FEBS Letters 348 (1994) 239–243

FEBS 14226

Cloning a novel human brain inward rectifier potassium channel and its functional expression in *Xenopus* oocytes

Weimin Tang[a], Xian-Cheng Yang[a,b,*]

[a]*Department of CNS Research, Medical Research Division, Lederle Laboratories, American Cyanamid Co., Pearl River, NY 10965, USA*
[b]*Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA*

Received 11 May 1994; revised version received 6 June 1994

Abstract

We have cloned a novel inward rectifier K^+ channel (hIRK2) from a human frontal cortex cDNA library. The amino acid sequence of hIRK2 shares 60% and 40% identity with the mouse IRK1 and the rat ROMK1 channels, respectively. *Xenopus* oocytes injected with hIRK2 cRNA showed an inwardly rectifying K^+ current that had a prominent 'N-shape' *I–V* curve and was blocked by extracellular Ba^{2+}. The hIRK2 channel has two unique features: (a) an 18 amino acid insertion between the first transmembrane region and the pore, and (b) restricted mRNA distribution found only in human brain and heart.

Key words: Inward rectifier K^+ channel; Human frontal cortex; cDNA; Tissue specificity; N-shape *I–V* curve; *Xenopus* oocyte

1. Introduction

Recently cloned inward rectifier K^+ (IRK) channels constitute a new family of K^+ channels, which contain two transmembrane regions and a pore region. When functionally expressed in *Xenopus* oocytes, the cloned IRK channels are either activated by membrane hyperpolarization [1] or by G protein [2,3], and regulated by intracellular ATP [4]. Physiological functions of IRK channels in peripheral tissues include [5] (a) maintaining the resting membrane potential by conducting on both directions near the K^+ equilibrium potential, E_K, (b) permitting long depolarization responses by shutting off at depolarization voltages, and (c) repolarizing the membrane at the late phase of the action potential as revealed in cardiac ventricular myocytes [6,7]. The IRK channels in central nervous system are suggested to play an important role in controlling membrane excitability in neurons [8] and in regulating neuronal K^+ homeostasis by glial cells [9]. Although hyperpolarization-activated and Ba^{2+}-sensitive IRK channels have been described in the central neurons [8,10–14], they are not as well characterized as their cardiac counterparts. In particular, little is known about human brain IRK channels, probably due to difficulties in accessing human brain tissue.

To facilitate studies of human brain IRK channels, we have cloned a novel IRK channel, hIRK2, from a human frontal cortex cDNA library and characterized its electrophysiological properties when expressed in *Xenopus* oocytes. This channel has a unique primary structure, restricted mRNA distribution only found in the heart and brain, and a prominent 'N-shape' *I–V* curve resembling the well characterized IRK channel (I_{K1}) in the heart. These properties distinguish this human IRK channel from any other IRK channels cloned to date, including two rodent brain IRK channels [15,16] and a rabbit cardiac IRK channel [17].

2. Materials and methods

2.1. Isolation and sequencing of cDNA

Deoxyoligonucliotide primers were synthesized based on the nucleotide sequence of the mouse IRK1 channel [1]: primer 1: 5′-AGGAAGATGGCATGAAGCTG-3′ (nucleotides 44–63) and primer 2: 5′-GAGCACTGGCTCATAGC GGT-3′ (nucleotides 971–990). A polymerase chain reaction (PCR) using DNA templates from a human hippocampus cDNA library (Strategene, La Jolla, CA) generated a fragment of ~ 1 kilobase (kb). Partial sequencing of the fragment indicated ~ 90% similarity with the corresponding nucleotide sequence of mouse IRK1 channel. We used this PCR fragment to screen a human frontal cortex cDNA library (Strategene). The probe was randomly labeled with ^{32}P and hybridization was carried out in a hybridization oven (Integrated Separation Systems, Natick, MA). Primary screening was performed under the following conditions: prehybridization at 55°C overnight with 25 ml buffer per bottle, and hybridization at 55°C for 4 h with 10 ml buffer per bottle. The membranes were washed twice with SSC containing 0.5% SDS at 55°C for 30 min and twice with 0.1 × SSC containing 1% SDS at 55°C for 30 min. Secondary hybridization was done under the same conditions except the temperature was 65°C. The same buffer was used for both prehybridization and hybridization, containing 1 M NaCl, 10% dextran sulphate (sodium salt, MW

*Corresponding author. Department of CNS Research, Lederle Laboratories, 200/3401, Pearl River, NY 10965, USA.
Fax: (1) (914) 732 5537.

Abbreviations: IRK channel, inward rectifier potassium channel; E_K, the K^+ equilibrium potential; *I–V* curve, current–voltage curve; SSC, saline-sodium citrate; SDS, sodium dodecyl sulfate; SSPE, saline-sodium phosphate EDTA.

SSDI 0014-5793(94)00612-Y

500,000), 1% SDS, and 100 μg/ml of denatured salmon sperm DNA. Isolated cDNA clones were sequenced using a dye terminator method with an automatic sequencer (Model 373A, Applied Biosystems, Foster City, CA). The full length hIRK2 clone was sequenced on both strands.

2.2. Northern blot analysis

The 2 kb *Eco*RI fragment of clone IRK1811, including the entire coding region and 5′ and 3′ untranslated regions, was labeled with ^{32}P by random priming (Life Technologies, Gaithersburg, MD). The probe was hybridized with a human multiple tissue northern blot (Clontech, Palo Alto, CA) under the following conditions: prehybridization for 3 h at 42°C, hybridization for 18 h at 42°C, and wash twice with 2 × SSC and 0.05% SDS for 40 min at room temperature and twice with 0.1 × SSC and 0.1% SDS for 40 min at 50°C. A buffer used for both prehybridization and hybridization contained 5 × SSPE, 0.1% SDS, 50% formamide, and 100 μg/ml freshly made, sheared salmon sperm DNA.

2.3. Expression of hIRK1 in Xenopus *oocytes*

The hIRK2 cDNA was in the pBluscript-SK vector (Stratagene). After linearization at the *Hin*dIII site in the polylinker region, cRNA was made in vitro using a T3 RNA polymerase kit (Ambion, Austin, TX).

Oocytes were taken from female *Xenopus* (purchased from Nasco, Fort Atkinson, WI). To remove the follicle cells, ocytes (stage 5–6) were first digested with freshly made collagenase (2 mg/ml, type IA; Sigma, St. Louis, MO), which was dissolved in a calcium-free ND96 solution (in mM: NaCl 96, KCl 2, $MgCl_2$ 1, HEPES-NaOH 5; pH 7.5), for two 40 min periods at room temperature (21–23°C). The collagenase-treated oocytes were then hand-picked and freed from follicle cells using two pairs of fine forceps. Before injection, the defolliculated oocytes were incubated at 18°C for 12–30 h in ND96 solution containing 1.8 mM $CaCl_2$ plus 2.5 mM Na-pyruvate and 10 μg/ml pennycillin/streptomycin. Each oocyte was injected with 50 nl hIRK2 cRNA (0.5 ng/nl) or H_2O using a microdispenser (Model 510 ×, Drummond, Broomall, PA). The injected oocytes were placed into wells of 24-well tissue culture plates with one oocyte per well bathed in 1.5 ml of ND96 solution containing pyruvate and antibiotics. The oocytes were viable for at least 7 days without medium change.

2.4. Electrophysiological studies

Microelectrodes were pulled from thick-wall, filamented, boroscilicate glass capillary tubings using a Flaming-Brown puller (Model P-80/PC, Sutter Instrument, Novato, CA). The resistance of microelectrodes ranged from 0.6–1.0 MΩ when filled with 3 M KCl. Whole-cell currents from oocytes were recorded with a cutoff frequency of 2 kHz (an 8-pole Bessel filter, Frequency Devices, Haverhill, MA) using a two-electrode voltage clamp (Axoclamp-2A, Axon Instruments, Foster City, CA). The pClamp software (Axon) was used for on-line data acquisition to the hard disk of a 486-based IBM clone and for later off-line data analysis. Bath solutions with various concentrations of KCl, named K0, K2, K5, K20, and K50, contained (in mM): KCl X, NaCl 98X, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, titrated with NaOH to pH 7.5, where X = 0, 2, 5, 20, and 50 mM, respectively. Recordings were made from oocytes 36 h to 10 days after injection. Temperature was 21–23°C.

A two-pulse voltage protocol was used in two-electrode voltage clamp experiments. The 1,600 ms first pulses ranged from −140 mV to +80 mV at a 20 mV increment. The 200 ms second pulse was always −90 mV. Holding potential was 0 mV. The interpulse interval was 5 s. The steady state current was measured at the end of the first pulse. A 1,000 ms-ramp protocol from −120 mV to +60 mV (HP = 0 mV) was used to obtain I–V curve for the reversal potential measurement. Both the steady state and ramp currents were measured after current traces were digitally subtracted from the background current, which was recorded in the absence of extracellular K^+, i.e. in K0 solution.

2.5. Sequence analysis and curve fitting

LaserGene for the Macintosh (DNASTAR, Madison, WI) was used for DNA and protein sequence analysis. A nonlinear least-square curve-fitting routing (SIGMAPLOT for WINDOWS, Jandel Scientific, San Rafael, CA) was used for fitting experimental data to theoretical models.

3. Results and discussion

3.1. cDNA isolation and sequence analysis

We obtained 28 positive clones after screening approximately 2×10^6 plaques of the human frontal cortex cDNA library. Two clones were further analyzed by sequencing and one was found to contain an open reading frame. We designated it hIRK2, which encodes a protein of 445 amino acids (Fig. 1) with a calculated molecular mass of 49,622 Da. The clone has ~200 bp in the 5′ untranslated region that has 77% GC content and 500 bp in the 3′ untranslated region that contains a poly(A) tail of 33 residues. The initiation codon was assigned to the first methionine in the open reading frame because the neighboring bases (CAGGACATGC) agree at 7 out of 10 bp with the consensus sequence of translation initiation site: GCCGCCATGG [18].

The amino acid sequence of hIRK2 shares 60%, 40%, and 38% identity with the mouse IRK1 [1], the rat ROMK1 [4], and the rat GIRK1/KGA [2,3] channels, respectively. Hydropathy analysis indicated that the hIRK2 channel contains two putative membrane-spanning regions (M1 and M2) and a pore (P) region, which are highly conserved with those of other IRK channels. In particular, the pore region is identical to that of mouse IRK1 channel except for one conserved amino acid change (Fig. 1B). A unique feature of hIRK2 is a stretch of 18 amino acids, which is hydrophobic and contains containing 4 proline and 7 glycine residues (the PG region; Fig. 1), between the M1 region and the pore. Such sequence is not present in any other IRK channels cloned to date. The functional significance of this structure is not known (but see below). Several consensus phosphorylation sites [19] for protein kinase A and protein kinase C are found in the hIRK2 channel (Fig. 1).

3.2. Tissue distribution of hIRK mRNA

Northern blot analysis of poly(A)$^+$ RNA from eight human tissues detected a single hIRK2 mRNA band of 2.7 kb that is present only in the heart and brain (Fig. 2). This pattern of mRNA expression is clearly distinct from that of other IRK channels. A faint band of ~5.7 kb was found in skeletal muscle (Fig. 2), which may result from cross-labeling a human homologue of the mouse IRK1 channel whose 5.5 kb transcript is expressed in skeletal muscle and other tissues in addition to brain and heart [1]. The rat GIRK1/KGA channel has at least two major mRNA bands of 4.5 and 6 kb [2,3]. Multiple transcripts of rat ROMK1 channel are expressed mainly in kidney [4]. Two major transcripts of the rat brain IRK2 channel are expressed in brain and multiple periphery tissues [16].

3.3. Functional expression of the hIRK2 channel in Xenopus *oocytes*

After injection with the hIRK2 cRNA, oocytes

A

```
                                                                                5'... CGTCTTCCCAGGTGACCACGCCGGCTTCAGGAC -1

ATGCACGGACACAGCCGCAACGGCCAGGCCCACGTGCCCCGGCGGAAGCGCCGCAACCGCTTCGTCAAGAAGAACGGCCAATGCAACGTG 90
M  H  G  H  S  R  N  G  Q  A  H  V  P  R  R  K  R  R  N  R  F  V  K  K  N  G  Q  C  N  V  30

TACTTCGCCAACCTGAGCAACAAGTCGCAGCGCTACATGGCGGACATCTTCACCACCTGCGTGGACACGCGCTGGCGCTACATGCTCATG 180
Y  F  A  N  L  S  N  K  S  Q  R  Y  M  A  D  I  F  T  T  C  V  D  T  R  W  R  Y  M  L  M  60

ATCTTCTCCGCGGCCTTCCTTGTCTCCTGGCTCTTTTTCGGCCTCCTCTTCTGGTGTATCGCTTTCTTCCACGGTGACCTGGAGGCCAGC 270
I  F  S  A  A  F  L  V  S  W  L  F  F  G  L  L  F  W  C  I  A  F  F  H  G  D  L  E  A  S  90
                          M1

CCAGGGGTGCCTGCGGCCGGGGGCCCCGGCCGCGGGTGGTGGCGGAGCAGACCCGGTGGCCCCCAAGCCCTGCATCATGCACGTGAACGGC 360
P  G  V  P  A  A  G  G  P  A  A  G  G  G  G  A  D  P  V  A  P  K  P  C  I  M  H  V  N  G  120
                PG

TTCCTGGGTGCCTTCCTGTTCTCGGTGGAGACGCAGACGACCATCGGCTATGGGTTCCGGTGCGTGACAGAGGAGTGCCCGCTGGCAGTC 450
F  L  G  A  F  L  F  S  V  E  T  Q  T  T  I  G  Y  G  F  R  C  V  T  E  E  C  P  L  A  V  150
                      H5                                                        

ATCGCTGTGGTGGTCCAGTCCATCGTGGGCTGCGTCATCGACTCCTTCATGATTGGCACCATCATGGCCAAGATGGCGCGGCCCAAGAAG 540
I  A  V  V  V  Q  S  I  V  G  C  V  I  D  S  F  M  I  G  T  I  M  A  K  M  A  R  P  K  K  180
                          M2

CGGGCGCAGACGTTGCTGTTCAGCCACCACGCGGTCATTTCGGTGCGCGACGGCAAGCTCTGCCTCATGTGGCGCGTGGGCAACCTGCGC 630
R  A  Q  T  L  L  F  S  H  H  A  V  I  S  V  R  D  G  K  L  C  L  M  W  R  V  G  N  L  R  210

AAGAGCCACATTGTGGAGGCCCACGTGCGGGCCCAGCTCATCAAGCCCTACATGACCCAGGAGGGCGAGTACCTGCCCCTGGACCAGCGG 720
K  S  H  I  V  E  A  H  V  R  A  Q  L  I  K  P  Y  M  T  Q  E  G  E  Y  L  P  L  D  Q  R  240

GACCTCAACGTGGGCTATGACATCGGCCTGGACCGCATCTTCCTGGTGTCGCCCATCATCATTGTCCACGAGATCGACGAGGACAGCCCG 810
D  L  N  V  G  Y  D  I  G  L  D  R  I  F  L  V  S  P  I  I  I  V  H  E  I  D  E  D  S  P  270

CTTTATGGCATGGGCAAGGAGGAGCTGGAGTCGGAGGACTTTGAGATCGTGGTCATCCTGGAGGGCATGGTGGAGGCCACGGCCATGACC 900
L  Y  G  M  G  K  E  E  L  E  S  E  D  F  E  I  V  V  I  L  E  G  M  V  E  A  T  A  M  T  300

ACCCAGGCCCGCAGCTCCTACCTGGCCAGCGAGATCCTGTGGGGCCACCGCTTTGAGTCTGTGGTCTTCGAGGAGAAGAGCCACTACAAG 990
T  Q  A  R  S  S  Y  L  A  S  E  I  L  W  G  H  R  F  E  S  V  V  F  E  E  K  S  H  Y  K  330

GTGGACTACTCGCGTTTTCACAAGACCTACGAGGTGGGCGGCACGCCCTGCTGCTCGGCCCGGGAGCTGCAGGAGAGTAAGATCACCGTT 1080
V  D  Y  S  R  F  H  K  T  Y  E  V  G  G  T  P  C  C  S  A  R  E  L  Q  E  S  K  I  T  V  360

CTGACCGCACCACCGCCCCCTCCCAGTGCCTTCTGCTACGAGAACGAGCTGGCCCTTATGAGCCAGGAGGAAGAGGAGATGGAGGAGGAG 1170
L  T  A  P  P  P  P  P  S  A  F  C  Y  E  N  E  L  A  L  M  S  Q  E  E  E  E  M  E  E  E  390

TCATCTGCGGCGTCCGCGGTGGCCGCAGGCCTGGGCCTGGAGGCGGGTTCCAAGGAGGAGACAGCCATCATCCGGATGCTGGAGTTCGGC 1260
S  S  A  A  S  A  V  A  A  G  L  G  L  E  A  G  S  K  E  E  T  A  I  I  R  M  L  E  F  G  420

AGCCACCTGGACCTGGAGCGCATGCAGGCTTCCCTCCCGCTGGACAACATCTCCTACCGCAGGGAGTCTGCCATCTGA              1338
S  H  L  D  L  E  R  M  Q  A  S  L  P  L  D  N  I  S  Y  R  R  E  S  A  I  ...              445

CCTCCAGGGCCGGCCCTCTC... 3'
```

B

```
hIRK2  MHGHSRNGQA ---------- ---------- -HVP------ ---------- ---------- RRKRRNRFVK  23
 IRK1  :GSVRT:RYS ---------- ---------- -I:SSEEDGM KLATMAVANG FGNGKSKVHT :QQC:S:::  49
ROMK1  ::ASERSVFR VLIRALTERM FKHLRRWFIT H:FG------ ---------- ---------- :SRQ:A:L:S  44

                  # #                                  M1
hIRK2  KNGQCNVYFA NLSNKSQ-RY MADIFTTCVD TRWRYMLMIF SAAFLVSWLF FGLLFWCIAF FHGDLEASPG  92
 IRK1  :D:H:::Q:I :VGE:G:-:: L:::::::::: I:::W::V:: CL::VL:::: ::CV::L::L L:::DT:--  116
ROMK1  :E:R::IE:G ::DAQSRFIF FV::W::VL: LK::YKMTV: IT::LG::FL ::LLWYVV:Y V:K::PEF--  112

       PG                                    P                                M2
hIRK2  VPAAGGPAAG GGGADPVAP- --KPCIMHVN GFLGAFLFSV ETQTTIGYGF RCVTEECPLA VIAVVVQSIV  159
 IRK1  ---------- ------KVS- --:A:VSE:: S:TA:::::I :::::::::: ::::D:::I: :FM::F::::  167
ROMK1  ---------- ------YPPD NRTP::ENI: GM:S:::::L :::V:::::: :F::EQ:AT: I:LLI::::L  166

                       x          #                    x
hIRK2  GCVIDSFMIG TIMAKMARPK KRAQTLLFSH HAVISVRDGK LCLMWRVGNL RKSHIVEAHV RAQLIKPYMT  229
 IRK1  ::I::A:I:: AV:::::K:: ::NE::V::: N:::AM::::  :::::::::: ::::L:::::  ::::L:SRI:  237
ROMK1  :V::NS:MC: :IL::ISR:: ::AK:IT::K ::::SK:G:: :::LI::A:: :::L:IGS:I YGK:::TT::  236


hIRK2  QEGEYLPLDQ RDLNVGYDIG LDRIFLVSPI IIVHEIDEDS PLYGMGKEEL ESEDFEIVVI LEGMVEATAM  299
 IRK1  S:::::I:::: I:I:::F:S: I:::::::::: T:::::::::: :::DLS:QDI DNA:::::::: ::::::::::  307
ROMK1  P:::T:I::: TN::FVV:A: NENL:FI::L ::Y:I::HN: :FFHMAAETL SQQ:::L::F :D:T::S:SA  306

          x                    x                                #          #
hIRK2  TTQARSSYLA SEILWGHRFE SVVFEEK-SH YKVDYSRFHK TYEVGGTPCC SARELQESKI TVLTAPPPPP  368
 IRK1  :::C:::::: N:::::::Y: P:L::::-HY :::::::::: ::::PN::L: :::D:A:K:Y I-----LSNA  317
ROMK1  :C:V:T::VP E:V:::Y:FV :IVSKT:EGK :R::FHN:GK :V::ETPHC- ---------- ----------  355

                                                                                  #
hIRK2  SAFCYENELA LMSQEEEEME EESSAASAVA AGLGLEAGSK EETAIIRMLE FGSHLDLERM QASLPLDNIS  438
 IRK1  NS:::::V: :T:K:::DS :NGVPE:TST DSPPGIDL-- ---------- --------HN :::V::EPRP  421
ROMK1  ---------- ---------- -AMCLYNEKD ARARMKRG-- ---------- --------YD NPNFV:SEVD  384

          x
hIRK2  YRRESAI                                                                    445
 IRK1  L::::E:                                                                    428
ROMK1  ETDDTQM                                                                    391
```

Fig. 1. (A) The nucleotide sequence and deduced single letter amino acid sequence of the hIRK2 cDNA. Positive numbers are assigned to nucleotides beginning with the A residue of the start codon. Negative numbers are assigned to nucleotides 5' to the start codon. Two putative membrane-spanning regions (M1 and M2) and the pore (P) are marked by lines below the sequence. The proline-glycine rich (PG) region is indicated by a dashed line below the sequence. (B) The alignment of amino acid sequences of hIRK2, mouse IRK1 and rat ROMK1, using the J. Hein method (LaserGene). Identical amino acids are indicated by colons. Dashes indicate gaps introduced to improve alignment. Two membrane spanning regions (M1 and M2), the pore (P) and the PG region are indicated by lines above the sequences. Potential phosphorylation sites for protein kinases A and C are indicated by x and #, respectively.

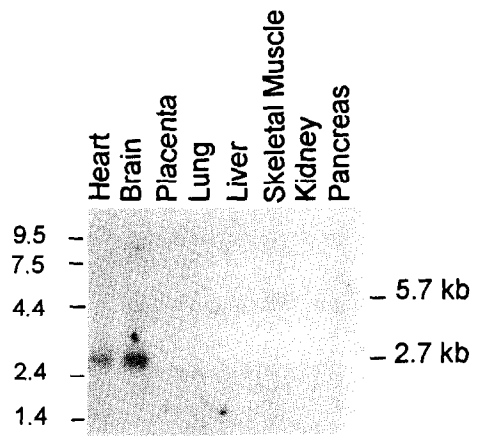

Fig. 2. Northern blot of the hIRK2 mRNA from eight human tissues. The positions of RNA size markers (kb) are shown on the left side. The size of hIRK2 mRNA is 2.7 kb, shown on the right side. See the text for possible interpretation of the 5.7 kb band in the skeletal muscle lane.

expressed a slowly activating and inactivating, inwardly rectifying current (Figs. 3 and 4). Water injected and noninjected oocytes had little inward current under the same recording conditions. An important feature of the hIRK2 channel was that the channel carried a substantial outward current for membrane potentials that were more positive than the K^+ equilibrium potential, E_K (Fig. 3A). As a result, the I–V curves of both ramp and steady state hIRK2 currents had an 'N-shape' (Figs. 3B and 4B) resembling that of the inward rectifier (I_{K1}) found in cardiac myocytes [20–22]. Other IRK channels cloned to date, including one cloned from rabbit heart [17], carry less outward currents than that observed for hIRK2 although the inward current amplitudes are similar. As noted above, the PG region between the M1 region and the pore of hIRK2 (Fig. 1), missing in all other IRK channels, might be the structural basis for the prominent outward current and the resulting N-shape I–V curve of hIRK2.

Ion selectivity of the hIRK2 channel was determined from the reversal potential measurement in various extracellular K^+ concentrations, $[K^+]_o$. The results determined from the steady state and ramp I–V curves agreed each other (Fig. 3B). The reversal potential of hIRK2 current was negative and changed 52.8 ± 0.8 mV per 10-fold change in $[K^+]_o$ as predicted for E_K (56.4 mV at 22°C) by the Nernst equation, indicating that the hIRK2 channel is selectively permeable to K^+ over Na^+.

Ba^{2+} block of the steady state and tail currents of hIRK2 was studied using a two-pulse protocol (see Section 2). Extracellular Ba^{2+} at concentrations of 20, 200, and 500 μM blocked the steady state current in the inward direction by accelerating the inactivation time course (Fig. 4), indicating Ba^{2+} is an open channel blocker. Ba^{2+} also blocked the outward current (Fig. 4B), as observed for cardiac IRK channels in humans [20] and in animals [21,22]. In addition, Ba^{2+}produced a voltage-dependent block of the tail current elicited by the second voltage pulse of −90 mV from various first-pulse voltages. For a first-pulse voltage that was more negative than E_K, for example −100 mV (Fig. 4), the tail current was fully blocked. This observation suggests that the channel that was blocked during the first pulse remained blocked and thus was unable to open in response to the second pulse. However, for a first-pulse voltage that was more positive than E_K, +80 mV for instance (Fig. 4), the tail current was completely activated then inactivated with an accelerated time course as seen for blocking the steady state current. This suggests that the channel was unblocked by the first pulse of +80 mV and was therefore ready to open in response to the second pulse. As soon as the channel opened during the second pulse, Ba^{2+} blocked it again. Enhancing the block by the negative first-pulse voltage and relieving the block by the positive first-pulse voltage of the tail current suggests that Ba^{2+} is an open channel blocker accessible from the extracellular side with an intra-channel binding site located within the membrane electric field.

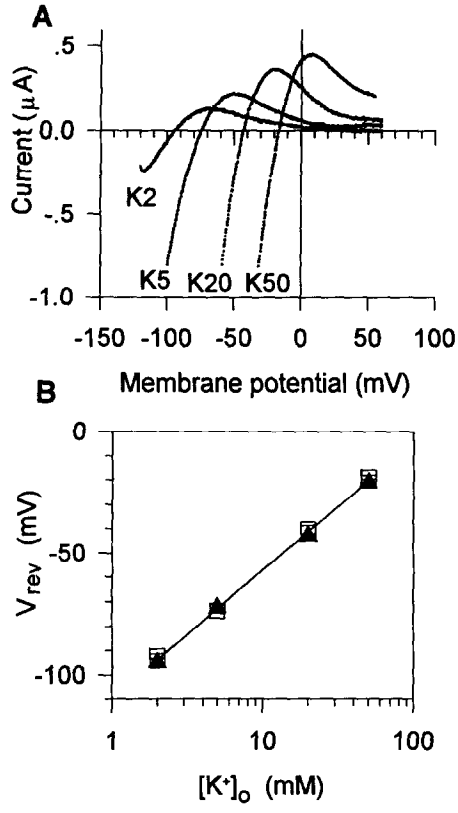

Fig. 3. Ionic selectivity of the hIRK2 channel expressed in *Xenopus* oocytes. (A) Whole-oocyte ramp currents recorded in various extracellular K^+ concentrations, $[K^+]_o$, from an oocyte 36 h after injection of hIRK2 cRNA. (B) The reversal potential (mean ± S.E.M.) of hIRK2 current as function of $[K^+]_o$ obtained from the ramp current (open squares, n = 4–10 oocytes) and from the steady state current (closed triangles, n = 3–6 oocytes, except n = 1 for $[K^+]_o$ = 50 mM). The straight line is the best fit of the data to the Nernst equation with a slope of 52.8 ± 0.8 mV and an intercept of −109.9 ± 0.9 mV.

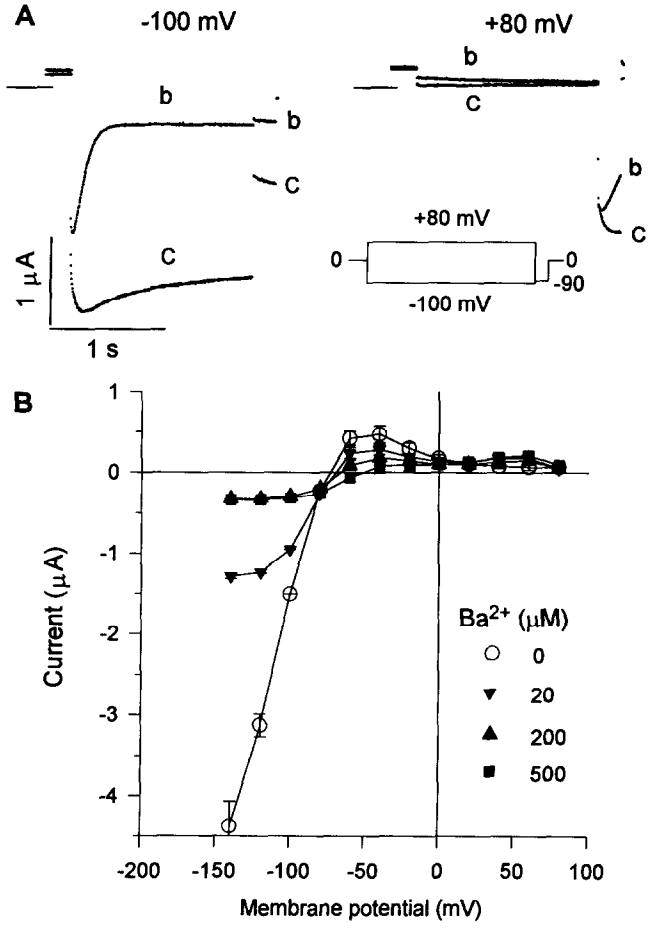

Fig. 4. Block of the steady state and tail currents of hIRK2 by Ba^{2+}. (A) Current waveforms recorded in K5 without (traces labeled with c) and with 500 μM Ba^{2+} (traces labeled with b). The two-pulse voltage protocol is shown for the first-pulse voltages of −100 mV and +80 mV. The short lines next to the current waveforms indicate the zero current levels. The holding current was outward at the holding potential of 0 mV. (B) Steady state *I–V* curves (mean ± S.E.M., 5 oocytes) obtained in K5 without (open circles) and with (filled symbols) Ba^{2+} at concentrations indicated in the figure. Each individual *I–V* curve was scaled relative to the corresponding *I–V* curve obtained from the oocyte 603 # 10 and the mean was calculated. A single scaling factor calculated at −100 mV was used for each individual *I–V* curve. The scaling factors ranged from 0.48–4.77.

The presence of hIRK2 mRNA in the heart and the functional similarity of the hIRK2 channel to cardiac IRK channels indicates that hIRK2 is one of the necessary structural components of cardiac IRK channels. However, IRK channels in neurons are not as well characterized as those in cardiac myocytes. Little is known about human brain IRK channels. The cloning and functional expression of the hIRK2 channel described here offers an opportunity to study structure-function relations, physiological functions, and pharmacology of IRK channels in human brain.

Acknowledgements: We thank Drs. James E. Barrett and J. Steven Jacobsen for critical reading of the manuscript and Kathleen O'Sullivan for her assistance in the preparation of this manuscript. We also thank Drs. Charles J. Homcy and Bernard Dubnick for their encouragement and support.

References

[1] Kubo, Y., Baldwin, T.J., Jan, Y.N. and Jan, L.Y. (1993) Nature 362, 127–133.
[2] Kubo, Y., Reuveny, E., Slesinger, P.A., Jan, Y.N. and Jan, L.Y. (1993) Nature 364, 802–806.
[3] Dascal, N., Schreibmayer, W., Lim, N.F., Wang, W., Chavkin, C., DiMagno, L., Labarca, C., Kieffer, B.L., Gaveriaux-Ruff, C., Trollinger, D., Lester, H.A. and Davidson, N. (1993) Proc. Natl. Acad. Sci. USA 90, 10235–10239.
[4] Ho, K., Nichols, C.G., Lederer, W.J., Lytton, J., Vassilev, P.M., Kanazirska, M.V. and Hebert, S.C. (1993) Nature 362, 31–38.
[5] Hille, B. (1992) Ionic channels of excitable membranes, Sinauer Associates, Sunderland.
[6] Ibarra, J., Morley, G.E. and Delmar, M. (1991) Biophys. J. 60, 1534–1539.
[7] Shimoni, Y., Clark, R.B. and Giles, W.R. (1992) J. Physiol. 448, 709–727.
[8] Yarom, Y. and Llinas, R. (1987) J. Neurosci. 7, 1166–1177.
[9] Brew, H., Gray, P.T.A., Mobbs, P. and Attwell, D. (1986) Nature 324, 466–468.
[10] Constanti, A. and Galvan, M. (1983) J. Physiol. 385, 153–178.
[11] Stanfield, P.R., Nakajima, Y. and Yamaguchi, K. (1985) Nature 315, 498–501.
[12] Williams, J.T., Colmers, W.F. and Pan, Z.Z. (1988) J. Neurosci. 8, 3499–3506.
[13] Williams, J.T., North, R.A. and Tokimasa, T. (1988) J. Neurosci. 8, 4299–4306.
[14] Grove, E.A. and Halliwell, J.V. (1990) J. Physiol. 424, 46P.
[15] Morishige, K.-I., Takahashi, N., Findlay, I., Koyama, H., Zanelli, J.S., Peterson, C., Jenkins, N.A., Copeland, N.G., Mori, N. and Kurachi, Y. (1993) FEBS Lett. 336, 375–380.
[16] Koyama, H., Morishige, K.-I., Takahashi, N., Zanelli, J.S., Fass, D.N. and Kurachi, Y. (1994) FEBS Lett. 341, 303–307.
[17] Ishii, K., Yamagishi, T. and Taira, N. (1994) FEBS Lett. 338, 107–111.
[18] Kozak, M. (1989) J. Cell Biol. 108, 229–241.
[19] Pearson, R.B. and Kemp, B.E. (1991) Methods Enzymol. 200, 62–81.
[20] Beuckelmann, D.J., Nabauer, M. and Erdmann, E. (1993) Circ. Res. 73, 379–385.
[21] Imoto, Y., Ehara, T. and Matsuura, H. (1994) Am. J. Physiol. 252, H325–H333.
[22] Tseng, G.-N. and Hoffman, B.F. (1989) Circ. Res. 64, 633–647.