladder (BRL) loaded on to and transferred from the same gel. The lambda fragments were hybridized with 32-P dCTP labelled lambda DNA using the techniques described above. Relative quantities of poly(A)+ RNA in each lane were determined by hybridizing with a 32-P dCTP labelled alpha tubulin cDNA provided by Dr. Frank Solomon. Autoradiography was performed on Kodak XRP paper at -80 degrees overnight.

RESULTS

Amplification of novel K+ channel sequences from rat heart cDNA using the polymerase chain reaction:

To isolate novel voltage-sensitive potassium channels in the heart, we used the polymerase chain reaction to amplify partial channel sequences from rat heart cDNA. Degenerate PCR primers were designed to correspond to regions in H5 and H6 that are highly conserved between the Shaker gene and its vertebrate homologs, Kv1.1- Kv1.6. These primers corresponded to the amino acids MTTVGYG and PVPVIVS, amino acids 440-447 and 473-480 of the Shaker A1 gene[55]. DNA fragments of the predicted 120 bp length were recovered from the PCR reaction, subcloned, and sequenced. Of a total of 27 fragments sequenced, 24 (89%) represented sequences identical or nearly identical to the corresponding region of RCK4 (Kv1.4), a channel gene that has been cloned from rat cortex[39] and from rat ventricle[53]. The remaining 4 sequences varied significantly from RCK4 and from other known potassium channels. Excluding the primer regions, the novel PCR fragment sequences (Fig. 1) are 71-74% identical to RCK4 at the nucleotide level, with most differences occuring at the degenerate third codon position. The novel sequences are over 95% identical to each other.

When compared to RCK4 at the amino acid level, all four novel sequences share substitutions K(533)-A, I(535)-V, and A(555)-S. PCR 21 contains only these common substitutions. The three other PCR fragments contain additional substitutions: PCR 4 contains D(531)-C, PCR 10 contains V(537)-E and V(551)-A, and PCR 27 contains A(549)-E. We felt that these PCR fragments may represent one or more novel potassium channels, and we used one of them, PCR 10, to screen a rat genomic library.

Isolation of genomic clones coding for RHK7:

Using the PCR 10 fragment as a probe, we screened approximately 500,000 genomic clones from a rat lambda-DASH library. This screen resulted in the isolation of 9 hybridizing clones. Four of these clones were

chosen for further analysis. Restriction analysis and subsequent Southern blotting demonstrated that all four clones shared a 2.3 Kb BamH1 restriction fragment that hybridized to the PCR10 probe. The 2.3 Kb fragment from one of these clones was then subcloned and sequenced. We refer to this clone as RHK7.

Sequencing of RHK7 revealed an 861 bp open reading frame coding for 287 amino acids (Fig. 2). The open reading frame is flanked by over 590 bp of non-coding sequence at the 3' end and over 850 bp of non-coding sequence at the 5' end. Interestingly, the RHK7 sequence does not exactly correspond to the sequence of the PCR10 probe. When compared to RHK7, PCR 10 contains two substitutions: T(1192)-A and T(1234)-C. The RHK7 sequence does correspond exactly to the sequence of another PCR fragment, PCR 21.

The deduced amino acid sequence of RHK7 contains five distinct hydrophobic domains as well as an S4-like sequence. An alignment of RHK7 with six other Shaker-related potassium channels, RCK1-5[38, 39], and Kv1[41], reveals that the RHK7 hydrophobic domains correspond to the H2, H3, H4, H5, and H6 domains of these channels (Fig. 2). The S4 sequence in RHK7 is identical to the S4 sequences of RCK1, RCK2, and RCK3, and differs by only one to two residues from the S4 sequences of RCK4, RCK5, and Kv1. Like the S4 regions in all of these channels, RHK7 contains five arginine residues and two lysine residues spaced evenly at every third position. Significant conservation also exists in the regions connecting H2 to H3 and S4 to H4. In the H2-H3 linking region, 5 of 10 residues are perfectly conserved and 2 are well conserved. In the S4-H4 region, 10 of 12 residues are perfectly conserved and the remaining two are well conserved.

RHK 7 diverges markedly from the other Shaker-like channels in the C-terminal region and in the H1-H2, H3-S4, and H4-H5 linking regions. The C-terminal region is shorter than that of the other channels (60 residues vs. 69-95 residues), and bears little homology to the other channels beyond a short region immediately following S6. The final three residues, TEV, are identical to the final three residues of RCK2 and similar to the TDV and TDL sequences of the other channels.

The RHK7 genomic sequence contains a putative 3' splice site:

The greatest difference between the RHK7 clone and other potassium channels is its lack of an amino terminus and H1 region. RHK7 also lacks the initiation consensus sequence ACCATGG[58]. The absence of these regions suggested that the RHK7 gene may contain an intron preceding the H2 domain. We thus searched for possible RNA splice acceptor sites in the RHK7 genomic sequence. We found that bp 627-642 of the clone closely match the consensus sequence for a 3' RNA splice acceptor (Fig. 4). The genomic sequence diverges from the consensus sequence only at the third postion and at the final position. This degree of identity to the consensus sequence matches that of the splice acceptor site in another vertebrate potassium channel, Kv3.3[49], which also diverges at two sites. The highly conserved "AG" sequence near the end of the consensus sequence is present in both channel splice sites.

The RHK7 splice site is located in the putative H1-H2 loop and would eliminate the first 16 amino acids of the open reading frame from the channel sequence, leaving 23 amino acids preceding H2. Assuming that the H1-H2 region in RHK7 is approximately the same length as that in the other Shaker-like channels, the splice site falls in the middle of the H1-H2 region.

RHK7 contains several consensus sites for post-translational modification:

The deduced amino acid sequence of RHK7 includes several sites that are candidates for phosphorylation by either Protein Kinase C or Casein Kinase II. In assigning these sites, we have assumed that the complete channel protein would contain a cytoplasmic N-terminus and C-terminus and would have six membrane-spanning domains. Such a configuration would leave only the H2-H3 region, the S4-H4 region, and the two terminal domains exposed to the cytoplasm and to potential phosphorylation. With this configuration, we used the "PC Gene" analysis program[59] to identify three potential protein kinase C phosphorylation sites at Thr-135, Ser-139 and Ser-279. We also found three potential casein kinase II phosphorylation

sites at Ser 139 (shared with protein kinase C), Thr-236,and Ser-245. Two of these sites are in the S4-H4 linking region and three are in the C-terminus (Fig. 5).

RHK7 shares significant homology with the Shaker-like subfamily of potassium channels.

To determine if RHK7 is a member of the Shaker, Shab, Shaw, or Shal-like subfamily, we compared the conserved domains of RHK7 to those of other cloned vertebrate potassium channels (Figs. 6a-f). Over the five hydrophobic domains H2-H6, RHK7 is 85% identical to other members of the Shaker-related subfamily. In contrast, RHK7 is only 55.6%, 55%, and 50% identical to members of the Shab, Shaw, and Shal-related subfamilies, respectively. In the S4 region, RHK7 is over 97% identical to the Shaker homologs and 46%, 61.5%, and 53.8% identical to the Shab, Shaw, and Shal homologs.

RHK7 shares the least homology to other Shaker-related channels over the H2, H3, and H5 domains, where the identity is 76.5%, 75.7%, and 82%, respectively. These are also the hydrophobic domains in which the other members of this family tend to diverge. In contrast, RHK7 is nearly identical to other members of the Shaker-like class in S4, H4, and H6, where the identity is 98%, 95.5%, and 95.6%, respectively. RHK7 contains a conservative substitution in H4, where a valine is substituted for what is an isoleucine in the other Shaker-like channels. A less conservative substitution occurs in H6, where the sole difference between RHK7 and the other Shaker-like channels is a serine substituted for an alanine. Remarkably, this alanine is conserved not only in the Shaker subfamily, but also in the Shab, Shaw, and Shal subfamilies.

RHK7 is expressed in the heart but not in the brain:

To determine if RHK7 is indeed expressed, we performed northern blots on poly-A selected RNA from rat heart. Because most voltage-sensive potassium channels so far cloned from the heart are also expressed in the brain, we included poly-A selected RNA from whole brain, forebrain,

hindbrain, and cerebellum. We found that a probe constructed from the RHK7 clone hybridized to three distinct bands in the heart, corresponding to transcripts of 4.5 Kb, 2.3 Kb, and 2.0 Kb (Fig. 7). Only faint bands were detected after a two-week exposure, indicating that the transcripts are expressed at relatively low levels. Remarkably, there was no apparent hybridization to brain RNA. A control using an alpha tubulin-specific probe revealed similar levels of RNA in all lanes after an overnight exposure.

DISCUSSION

We have used the polymerase chain reaction to isolate DNA fragments representing potassium channel genes expressed in the rat heart. The use of one of these fragments to screen a rat genomic library resulted in the isolation of a genomic clone, RHK7, representing the partial sequence of a putative novel potassium channel.

An analysis of the nucleotide and deduced amino acid sequence of this clone indicates that it is likely to represent part of a novel Shaker-like potassium channel. RHK7 contains an 861-bp open reading frame that codes for 287 amino acids. These residues form five distinct hydrophobic domains as well as an S4 sequence. The five hydrophobic domains are highly homologous to the conserved hydrophobic domains of other voltage-sensitive potassium channels, and correspond to the domains H2, H3, H4, H5, and H6. A direct comparison of these domains to those of other potassium channels reveals that RHK7 is most similar to the Shaker-related subfamily of potassium channels. RHK7 shares, on average, over 85% identity in the hydrophobic domains with other Shaker-related channels, whereas the identity with the Shab, Shaw, and Shal subfamilies is only 50-55% in these same regions. A comparison of the RHK7 S4 sequence to the S4 sequences of the Shaker-related channels further supports a close relationship to this subfamily. The RHK7 sequence is in fact identical to three of the S4 sequences in the Shaker-like channels. Like all of the Shaker-related genes, the RHK7 S4 domain contains 7 positively charged amino acid residues spaced at every third position. In contrast, the Shab-related channels contain 6 positively charged residues in this domain, the Shal-related channels 5, and the Shaw-related channels 4[35].

It is unlikely that the RHK7 clone codes for an entire potassium channel polypeptide. Although it is similar to other potassium channels from the H2 domain onward, it does not contain an amino terminus or an H1 domain. Moreover, we were unable to identify an initiation consensus sequence in the 5' portion of the open reading frame. One explanation for these observations is that the 5' region of our clone is an intron and that the

region containing the open reading frame represents the 3' exon of the channel. Our discovery of a site that closely matches the consensus sequence for a 3' splice acceptor site supports this idea. This site would allow an upstream exon or exons representing the N-terminus and the H1 region to be spliced to the region prior to H2. The existence of introns in potassium channel genes is certainly not unprecedented. The Shaker gene in Drosophila, for example, contains multiple introns[26]. However, introns in vertebrate potassium channel genes appear to be less common. Introns have been described in two Shaw-related channels[60 49], but all Shaker-related channels so far cloned are believed to have intronless coding regions[39, 41, 42, 43].

We have considered alternate explanations for the lack of an amino terminal region or initiation sequences in RHK7. One possibility is that RHK7 is a pseudogene. This explanation, however, seems unlikely given the existence of a splice acceptor sequence and the fact that we detected hybridizing transcripts in the heart. Another possibility is that the gene represents an artifact created during the synthesis of the genomic DNA library or during subsequent manipulations. This again seems unlikely in light of our isolation of four independent clones with identical restriction sites. However, conclusive evidence for the intron hypothesis will rest on the isolation of a cDNA and the discovery of the N-terminal exon or exons.

In addition to possibly containing an intron, the RHK7 sequence differs from its Shaker-like homologs in other important respects. The C-terminal region of RHK7 differs significantly from other Shaker-like channels. With the exception of a short sequence immediately following H6 and a region encompassing the final three residues of the sequence, we found little similarity between RHK7 and the other channels. The RHK7 C-terminus is also shorter than that of its homologs. This divergence, however, is not unexpected. Sequence divergence in these regions is ubiquitous among voltage-sensitive potassium channels, and probably contributes to their different electrophysiologic characteristics. Indeed, experiments with chimeric channels in which terminal regions were exchanged have demonstrated that these regions strongly affect channel kinetics[27].

Sequence analysis of RHK7 further revealed that this putative channel protein contains several consensus sites for phosphorylation. We found a total of five sites: three potential protein kinase C phosphorylation sites and three potential casein kinase II phosphorylation sites, with one site shared between the two enzymes. Two of these sites are in the S4-H4 loop, a region that is highly conserved between channels in the Shaker subfamily, and three are in the channel-specific C-terminal region. Although we do not know if these sites are used *in vivo*, it is well established that phosphorylation plays an important role in ion channel modulation[61]. The role of protein kinase C dependent phosphorylation in the heart is particularly intriguing. Stimulation of alpha adrenergic receptors has been shown to have positive inotropic effects on the heart[62]. It is also known that alpha-1 receptor activation results in the production of diacylglycerol, which in turn activates protein kinase C. One can therefore speculate that the increased contractility caused by alpha adrenergic stimulation is in part due to phosphorylation of potassium channels. For this to occur, phosphorylation would have to inhibit potassium channels, thereby facilitating membrane depolarization, calcium influx, and cell contraction. Honore et. al. have demonstrated that application of protein kinase C does indeed inhibit mIsK, a delayed rectifier primarily expressed in neonatal mouse heart[55]. It would be interesting to determine whether RHK7 and other Shaker-related channels display similar properties.

In order to check that RHK7 is expressed, we performed Northern blots on both heart and brain RNA. We detected transcripts in the heart only after a two week exposure, suggesting that RHK7 is expressed only at low levels in the heart. Surprisingly, we detected no expression of RHK7 in the brain. While it is certainly possible that our Northern blot analysis was not sensitive enough to detect brain transcripts, this expression pattern clearly differs from that of most other potassium channels. The channels in the Shaker subfamily, as well as the channels in the Shab, Shal, and Shaw subfamilies are all expressed relatively abundantly in brain.

The presence of three distinct transcripts in the heart raises the possibility that the RHK7 sequence contributes to several related heart channels through alternative splicing. Luneau et. al. have reported alternative splicing of Shaw homologs in the rat central nervous system and demonstrated that the delayed rectifier Kv4 (Kv3.1) arises by alternative exon usage from the same gene that encodes NGK2[60]. In this case, the alternative splice site is located in the C-terminal region, outside of the H1-H6 core region. In contrast, alternative splicing in RHK7 could involve the N terminal region and the H1 region of the channel. The alternative splicing of hydrophobic domains has not been described in vertebrate potassium channels, although it does occur in the Shaker gene[26]. Other explanations for the hybridization to multiple transcripts include cross-hybridization with other Shaker-related channels. The fact that we did not detect any transcripts in brain RNA, which contains multiple Shaker-like transcripts, makes this unlikely. A possibility that we cannot rule out is that these transcripts arise from multiple transcription start sites or that they are the result of RNA processing. Firmer evidence for alternative splicing would require the isolation of divergent cDNA's.

Experiments in other laboratories indicate that RHK7 may also be expressed in other tissues. Betsholtz et. al.[63] have used the polymerase chain reaction to detect potassium channels expressed in insulin-producing cells. One of the PCR fragments isolated from a rat insulinoma cell line closely resembles the corresponding sequence in RHK7. This fragment, RK6, differs from RHK7 at only 5 bp over a 171 bp region spanning H4 and H6, and by only one amino acid (A169-V) in the H4-H5 region. Similar 171 bp fragments were isolated from mouse and hamster insulin-producing cell lines. The high degreee of identity between the rat PCR sequence and the RHK7 genomic sequence indicates that these differences may be due to allelic variations or PCR error, and that the two sequences represent the same gene. Indeed, allelic variants have been found in nearly all channels so far identified[36]. The isolation of these fragments provides evidence that, in addition to being expressed in the heart, RHK7 may be expressed in pancreatic islet cells.

The possible expression of RHK7 in both heart tissue and in insulin-producing cells raises interesting questions about its function. It is possible that RHK7 serves the same function in different cell types. Pancreatic beta cells, like heart cells, contain voltage-gated potassium currents that include both transient outward currents[64] and delayed rectifiers[65]. These cells might therefore express identical channels. Another possibility is that RHK7 contributes to different potassium currents depending on the requirements of the cell. A myocyte, for example, may require a short action potential whereas a beta cell might tolerate longer lasting depolarizations. Changes in channel function could be mediated by differences in post-translational modification or by association with other channel subunits[66].

Further insight into the function of RHK7 will require both a more detailed understanding of its expression pattern and an analysis of its electrophysiologic characteristics. The successful use of the polymerase chain reaction to detect RHK7 in pancreatic beta cells indicates that PCR may be an effective way to detect its expression in other tissues as well. Quantitation of RHK7 mRNA using PCR should also be possible[67]. It would be particularly interesting to compare the levels of RHK7 expressed in various parts of the heart. Selective expression in atrium or ventricle, for example, could have significant clinical implications. The electophysiologic characteristics of this channel could be determined by expressing it in Xenopus oocytes. These experiments would first require the isolation of a full-length cDNA or a complete genomic clone. In addition to providing valuable information on the physiology of normal channels, such a system would facilitate the search for pharmacologic agents that modify channel function and have therapeutic value.

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Fig. 1: PCR sequences obtained from rat heart cDNA.

Novel PCR sequences are compared to the RCK4 sequence. Numbering of RCK4 amino acids is from ref. 39. Regions corresponding to primer sequences are not shown for novel PCR fragments.

	M	*T*	*T*	*V*	*G*	*Y*	*G*	*D*	*M*	*K*	*P*	(534)
RCK4	ATG	ACA	ACT	GTG	GGC	TAC	GGG	GAC	ATG	AAG	CCC	
PCR4								TG*	**C	GCA	**T	
PCR10								***	**C	GCA	**T	
PCR21								***	**C	GCA	**T	
PCR27								***	**C	GCA	**T	

	I	*T*	*V*	*G*	*G*	*K*	*I*	*V*	*G*	*S*	*L*	(545)
RCK4	ATC	ACA	GTG	GGA	GGA	AAG	ATT	GTG	GGG	TCC	CTG	
PCR4	G**	**C	***	**T	**C	***	**C	***	**C	**T	***	
PCR10	G**	**C	*A*	**T	**C	***	**C	***	**C	**T	***	
PCR21	G**	**C	***	**T	**C	***	**C	***	**C	**T	***	
PCR27	G**	**C	***	**T	**C	***	**C	***	**C	**T	***	

	C	*A*	*I*	*A*	*G*	*V*	*L*	*T*	*I*	*A*	*L*	(556)
RCK4	TGT	GCC	ATT	GCT	GGT	GTC	TTA	ACC	ATT	GCT	TTG	
PCR4	***	***	***	**A	**C	**G	C*C	***	**C	T**	C**	
PCR10	***	***	***	**A	**C	*CG	C*C	***	**C	T**	C**	
PCR21	***	***	***	**A	**C	**G	C*C	***	**C	T**	C**	
PCR27	***	***	***	*AG	**C	**G	C*C	***	**C	T**	C**	

	P	*V*	*P*	*V*	*I*	*V*	*S*	(563)
RCK4	CCC	GTG	CCG	GTG	ATT	GTG	TCT	
PCR4								
PCR10								
PCR21								
PCR27								

FIG.2: NUCLEOTIDE SEQUENCE AND DEDUCED AMINO ACID SEQUENCE OF CLONE RHK7. Nucleotides are numbered from the 5'to 3' direction, beginning in the 5' untranslated region of the cloned genomic DNA fragment. Amino acids are shown beginning at the outset of the open reading frame. Putative H2-H6 domains are underlined and labelled.

```
        10        20        30        40        50        60
         |         |         |         |         |         |
AGCTGTTCTGAGTGCTGGGGTTGAAGGTGTCTACCACAACAATTGACCATGTTTTAATCT

        70        80        90       100       110       120
         |         |         |         |         |         |
TTGATATGCTGTGGTACAACCCAAGGCATTAGGCAAACGCTTTACTACTAATCCAAGTCT

       130       140       150       160       170       180
         |         |         |         |         |         |
GCAGATCCTCCCTGGGGGAGGCTAGACAGGAGTGCTACCTTTGAACCACACCACCCCCCT

       190       200       210       220       230       240
         |         |         |         |         |         |
CTCTGGGGAGTCTAGTGAGGAACTGCTATCACTGAGTCATACCCTGAGCCTCTTCTCTGG

       250       260       270       280       290       300
         |         |         |         |         |         |
GACGTGTAGAACAGGGCTCCACCATCCAGCCACACCACAAACCGGAGGGCTTCTAAGCAG

       310       320       330       340       350       360
         |         |         |         |         |         |
GATTTGTGCCAATGAACTCCACCTCCAACCTTCATTTCGCTTTCTATTTTGAGAGGGTCT

       370       380       390       400       410       420
         |         |         |         |         |         |
CATTAGTTGTCCAGATCTGCCTTGAAATTACCTGTCCTCCCTCAGGCTCCTGAGTGTGGA

       430       440       450       460       470       480
         |         |         |         |         |         |
ACGACAGGCTTGTGTCACTAGGCCCAGGAGGCACCACCTTTTCTGCCCATGGGTCTGAAG

       490       500       510       520       530       540
         |         |         |         |         |         |
AACGTTCAGCTACCCTAAGGCAGTCAGTCACTCGCTGGGGTCTTGAGTTGTGTAGGACAG

       550       560       570       580       590       600
         |         |         |         |         |         |
TGAAGTCACCCCTCAGGAACAGTGGTACTGTTACTTTCACATTGAAACGGTGACTAGAGG
                                                         L  E

       610       620       630       640       650       660
         |         |         |         |         |         |
TGTTGACCCAGCTCTCTGACTCCTTTCTGTCCCCTCCACAGTTCCTCGCTCGGCTCAATG
V  L  T  Q  L  S  D  S  F  L  S  P  P  Q  F  L  A  R  L  N
```

```
         670       680       690       700       710       720
           |         |         |         |         |         |
GCTCCAGTCCCATGCCCGGAGCTCCTTCCCGACAGCCCTTCAACGATCCATTCTTTGTGG
 G  S  S  P  M  P  G  A  P  S  R  Q  P  F  N  D  P  F  F  V
                                                   ----------

         730       740       750       760       770       780
           |         |         |         |         |         |
TGGAGACCCTGTGTATCTGCTGGTTCTCCTTTGAGCTGCTGGTGCGTCTGGCGGCGTGTC
 V  E  T  L  C  I  C  W  F  S  F  E  L  L  V  R  L  A  A  C
-------------------H2---------------------------------

         790       800       810       820       830       840
           |         |         |         |         |         |
CAAGCAAAGCTGTATTTTTCAAGAATGTGATGAACCTTATTGACTTCGTGGCCATCCTGC
 P  S  K  A  V  F  F  K  N  V  M  N  L  I  D  F  V  A  I  L
                           -----------------------H3------

         850       860       870       880       890       900
           |         |         |         |         |         |
CTTACTTTGTGGCCCTGGGCACAGAGTTAGCCCGTCAGCGGGGCGTGGGCCAGCCAGCTA
 P  Y  F  V  A  L  G  T  E  L  A  R  Q  R  G  V  G  Q  P  A
--------------------------------

         910       920       930       940       950       960
           |         |         |         |         |         |
TGTCCCTGGCCATCCTAAGGGTCATCAGATTGGTGCGTGTTTTCCGCATCTTCAAGCTAT
 M  S  L  A  I  L  R  V  I  R  L  V  R  V  F  R  I  F  K  L
       --------------------------------S4-------------------

         970       980       990      1000      1010      1020
           |         |         |         |         |         |
CCAGGCATTCGAAGGGCCTGCAGATCTTGGGGCAGACACTGCGGGCTTCCATGCGAGAGC
 S  R  H  S  K  G  L  Q  I  L  G  Q  T  L  R  A  S  M  R  E
-----------------------

        1030      1040      1050      1060      1070      1080
           |         |         |         |         |         |
TAGGTCTCCTCATCTTCTTCCTCTTCATCGGCGTGGTCCTCTTTTCCAGCGCAGTCTACT
 L  G  L  L  I  F  F  L  F  I  G  V  V  L  F  S  S  A  V  Y
----------------------------------H4------------------------

        1090      1100      1110      1120      1130      1140
           |         |         |         |         |         |
TTGCTGAAGTGGACCGGGCGGACACCCATTTCACCAGCATCCCAGAGTCCTTTTGGTGGG
 F  A  E  V  D  R  A  D  T  H  F  T  S  I  P  E  S  F  W  W
----                                            -------------

        1150      1160      1170      1180      1190      1200
           |         |         |         |         |         |
CAGTGGTCACCATGACCACGGTCGGCTACGGGGACATGGCACCTGTCACCGTGGGTGGCA
 A  V  V  T  M  T  T  V  G  Y  G  D  M  A  P  V  T  V  G  G
-------------------H5--------------------------------------

        1210      1220      1230      1240      1250      1260
           |         |         |         |         |         |
AGATCGTGGGCTCTCTGTGTGCCATTGCAGGCGTGCTCACCATCTCTCTGCCGGTGCCTG
 K  I  V  G  S  L  C  A  I  A  G  V  L  T  I  S  L  P  V  P
   -------------------------H6-----------------------------
```

```
      1270      1280      1290      1300      1310      1320
         |         |         |         |         |         |
TCATCGTCTCCAACTTCAGCTACTTTACCACCGGGAGACAGAGGGCGAAGAGGCAGGGA
V  I  V  S  N  F  S  Y  F  Y  H  R  E  T  E  G  E  E  A  G
----------

      1330      1340      1350      1360      1370      1380
         |         |         |         |         |         |
TGTACAGCCATGTGGACACACAGCCCTGCGGTACACTGGAGGGCAAGGCCAATGGGGGTC
M  Y  S  H  V  D  T  Q  P  C  G  T  L  E  G  K  A  N  G  G

      1390      1400      1410      1420      1430      1440
         |         |         |         |         |         |
TGGTGGACTCTGAGGTGCCTGAACTCCTTCCACCACTCTGGGCCCCTTCTGGGAAACACA
L  V  D  S  E  V  P  E  L  L  P  P  L  W  A  P  S  G  K  H

      1450      1460      1470      1480      1490      1500
         |         |         |         |         |         |
TGGTGACCGAGGTGTGAGGGACAGCTGGGGTCTCCAGGAAGCAGTGGGGTGGGAGGGAGG
M  V  T  E  V

      1510      1520      1530      1540      1550      1560
         |         |         |         |         |         |
CGGGAAGGCAGGGTCAGGTGCTGGGTTAAGGACTAAGGTGGTGACGAGAGGGCACAGAAT

      1570      1580      1590      1600      1610      1620
         |         |         |         |         |         |
CTGAATTTGAAGGCATGTCACATGGTAGCTTCTAGGGGGACCTTATGTGACACCTTGGCC

      1630      1640      1650      1660      1670      1680
         |         |         |         |         |         |
AGGATTTGGATTTCGTCCAGGGCTTCCTCATTGGTTGTGCAATGTTGCAGAGCTGTCCAG

      1690      1700      1710      1720      1730      1740
         |         |         |         |         |         |
ATCTCCAGGGACTTGGTCATATATTGTGAGTTTTCTAGGTGTGTATGGGGTCCCACTGGG

      1750      1760      1770      1780      1790      1800
         |         |         |         |         |         |
GGCAAGTGTGGCCTCAGTCAAGTGGATGCTAAGTAGTTACGGAGCTTTGAGTCCTCTGGG

      1810      1820      1830      1840      1850      1860
         |         |         |         |         |         |
GCCAAGTTGGCCCTGCAGGTCTCTATTCGCACTGTCAAGAGGGAGCATACGGCTCCACGG

      1870      1880      1890      1900      1910      1920
         |         |         |         |         |         |
CCACACACACGGAGGTTATGTGGAAATACGCAGCAGATTGTATGGACTCGCGTTTCTTCT

      1930      1940      1950      1960      1970      1980
         |         |         |         |         |         |
AGGACTCAAGTTATTTAAGATTCTGTAAGCTCTGGAGTCTTGAAGGCAGAGCGAGATCAC

      1990      2000      2010      2020      2030      2040
         |         |         |         |         |         |
CCTGGGTCCTGATGGAAAGGGTTAGGGTATAAAGGAGAGAAAGAAGCAGAGAGAGCAGCA

      2050      2060      2070      2080      2090      2100
         |         |         |         |         |         |
GAGACTCGGAGAAAGAGACAGAGACACCCCCCCCCCAAAACTACAGTTCTCTGTGTTGTG
```

```
        2110      2120      2130      2140      2150      2160
           |         |         |         |         |         |
CAGGGCTATAGGGCTGGAACCCGTTCAGGGCTATGCAGAGCTGAGATGTGTGACCGACTA

        2170      2180      2190      2200      2210      2220
           |         |         |         |         |         |
CATAAGTCTCCTAACAGTTGAGTCTGATGACTTCTTTGGTCTTGTGTGACCATGCTGACT

        2230      2240      2250      2260      2270      2280
           |         |         |         |         |         |
CATGTCACCCTGAGTCCTGCGGAAATATTGGTTGCATTTGACTGTATGTGAGGGCCATAG

        2290      2300      2310
           |         |         |
GGACATGGGGGCCATTTTAAGTGGTAGCATCA
```

FIG.3: ALIGNMENT OF DEDUCED RHK7 AMINO ACID SEQUENCE WITH OTHER SHAKER-RELATED CHANNELS. Sequences were initially aligned using the "PC Gene" program as described. Alignments were later modified by eye. A "*" indicates that the position is perfectly conserved; a "." indicates that the position is well conserved. The location of the putative RHK7 splice site is marked with a "#".The region upstream of this site is postulated to be coded for by an intron and therefore may not be translated.

```
RCK1 MTVM---------------------------------------SGENA---DEAS    13
RCK5 MTVA---------------------------------------TGDPV---DEAA    13
RCK3 MTVV---------------------------------------PGDHLLEPEAAG    16
RCK4 MEVAMVSAESSGCNSHMPYGYAAQARARERERLAHSRAAAAAAVAAATAAVEGTG    55
KV1  MEISLVPLENGS-----------------------------------AMTLRGGG    20
RCK2 M------------------------------------------RSEKSLTLAAPG    13


RCK1 AAPGHPQD-----GSYPRQADHD--------------------------------    31
RCK5 ALPGHPQD------TYDPEADH---------------------------------    29
RCK3 GGGGDPPQ-----GGCVSGGGCDR-------------------------------    35
RCK4 GSGGGPHHHHQTRGAYSSHDPQGSRGSREEEATRTEKKKKLHHRQSSFPHCSDLM   110
KV1  EAGASCVQTPRGECGCPPTSGLNNQS----------KETLLRGR-------TTLE    58
RCK2 EVRGP--------------------------------------------------    18


RCK1 -------------------------------------------------------    31
RCK5 -------------------------------------------------------    29
RCK3 -------------------------------------------YEPLPPALPAAG    47
RCK4 PSGSEEKILRELSEEEEDEEEEEEEEEEGRFYYSEEDHGDGCSYTDLLPQDDGGG   165
KV1  DANQGGRPLPPMAQELPQPRRLSAEDEEG-----EGDPGLGTVEEDQAPQDAGSL   108
RCK2 ---------------------EGEQQDAGEF--QEAEGGGGC-------------    37


RCK1 DH-------ECC-ERVVINISGLRFETQLKTLAQFPNTLLGNPKKRMRYFDPLRN    78
RCK5 ---------ECC-ERVVINISGLRFETQLKTLAQFPETLLGDPKKRMRYFDPLRN    74
RCK3 EQ-------DCCGERVVINISGLRFETQLKTLCQFPETLLGDPKRRMRYFDPLRN    95
RCK4 GGYSSVRYSDCC-ERVVINVSGLRFETQMKTLAQFPETLLGDPEKRTQYFDPLRN   219
KV1  HH-----------QRVLINISGLRFETQLGTLAQFPNTLLGDPAKRLHYFDPLRN   152
RCK2 ----------CSSERLVINISGLRYETQLRTLSLFPDTLLGDPGRRVRFFDPLRN    82


RCK1 EYFFDRNRPSFDAILYYYQSGGRLRRPVNVPLDMFSEEIKFYELGEEAMEKFRED   133
RCK5 EYFFDRNRPSFDAILYYYQSGGRLRRPVNVPLDIFSEEIRFYELGEEAMEMFRED   129
RCK3 EYFFDRNRPSLDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEKFRED   150
RCK4 EYFFDRNRPSFDAILYYYQSGGRLKRPVNVPFDIFTEEVKFYQLGEEALLKFRED   274
KV1  EYFFDRNRPSFDGILYYYQSGGRLRRPVNVSLDVFADEIRFYQLGDEAMERFRED   207
RCK2 EYFFDRNRPSFDAILYYYQSGGRLRRPVNVPLDIFMEEIRFYQLGDEALAAFRED   137
```

```
RCK1 EGFIKE--EE-RPLPEKEYQRQVWLLFEYPESSGPARVIAIVSVMVILISIVIFC   185
RCK5 EGYIKE--EE-RPLPENEFQRQVWLLFEYPESSGPARIIAIVSVMVILISIVSFC   181
RCK3 EGFLRE--EE-RPLPRRDFQRQVWLLFEYPESSGPARGIAIVSVLVILISIVIFC   202
RCK4 EGFVRE--EEDRALPENEFKKQIWLLFEYPESSSPARGIAIVSVLVILISIVIFC   327
KV1  EGFIKE--EE-KPLPRNEFQRQVWLIFEYPESSGSARAIAIVSVLVILISIITFC   259
RCK2 EGCLPEGGEDEKPLPSQPFQRQVWLLFEYPESSFPARGIAIVSVLVILISIVIFC   192
                                           -----------H1----

                                                    #
RHK7 LEVL--------------------------------TQLSDSFLSPPQFLARLNG    23
RCK1 LETLPELK-------DDK-------DFTG---TIHRIDNT-------TVIY---T   213
RCK5 LETLPIFR-------DENE------DMHGGGVTFHTYSNS-------TIGY---Q   213
RCK3 LETLPEFR-------DEKDY----PASPSQDVFEAANNST-------SGAS---S   236
RCK4 LETLPEFR-------DDRDL----IMALSAGGHSRLLNDT-------SAPHLENS   364
KV1  LETLPEFR-------DERELLRHPPVPPQPPAPAPGINGS-------VSGALSSG   300
RCK2 LETLPQFRADGRGGSNEGSGTRMSPASRGSHEEEDEDEDSYAFPGSIPLGGLGTG   247
     **.*
     --

RHK7 SSPMPGAPSRQPFNDPFFVVETLCICWFSFELLVRLAACPSKAVFFKNVMNLIDF    78
RCK1 -SN--------IFTDPFFIVETLCIIWFSFELVVRFFACPSKTDFFKNIMNFIDI   259
RCK5 QST--------SFTDPFFIVETLCIIWFSFEFLVRFFACPSKSCFFTNIMNIIDI   260
RCK3 GAS--------SFSDPFFVVETLCIIWFSFELLVRFFACPSKATFSRNIMNLIDI   283
RCK4 GHT--------IFNDPFFIVETVCIVWFSFEFVVRCFACPSQALFFKNIMNIIDI   411
KV1  PTVA--PLLPRTLADPFFIVETTCVIWFTFELLVRFFACPSKAEFSRNIMNIIDV   353
RCK2 GTSSFSTLGGSFFTDPFFLVETLCIVWFTFELLVRFSACPSKAAFFRNIMNIIDL   302
                 ..****.*** *. **.**..**  ****.. *  *.**.**
                     ----------H2----------          -------

RHK7 VAILPYFVALGTELARQRG----------VGQPAMSLAILRVIRLVRVFRIFKLS   123
RCK1 VAIIPYFITLGTEIAEQEG-------NQKG-EQATSLAILRVIRLVRVFRIFKLS   306
RCK5 VTIIPYFITLGTELAEKPE-------DAQH-TNRMSLATLRVIRLVRVFRIFKLS   307
RCK3 VAIIPYFITLGTELAERQG-------NGQ---QAMSLAILRVIRLVRVFRIFKLS   328
RCK4 VSILPYFITLGTDLAQQQG-----GGNGQQ-QQAMSFAILRIIRLVRVFRIFKLS   460
KV1  VAIFPYFITLGTELAEQQP-----GGGGQNGQQAMSLAILRVIRLVRVFRIFKLS   403
RCK2 VAIFPYFITLGTELVQRHEQQPVSGGSGQNGQQAMSLAILRVIRLVRVFRIFKLS   357
     *.*.***..***... .                  *.*.**.*************
     -H3------------                     ---------------S4--

RHK7 RHSKGLQILGQTLRASMRELGLLIFFLFIGVVLFSSAVYFAEVDRADTHFTSIPE   178
RCK1 RHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEAEEAESHFSSIPD   361
RCK5 RHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADERDSQFPSIPD   362
RCK3 RHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPSSGFNSIPD   383
RCK4 RHSKGLQILGHTLRASMRELGLLIFFLFIGVILFSSAVYFAEADEPTTHFQSIPD   515
KV1  RHSKGLQILGKTLQASMRELGLLIFFLFIGVILFSSAVYFAEADNHGSHFSSIPD   458
RCK2 RHSKGLQILGKTLQASMRELGLLIFFLFIGVILFSSAVYFAEADDVDSLFPSIPD   412
     **********.**.*****************.**********..  .. * ***.
     -------            ----------H4----------             -
```

```
RHK7 SFWWAVVTMTTVGYGDMAPVTVGGKIVGSLCAIAGVLTISLPVPVIVSNFSYFYH   233
RCK1 AFWWAVVSMTTVGYGDMYPVTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   416
RCK5 AFWWAVVSMTTVGYGDMVPTTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   417
RCK3 AFWWAVVTMTTVGYGDMHPVTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   438
RCK4 AFWWAVVTMTTVGYGDMKPITVGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   570
KV1  AFWWAVVTMTTVGYGDMRPITVGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   513
RCK2 AFWWAVVTMTTVGYGDMYPMTVGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYH   467
     .******.********* * *.*****************.**********.****
     ---------H5------------- ------------H6---------

RHK7 RETEGEEAGMYSHVD-TQPCGTLEGKANG----------------------GLV   264
RCK1 RETEGEEQAQLLH-V--SS-PNLASDSD-LSR-RSSSTIS---KSEYMEIEEDMN   462
RCK5 RETEGEEQAQYLQ-V--TSCPKIPSSPD-LKKSRSASTIS---KSDYMEIQEGVN   465
RCK3 RETEGEEQAQYMH-V--GSCQHLSSSAEELRKARSNSTLS---KSEYMVIEEG-G   486
RCK4 RETENEEQTQLTQNA--VSCPYLPSNL--LKKFRSSTSSSLGDKSEYLEMEEGVK   621
KV1  RETDHEEQAALKEEQ--GNQRRESGLDTGGQRKVSCSKASFCKTGGSLESSDSIR   566
RCK2 RETEQEEQGQYTHVTCGQPTPDLKATDNGLGKP---------------DFAEASR   507
     ***. **..   .           .                            .

RHK7 DSEVPELLPPLWAPSG----------------KHMVTEV   287
RCK1 NSIAHYRQANIRTGN----CTATDQ--NCVNKSKLLTDV   495
RCK5 NSNEDFREENLKTAN----CTLANT--NYVNITKMLTDV   498
RCK3 MNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV   525
RCK4 ESLCGKEEKCQGKGDDSET-----DKNNCSNAKAVETDV   655
KV1  RGSCPLEKCHLKAKSNVD--LRRSLYALCLDTSR-ETDL   602
RCK2 ERRSSYLPTPHRAYAE----------------KRMLTEV   530
                   .                 .   *..
```

Fig. 4: RHK7 putative splice site.

The RHK7 putative splice site is compared to the consensus sequence for a 3' RNA splice acceptor. The highly conserved "AG" sequence is highlighted. The RHK7 sequence shown corresponds to nucleotides 627-642 of the RHK7 clone (see fig. 2).

Consensus sequence for 3' RNA splice site acceptor*

```
                                          3' exon
                                          __________________________________
T T T T T T T T T T T N T A G G
C C C C C C C C C C C   C     A
```

RHK7 genomic sequence

```
C T G T C C C C T C C A C A G T T C C T C G C T C G G C T C
                                F     L     A     R     L
```

* Adapted from Molecular Biology of the Cell, second edition, p. 534, ed. by B. Alberts, D. Bray, J. Lewis, M. Ragg, K. Roberts, and J. D. Watson. Garland Publishing Inc., New York (1989).

FIG. 5: POST-TRANSLATIONAL MODIFICATION OF RHK7

Potential phosphorylation sites are shown for the H2 to C-terminal regions of the putative RHK7 potassium channel protein. Consensus sites for post-translational modification were identified using the "PC gene" analysis program as described. The N-terminal region and the H1 domain are postulated to be coded for by a separate exon or exons, and are shown by a dotted line.

Protein Kinase C phosphorylation sites:	Thr-135 (S4-H4) Ser-139 (S4-H4) Ser-279 (C-terminus)
Casein Kinase II phosphorylation sites:	Ser-139 (S4-H4) Thr-236 (C-terminus) Ser-245 (C-terminus)

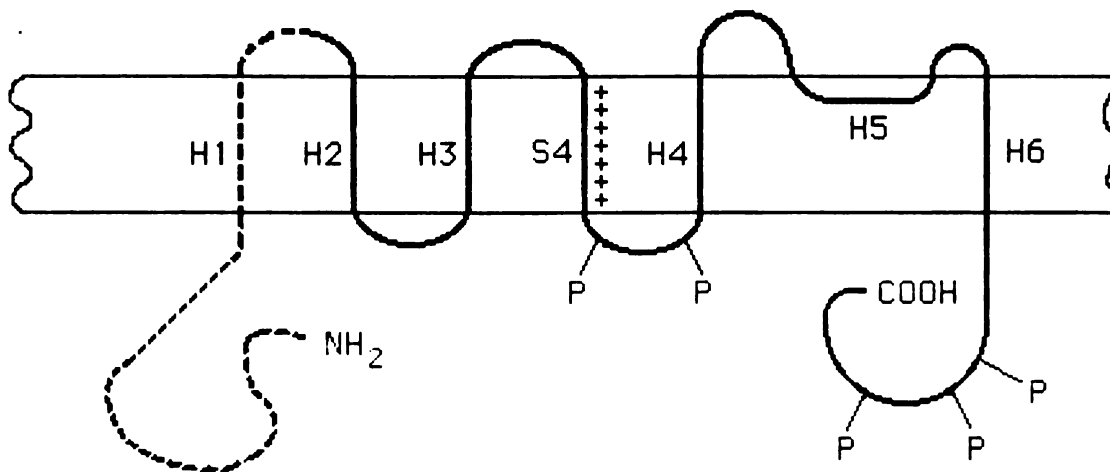


Fig 6a: **Alignment of the RHK7 H2 domain with the H2 domains of other potassium channels.**

Shaker-related subfamily

Kv1.7/ RHK7	F	F	V	V	E	T	L	C	I	C	W	F	S	F	E	L	L	V	R	L	A	A
Kv1.1/RCK1	-	-	I	-	-	-	-	-	-	I	-	-	-	-	-	-	V	-	-	F	F	-
Kv1.2/RCK5	-	-	I	-	-	-	-	-	-	I	-	-	-	-	-	F	-	-	-	F	F	-
Kv1.3/RCK3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F	F	-
Kv1.4/RCK4	-	-	I	-	-	-	V	-	-	V	-	-	-	-	-	F	V	-	-	C	F	-
Kv1.5/Kv1	-	-	I	-	-	-	T	-	V	I	-	-	T	-	-	-	-	-	-	F	S	-
Kv1.6/RCK2	-	-	L	-	-	-	-	-	-	V	-	-	T	-	-	-	-	-	-	F	S	-

Shab-related subfamily

Kv2.1/Drk1	L	A	H	-	-	A	V	-	-	A	-	-	T	M	-	Y	-	L	-	F	L	S

Shaw-related subfamily

Kv3.1/ NGK2	L	T	Y	I	-	G	V	-	V	V	-	-	T	-	-	F	-	M	-	V	V	F
Kv3.2/ RKSHIIIA	L	T	Y	-	-	G	V	-	V	V	-	-	T	-	-	F	-	-	-	I	V	F
Kv3.3(rat)	L	T	Y	-	-	G	V	-	V	V	-	-	T	-	-	F	-	M	-	V	T	F
Kv3.4(rat)	L	T	Y	I	-	G	V	-	V	M	-	-	T	L	-	F	-	-	-	I	V	C

Shal-related subfamily

Kv4.1/ mShal1	-	-	C	M	D	-	A	-	V	L	I	-	T	G	-	Y	L	L	-	-	F	-
Kv4.2/ RShal1	-	-	C	L	D	-	A	-	V	M	I	-	T	V	-	Y	L	L	-	-	-	-

Percent identity to Kv1.7(RHK7):

Kv1.1: 77.3
Kv1.2: 77.3
Kv1.3: 90.9
Kv1.4: 68.2
Kv1.5: 68.2
Kv1.6: 77.3

Overall: 76.5%

Kv2.1: 40.1

Overall: 40.1%

Kv3.1: 36.4
Kv3.2: 45.5
Kv3.3: 40.1
Kv3.4: 36.4

Overall: 39.6%

Kv4.1: 40.1
Kv4.2: 45.5

Overall: 42.8%

Fig. 6b: **Alignment of the RHK7 H3 domain with the H3 domains of other potassium channels.**

Shaker-related subfamily

Kv1.7/ RHK7	I	M	N	L	I	D	F	V	A	I	L	P	Y	F	V	A	L	G	T	E	L	A
Kv1.1/RCK1	-	-	-	F	-	-	I	-	-	-	I	-	-	-	I	T	-	-	-	-	I	-
Kv1.2/RCK5	-	-	-	I	-	-	I	-	-	-	I	-	-	-	I	T	-	-	-	-	-	-
Kv1.3/RCK3	-	-	-		-	-	I	-	-	-	I	-	-	-	I	T	-	-	-	-	-	-
Kv1.4/RCK4	-	-	-	F	-	-	I	-	S	-	-	-	-	-	I	T	-	-	-	D	-	-
Kv1.5/Kv1	-	-	-	I	-	-	V	-	-	-	F	-	-	-	I	T	-	-	-	-	-	-
Kv1.6/RCK2	-	-	-	I	-	-	L	-	-	-	F	-	-	-	I	T	-	-	-	-	-	V

Shab-related subfamily

Kv2.1/Drk1	P	L	-	A	-	-	L	L	-	-	-	-	-	Y	-	T	I	F	L	T	E	S

Shaw-related subfamily

Kv3.1/ NGK2	S	L	-	I	-	-	-	-	-	-	-	-	F	Y	L	E	V	-	L	S	G	L
Kv3.2/ RKSHIIIA	L	L	-	I	-	-	-	-	-	-	-	-	F	Y	L	E	V	-	L	S	G	L
Kv3.3 (rat)	S	L	-	I	-	-	C	-	-	-	-	-	F	Y	L	E	V	-	L	S	G	L
Kv3.4 (rat)	L	L	-	I	-	-	-	-	-	-	-	-	F	Y	L	E	V	-	L	S	G	L

Shal-related subfamily

Kv4.1/ mShal1	V	-	S	-	-	-	V	-	-	-	-	-	-	Y	I	G	-	F	V	P	K	N
Kv4.2/ RShal1	V	-	S	I	-	-	V	-	-	-	-	-	-	Y	I	G	-	V	M	T	D	N

Percent identity to Kv1.7(RHK7):

Kv1.1: 72.7
Kv1.2: 77.2
Kv1.3: 81.8
Kv1.4: 72.7
Kv1.5: 77.2
Kv1.6: 72.7

Overall: 75.7%

Kv2.1: 40.9

Overall: 40.9%

Kv3.1: 45.4
Kv3.2: 45.4
Kv3.3: 40.9
Kv3.4: 45.4

Overall: 44.3%

Kv4.1: 50
Kv4.2: 45.4

Overall: 47.7%

Fig. 6c: **Alignment of the RHK7 S4 domain with the S4 domains of other potassium channels.**

Shaker-related subfamily

Kv1.7/RHK7	L	A	I	L	R	V	I	R	L	V	R	V	F	R	I	F	K	L	S	R	H	S	K	G	L	Q
Kv1.1/RCK1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kv1.2/RCK5	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kv1.3/RCK3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kv1.4/RCK4	F	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kv1.5/KV1	-	-	-	-	-	-	-	-	-	-	-	-	E	-	-	-	-	-	-	-	-	-	-	-	-	-
Kv1.6/RCK2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Shab-related subfamily

Kv2.1/Drk1	R	R	V	V	Q	I	F	-	I	M	-	I	L	-	-	L	-	-	A	-	-	-	T	-	-	-

Shaw-related subfamily

Kv3.1/NGK2	-	G	F	-	-	-	V	-	F	-	-	I	L	-	-	-	-	-	T	-	-	F	V	-	-	R
Kv3.2/ RKShIIIA	-	G	F	-	-	-	V	-	F	-	-	I	L	-	-	-	-	-	T	-	-	F	V	-	-	R
Kv3.3(rat)	-	G	F	-	-	-	V	-	F	-	-	I	L	-	-	-	-	-	T	-	-	F	V	-	-	R
Kv3.4(rat)	-	G	F	-	-	-	V	-	F	-	-	I	L	-	-	-	-	-	T	-	-	F	V	-	-	R

Shal-related subfamily

Kv4.1/ mshal1	S	G	A	F	V	T	L	-	V	F	-	-	-	-	-	-	-	F	-	-	-	-	Q	-	-	R
Kv4.2/ RShal1	S	G	A	F	V	T	L	-	V	F	-	-	-	-	-	-	-	F	-	-	-	-	Q	-	-	R

Percent identity to Kv1 (RHK7):

Kv 1.1:100
Kv 1.2:100
Kv 1.3:100
Kv 1.4: 92.3
Kv 1.5: 96.1
Kv 1.6: 100

Overall: 97.4%

Kv 2.1: 46

Overall: 46%

Kv 3.1-3.3: 61.5

Overall: 61.5%

Kv 4.1-4.2: 53.8%

Overall: 53.8%

Fig. 6d: Alignment of the RHK7 H4 domain with the H4 domain of other potassium channels.

Shaker-related subfamily

Kv1.7/ RHK7	L	G	L	L	I	F	F	L	F	I	G	V	V	L	F	S	S	A	V	Y	F	A
Kv1.1/RCK1	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Kv1.2/RCK5	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Kv1.3/RCK3	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Kv1.4/RCK4	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Kv1.5/Kv1	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Kv1.6/RCK5	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-

Shab-related subfamily

Kv2.1/Drk1	-	-	-	-	-	L	-	-	A	M	-	I	M	I	-	-	-	L	-	F	-	-

Shaw-related subfamily

Kv3.1/ NGK2	F	L	-	-	-	I	-	-	A	L	-	-	L	I	-	A	T	M	I	-	Y	-
Kv3.2/ RKSHIIIA	F	L	-	-	-	I	-	-	A	L	-	-	L	I	-	A	T	M	I	-	Y	-
Kv3.3(rat)	F	L	-	-	-	I	-	-	A	L	-	-	L	I	-	A	T	M	I	-	Y	-
Kv3.4(rat)	F	L	-	-	-	I	-	-	A	L	-	-	L	I	-	A	T	M	I	-	Y	-

Shal-related subfamily

Kv4.1/ Mshal1	-	-	F	-	V	-	S	-	A	M	A	I	I	I	-	A	T	V	M	F	Y	-
Kv4.2/ RShal1	-	-	F	-	L	-	S	-	T	M	A	I	I	I	-	A	T	V	M	F	Y	-

<u>Percent identity to Kv1.7/RHK7:</u>

Kv1.1-1.6: 95.5% Kv2.1: 63.6% Kv3.1-3.3: 45.45% Kv4.1-4.2: 31.8%

Fig. 6e: **Alignment of the RHK7 H5 domain with the H5 domains of other potassium channels.**

Shaker-related subfamily

Kv1.7/RHK7	E	S	F	W	W	A	V	V	T	M	T	T	V	G	Y	G	D	M	A	P	V	T	V	G	G
Kv1.1/RCK1	D	A	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	Y	-	-	-	I	-	-
Kv1.2/RCK5	D	A	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	V	-	T	-	I	-	-
Kv1.3/RCK3	D	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-	-	-	I	-	-
Kv1.4/RCK4	D	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	-	I	-	-	-	-
Kv1.5/KV1	D	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	T	-	-	-	-
Kv1.6/RCK2	D	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	M	-	-	-	-

Shab-related subfamily

Kv2.1/Drk1	A	S	-	-	-	-	T	I	-	-	-	-	-	-	-	-	-	I	Y	-	K	-	L	L	-

Shaw-related subfamily

KV3.1/ NGK2	I	G	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	Y	-	Q	-	W	S	-
KV3.2/ RKShIIIA	I	G	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	Y	-	Q	-	W	S	-
Kv3.3 (rat)	I	G	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	Y	-	K	-	W	S	-
Kv3.4(rat)	I	G	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	Y	-	K	-	W	S	-

Shal-related subfamily

Kv4.1/ mShal1	A	A	-	-	Y	T	I	-	-	-	-	-	L	-	-	-	-	-	V	-	S	-	I	A	-
Kv4.2/ RShal 1	A	A	-	-	Y	T	I	-	-	-	-	-	L	-	-	-	-	-	V	-	K	-	I	A	-

Percent identity to Kv1.7(RHK7):

Kv1.1: 80
Kv1.2: 76
Kv1.3: 84
Kv1.4: 84
Kv1.5: 84
Kv1.6: 84

Overall: 82%

Kv2.1: 64

Overall: 64%

Kv3.1-3.4: 72

Overall: 72%

Kv4.1-4.2: 60

Overall: 60%

Fig 6f: Alignment of the RHK7 H6 domain with the H6 domains of other potassium channels.

Shaker-related subfamily

Kv1.7/ RHK7	I	V	G	S	L	C	A	I	A	G	V	L	T	I	S	L	P	V	P	V	I	V	S
Kv1.1/RCK1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-
Kv1.2/RCK5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-
Kv1.3/RCK3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-
Kv1.4/RCK4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-
Kv1.5/KV1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-
Kv1.6/RCK2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-

Shab-related subfamily

Kv2.1/Drk1	-	-	-	G	-	-	C	-	-	-	-	-	V	-	A	-	-	I	-	I	-	-	N

Shaw-related subfamily

Kv3.1/ NGK2	L	-	-	A	-	-	-	L	-	-	-	-	-	-	A	M	-	-	-	-	-	-	N
Kv3.2/ RKShIIIA	L	-	-	A	-	-	-	L	-	-	-	-	-	-	A	M	-	-	-	-	-	-	N
Kv3.3(rat)	L	-	-	G	-	-	-	L	-	-	-	-	-	-	A	M	-	-	-	-	-	-	N
Kv3.4(rat)	L	-	-	A	-	-	-	L	-	-	-	-	-	-	A	M	-	-	-	-	-	-	N

Shal-related subfamily

Kv4.1/ mShal1	-	F	-	-	I	_	S	L	S	-	-	-	V	-	A	-	-	-	-	-	-	-	-
Kv4.2/ RShal1	-	F	-	-	I	-	S	L	S	-	-	-	V	-	A	-	-	-	-	-	-	-	-

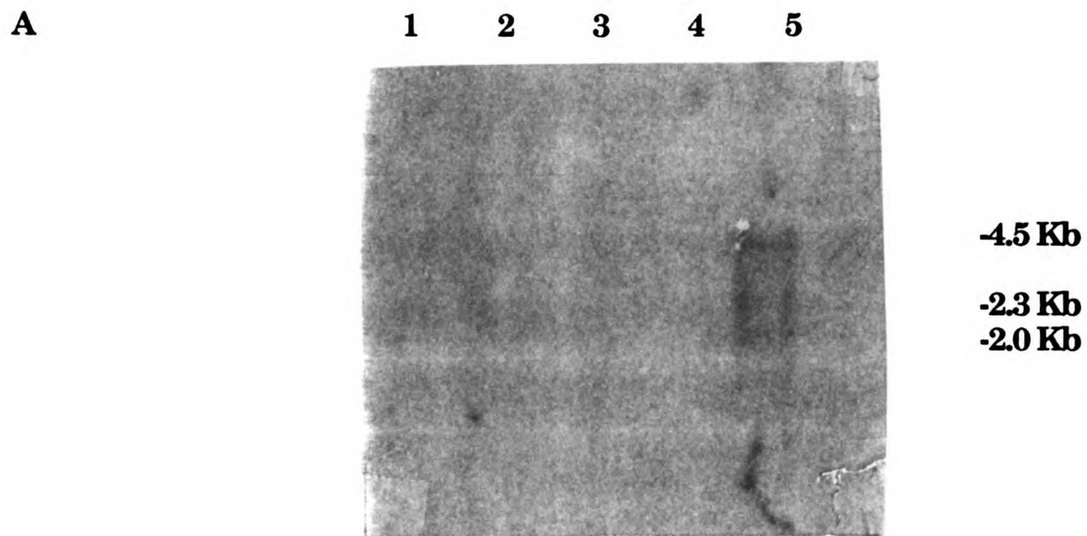
Percent identity to Kv1.7(RHK7):

Kv1.1-1.6: 95.6% Kv2.1: 69.6% Kv3.1-3.3: 73.9% Kv4.1-4.2: 69.6%

Fig. 7: Expression of RHK7 in rat heart and rat brain

A: Northern blot analysis of RHK7 expression in (1) whole rat brain, (2) rat forebrain, (3) rat hindbrain, (4) rat cerebellum, and (5) rat heart. Each lane contains approximately 5 ug of poly(A)+ selected mRNA. Blot was autoradiographed for two weeks.

B: The same blot, probed with rat alpha-tubulin, to demonstrate roughly equal levels of mRNA in each lane. Blot was autoradiographed overnight.



B

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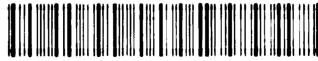

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