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The voltage-dependent K^+ channel (Kv1.5) cloned from rabbit heart and facilitation of inactivation of the delayed rectifier current by the rat β subunit

Yoshiteru Sasaki, Kuniaki Ishii*, Kazuo Nunoki, Toshio Yamagishi, Norio Taira

Department of Pharmacology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan

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Abstract **We have isolated a cDNA coding for a delayed rectifier K^+ channel (RBKV1.5) from rabbit heart. The amino acid sequence of RBKV1.5 displays a homology to that of other K^+ channels of Kv1.5 class. Overall amino acid identity between RBKV1.5 channel and Kv1.5 channel of other species is about 85%. RNA blot analysis revealed the expression of the primary transcript in various rabbit tissues, at the highest level in both the atrium and ventricle. When expressed in *Xenopus* oocytes, RBKV1.5 current showed a delayed rectifier type characteristics, which was converted to rapidly inactivating currents upon coexpression with a β subunit.**

Key words: K^+ channel; Kv1.5; β subunit; Rabbit heart

1. Introduction

Voltage-dependent K^+ channels play important roles in cardiac excitability as they contribute to the various repolarization phases of the action potential. They form the most diversified class of ion channels and at present at least six different voltage-dependent K^+ currents have been described in mammalian heart [1–6]. In order to study the current of interest, such techniques as ion substitution and pharmacological dissection have been used to eliminate the overlapping currents. However, it is not easy to isolate and characterize the current of interest because the overlapping currents have similar sensitivities to pharmacological agents and the changes in its kinetics may be due to ion substitution. Another attempt to isolate the individual channel components has been made by molecular biological approach. Several voltage-dependent K^+ channel clones (pore-forming α subunits) have been isolated from the heart of various species including human [7–10]. In addition to α subunits, two β subunits (Kvβ1 and Kvβ2) of Shaker-related Kv1 channel family have recently been cloned from rat brain [11]. Kvβ1 is reported to convert a delayed rectifier type K^+ channel (Kv1.1) into a rapidly inactivating A-type channel and to accelerate the inactivation of an A-type channel (Kv1.4) upon coexpression [11]. Very recently a third type of K^+ channel β subunits (Kvβ3) has been cloned from human atrium, which modulates the kinetics of some Kv1 type channels [12]. These K^+ channel β subunits are thought to increase the complexity and the diversity of voltage-dependent Kv1 channels.

It is obvious that careful comparison of the properties of any cloned channel and the native channel is necessary to evaluate the physiological importance of the results obtained from the cloned channel. Therefore, we must have both cloned channels and native channels of easily obtainable animal cells from the same species to compare the two systems. Rabbit heart may be a suitable preparation because the types and sizes of ionic currents in rabbit atrium are known to be very similar to those in human atrium. In the present study we show the cloning a voltage-dependent K^+ channel from rabbit heart and the influence of rat β subunit on the currents flowing through the K^+ channel.

2. Materials and methods

2.1. cDNA cloning and sequencing

Rabbit heart cDNA library was constructed in λGEM2 (Promega) according to the standard methods [14]. A *Kpn*I-*Pst*I-digested fragment of rat Kv1.2 [15] was labeled with ^{32}P by the random priming method and used to screen the cDNA library. Approximately 6.0×10^5 plaques were screened under low stringency conditions and 11 positive clones were isolated. The cDNA inserts were subcloned into EcoRI site of pBluescript SK(+) vector (Stratagene). We selected one clone (RBKV1.5) containing the longest insert (2.7 kb) for further characterization. DNA sequencing of RBKV1.5 was performed on both strands by the dideoxy chain termination method using A.L.F. DNA Sequencer II (Pharmacia).

Polymerase chain reaction (PCR) was used to obtain rat Kvβ1. Two oligonucleotide primers were synthesized according to non-coding region of Kvβ1 mRNA [11]. The sense primer corresponded to nucleotides −160 to −141 and the antisense primer corresponded to nucleotides 1346–1365 (the first residue of the initiating ATG is number 1). The 5′ part of both primers was designed to contain EcoRI site to facilitate subcloning. PCR was carried out using rat brain cDNA as a template under the following conditions: 94°C, 1 min; 54°C, 2 min; 72°C, 3 min for 30 cycles. The amplified products were digested with *Eco*RI and subcloned into pBluescript SK(+) vector (Stratagene). DNA sequencing confirmed the PCR clone as Kvβ1.

2.2. RNA blot analysis

Total RNAs were isolated from various tissues by the guanidinium thiocyanate method and poly(A)$^+$ RNAs were purified using mRNA Purification Kit (Pharmacia). Five μg poly(A)$^+$ RNAs from each tissue were electrophoresed on 1% agarose gel containing formaldehyde and transferred by capillary action to GeneScreen Plus membrane (NEN). A 2.7 kb fragment specific for RBKV1.5 and 0.5 kb fragment specific for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as hybridization probes. The probes were labeled by ^{32}P using BcaBEST Labeling Kit (Takara). The filter was hybridized for 24 h in 50% formamide, 5 × SSPE, 0.1% SDS, 5 × Denhardt's solution and 200 μg/ml herring testis DNA at 42°C. The filter was briefly washed with 0.2 × SSC/0.1% SDS at room temperature, followed by two 15-min washes with 0.2 × SSC/0.1% SDS at 65°C. Autoradiography was per-

*Corresponding author. Fax: (81) (22) 273-6996.

The nucleotide sequence data of RBKV1.5 will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession number D45025.

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formed on Kodak X-AR film for 88 h at −80°C with an intensifying screen.

2.3. *Functional expression in* Xenopus *oocytes*

Electrophysiological measurements were carried out essentially the same as those reported previously [15]. The plasmid containing RBKV1.5 or Kvβ1 was linearized by digestion with appropriate restriction enzyme and capped run-off cRNA was synthesized in vitro with T7 RNA polymerase (Stratagene). Transcribed RNAs were dissolved in water at a final concentration of 0.2 μg/μl for oocyte injection. When cRNAs specific for RBKV1.5 and Kvβ1 were mixed; weight ratio of 1 : 1 was employed. *Xenopus laevis* oocytes (stage V and VI) were treated for 2 h with collagenase (2 mg/ml; Wako) in modified Barth's medium and defolliculated manually with fine forceps. The oocytes were injected with the transcribed RNA (40–50 nl) and incubated at 19°C for 2–4 days in modified Barth's medium before electrophysiological measurements. Whole cell currents were recorded by a conventional two-microelectrode voltage clamp method with 3 M KCl-filled electrodes. The bath recording solution consisted of 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96). All electrophysiological measurements were carried out at room temperature.

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RBKV1.5  1 MEIALGPLENGGAMTIRGGGEETAGCS  QAA      P TAGLGDGSQEPAPRGR   47
 HK2     1 -----V---------V---D-AR---G  --TGGELQC-P----S--PK----K--  54
 Kv1     1 ---S-V------S---L-----  --A-CV-TPRGECGC-P-S--NNQ-K-TLL---  54
mKv1.5   1 ---S-V-T---S---L-----AV-S-V  -SPRGECGC-P----NNQ-K-TS--  -  53
cKv1.5   1 --L--V---------V-----AR---G  --VGGELQC- --AR-A-PK-RE--E-  53

        48 GCS  ARRGA  EP   GERPLPP  QP P         E LPQSRR SPLE E E   80
        55      -Q-D-  DS   -V-----  L-D-GVRPLPPLPEE--RP-- P-P-D-  -   96
        55 TTLED-NQ-         - -----MA-              - ---P--L-A - D -  85
        54       ~  -TH-DAAQ-G----  M- Q             - ---P--  -SA -D-  85
        54 -PP   ~ --D  -   -A----A  L-                --  -- L-PG D -  80

        81 EGEGDP  GLS VAEE QTLG A    GA L HHQRVLINISGLRFETQLGTLAQF  125
        97 - ----  --GT~ -D -A-- T     -S- -----H-------------------  140
        86 ------  --GT~ --D-APQD-    -S ~ ----------------------    132
        86 ------  --GT~ --D-     -PQDS-S ~ ------------------D--V~-  132
        81 --D---RL--   --D -CR  ~    R-RVF----------------A--------  126

       126 PNTLLGDPAKRLRYFDPLRNEYFFDRNRPSFDGILYYYQSGGRLRRPVNVSLDVFA  181
       141 ------~-----P-------------------------------------G ----  195
       133 ------~-----H-------------------------------------------  188
       133 --N-----V-------------------------------------------------  188
       127 -D-------------------------------------------------------  182

       182 DEIRFYQLGDEAMERFREDEGFIKDEEKPLPRNEFQRQVWLIFEYPESSGSARAIA  237
       196 ----------------------E-------------------------------  251
       189 ----------------------E-------------------------------  244
       189 ----------------------E-------------------------------  244
       183 ----------L-----------E-------------------------------  238
                S1
       238 IVSVLVLILSIITFCLETLPEFK DERELLRHPPVP HQPPAAPALGAN  GSGAV  289
       252 ---------------------R ----------A- -----  --P---  --- -  301
       245 ---------------------R ------------ P----  --P-I-  --  -  293
       245 ---------------------RV- ----L-----  P----  --P-T-AS--  -  296
       239 ---------------------R  -----V----G-P-  --G  --R-PTQR-R-   289
                                      S2
       290  APA S GSTVAPLLPRTLADPFFIVETTCVIWFTFELLVRFFACPSKAEFSRNIM  342
       302 M---  -  -P-------------------------------------G------  355
       294  SG-L-S-P-------------------------------------------  348
       297 LS   - -T-------------------------------------------  348
       290  -      ---------------------------------------------  338
              S3                                          S4
       343 NLIDIVAIFPYFITLGTELAEQQPGGGGGGQNGQQAMSLALLRVIRLVRVFRIFKL  398
       356 ----V-----------------------------------------------  411
       349 ----V-----------------------  ----------------------  402
       349 ------------------------------  --------------------  402
       339 ----V-------------------------------------------------  394
                                   S5
       399 SRHSKGLQILGKTLQASMRELGLLIFFLFIGVILFSSAVYFAEADNQGTHFSSIPD  454
       412 ------------------------------------------------------  467
       403 ------------------------------------------H-S-------  458
       403 ------------------------------------------SQF-------  458
       395 ------------------------------------------E---------  450
                                         S6
       455 AFWWAVVTMTTVGYGDMRPITVGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHR  510
       468 ------------------------------------------------------  523
       459 ------------------------------------------------------  514
       459 ------------------------------------------------------  514
       451 ----------V-------------------------------------------  506

       511 ETDHEEQAALKEEPGSQSRGTS LDAGGQRKASWSKASLCKAGGSLE TADSVRRG  564
       524 ------P-V----Q-T--Q-PG --R-V---V-G-RG-F-----T-- N---A---  577
       515 ------------Q-N-R-E -G--T-----V-C----F--T----- SS--I---  568
       505 ------------Q-I-R-E -G--T-----V-C----FH-T--P--S- --I---  568
       507 ------------Q----Q--G --S--P--T----G------V--- N--GA--Q  560

       565 SCL LEKYNLKAKSNVDLRRSLYALCLDTSRETDL*                    598
       578 --P ---C-V-------------------------*                    612
       569 --P ---CH-------------------------*                     603
       569 --P ---CH-------------------------*                     603
       561   -P---C--------H-----------------*                     594
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Fig. 1. Amino acid comparison of rabbit Kv1.5 (RBKV1.5), human Kv1.5 (HK2), rat Kv1.5 (Kv1), mouse Kv1.5 (mKv1.5) and canine Kv1.5 (cKv1.5). Amino acid sequence differences among the five channels are indicated. Horizontal dash lines indicate identical amino acids among the five channels and blank spaces represent gaps inserted to maximize alignment.

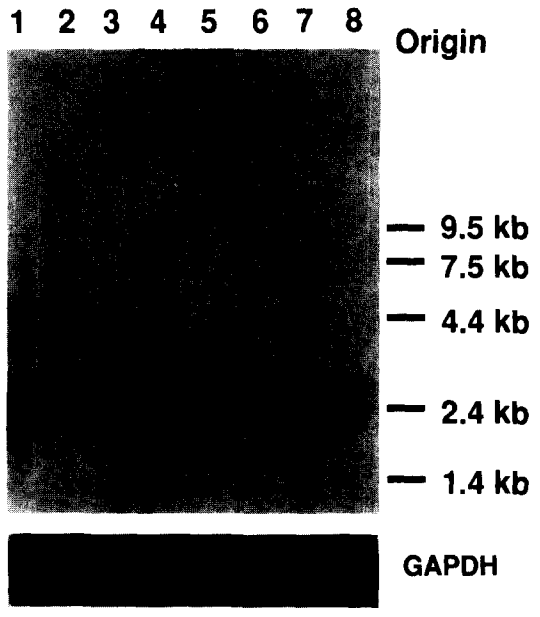

Fig. 2. RNA blot analysis of RBKV1.5 mRNA. Poly(A)$^+$ RNAs analysed were as follows: (1) cerebrum, (2) cerebellum, (3) atrium, (4) ventricle, (5) kidney, (6) liver, (7) lung, (8) skeletal muscle. The positions of RNA size markers (BRL) are shown on the right. After hybridization with RBKV1.5 probe, the blots were rehybridized with the GAPDH probe as control.

3. Results and discussion

3.1. *Primary structure of RBKV1.5*

Nucleotide sequence analysis of RBKV1.5 revealed one long open reading frame encoding a protein of 598 amino acid residues with calculated M_r of 65,472. The deduced amino acid sequence of RBKV1.5 is 83–86% identical to that of other species [10,16–19] (Fig. 1). The percentage of identity is not as high as it is found in the other members of *Shaker* K channel subfamily. For example, the identity among Kv1.2 channels of different species is about 98% and the identity among Kv1.4 channels of different species is about 95%. The highest identity among the Kv1.5 channels is found within the region containing the subunit assembly domain and the putative transmembrane segments. Within this region, RBKV1.5 is about 95% identical to other Kv1.5 channels. However, the identity among the channels decreases significantly within the amino- and carboxyl-terminal regions. RBKV1.5 showed only 52–57% identity within the amino-terminal region (the residues 1–101) and about 70% identity within the carboxyl-terminal region (the residues 517–598) to other Kv1.5 channels. The low percentage of identity in the amino- and carboxyl-terminal regions may reflect the less important role of these regions in forming functional K^+ channels. In fact, it is reported that mouse Kv1.5

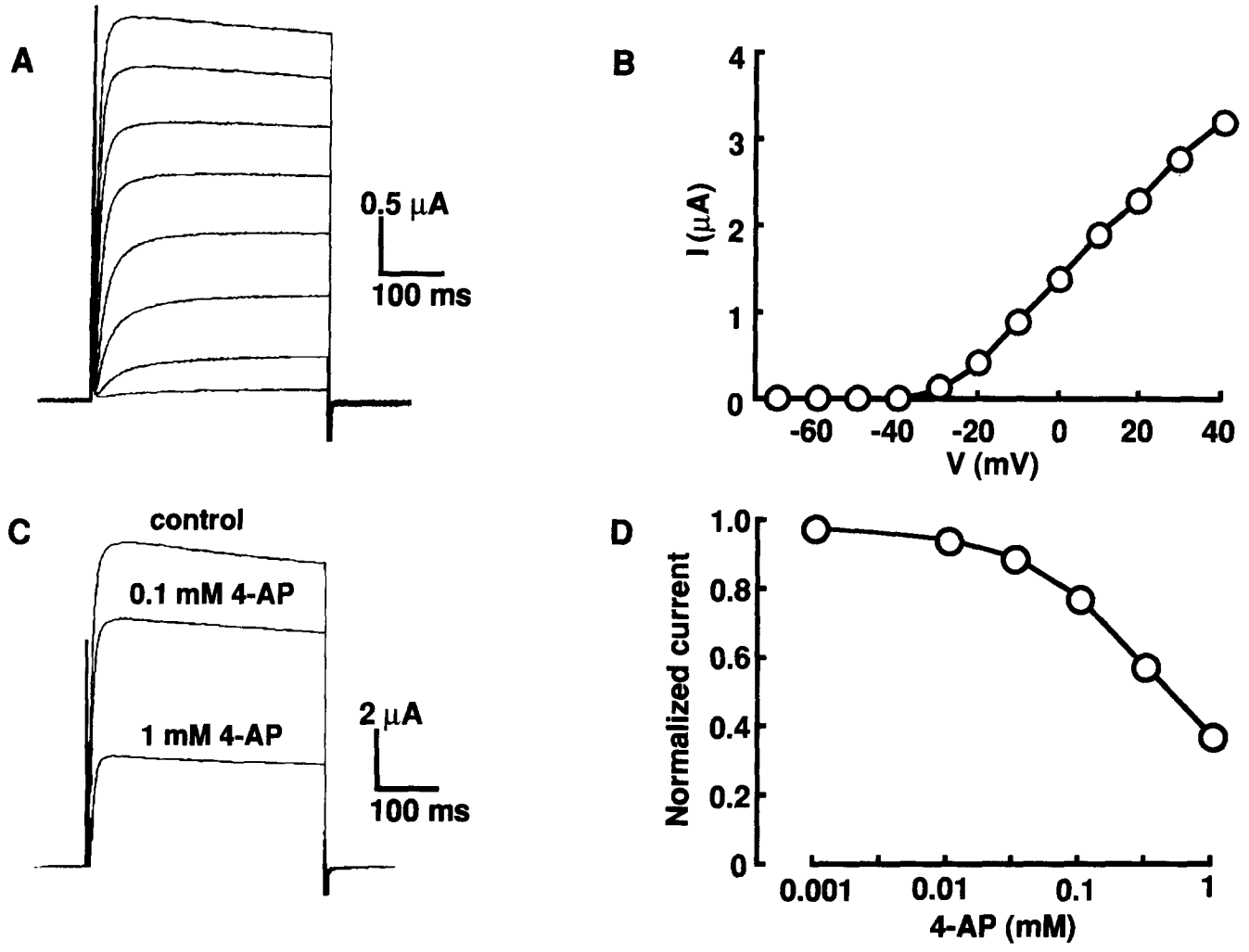

Fig. 3. Volatage-dependent currents recorded from oocytes injected with RBKV1.5 cRNA. (A) Outward currents were elicited by depolarizing voltage steps from a holding potential of −80 mV to test potentials ranging from −70 to +40 mV in 10 mV increments. Peak current–voltage relationship is shown in (B). (C) Typical current traces recorded in the absence (control) and the presence of 0.1 and 1 mM 4-AP. The currents were elicited by test pulses to +20 mV from a holding potential of −80 mV. Concentration–response curve for 4-AP is shown in (D) (n = 12). Current amplitudes are normalized to the initial values before application of 4-AP.

isoform in which the first 200 amino acids are deleted can form functional K^+ channel although with less efficacy [18]. It is also reported that deletion of 57 carboxyl-terminal residues of HK2 does not affect the channel function [20]. However, there are several potential phosphorylation sites for protein kinase A (the residue 565) and protein kinase C (the residues 50, 72, 232, 560 and 592) in the amino-and carboxyl-termini of RBKV1.5 channel. And Kv1 type K^+ channels have been reported to be modulated by protein kinase A and protein kinase C [21–27]. Therefore, the amino- and carboxyl-termini may be related to modulation of the channels by phosphorylation.

3.2. Distribution of RBKV1.5 mRNA in rabbit tissues

To investigate the tissue distribution of RBKV1.5, RNA blot analysis was performed with a specific probe for RBKV1.5. The major 2.8 kb band was detected in all tissues examined except the liver (Fig. 2). The mRNA was expressed at the highest abundance in the heart, at high levels in the lung and at moderate to low levels in the cerebrum, cerebellum, kidney and skeletal muscle. In the heart, the primary transcript is almost equally abundant in the atrium and ventricle. For human Kv1.5 channel mRNA (HK2 and fHK mRNA), two different expression patterns in the heart are reported although HK2 and fHK differ by only 4 amino acids. Whereas HK2 is much more abundant in the atrium than in the ventricle, fHK is expressed both in the atrium and ventricle at easily detectable level [10,16]. Therefore, as to the expression pattern in the heart, RBKV1.5 is similar to fHK. The minor 5.5 kb band was detected in cerebrum and cerebellum and the faint 3.1 kb band was detected in skeletal muscle. No further analysis was made on these faint bands.

3.3. Electrophysiological properties of RBKV1.5

The electrophysiological properties of RBKV1.5 channel were examined using *Xenopus* oocyte expression system. Depolarizing voltage pulses elicited large outward currents in oocytes injected with RBKV1.5 cRNA, which showed rapid activation and little inactivation during 400 ms pulses (Fig. 3A). With the

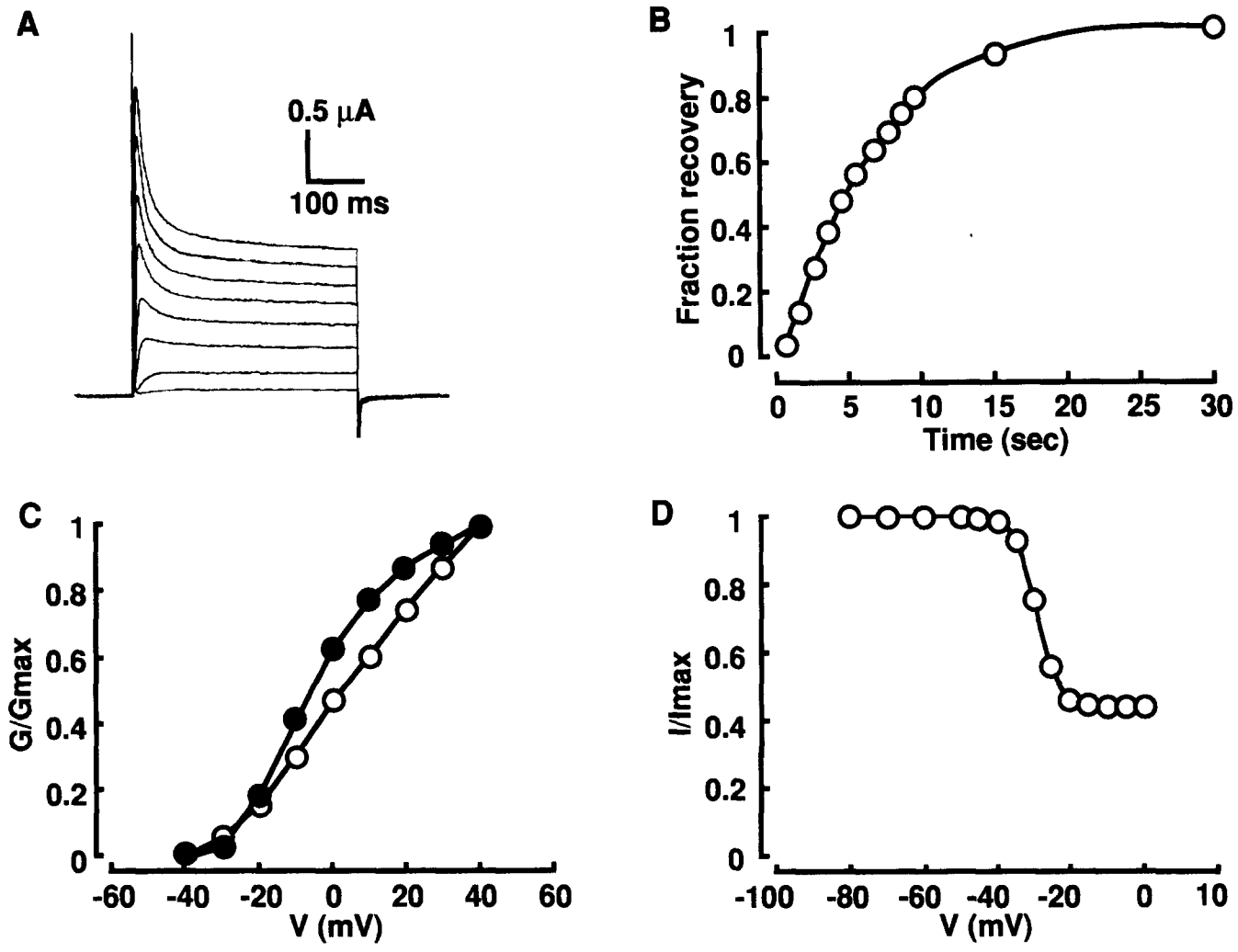

Fig. 4. Properties of voltage-dependent currents recorded from oocytes injected with RBKV1.5 and rat Kvβ1 cRNA. (A) Outward currents were elicited by depolarizing voltage steps from a holding potential of -80 mV to test potentials ranging from −70 to +40 mV in 10 mV increments. (B) Time course of recovery from inactivation. A control 400-ms pulse to +20mV from a holding potential of −80mV was given and followed by a second identical pulse after an interval of increasing duration at −80 mV. The peak amplitude of the current elicited by the second voltage pulse was expressed as a fraction of the corresponding values obtained by control pulse. Fraction recovery was plotted as a funtion of the interpulse interval. (C) Steady-state activation curve of RBKV1.5 current (○) and RBKV1.5 + rat Kvβ1 (●). Peak currents were converted into conductance (G) by the formula $G = I/(V_m - V_{rev})$, assuming a reversal potential (V_{rev}) of −85 mV, where V_m is the membrane voltage of depolarization pulse. The steady-state activation curves were fitted to Boltzmann distribution, $G/G_{max} = 1/[1 + \exp(-(V_m - V_a)/a_n)]$, where G_{max} is the maximal conductance, V_a is the voltage for half-activation and a_n is the slope factor. (D) Steady-state inactivation curve of the current of RBKV1.5 + rat Kvβ1. Membrane potential was stepped from a holding potential of −80 mV to a conditioning prepulse voltage (V_p) ranging from −100 to 0 mV for 400 ms and then to a test pulse of +20 mV. The steady-state inactivation curves were fitted to Boltzmann distribution, $I/I_{max} = (1 - \alpha)/[1 + \exp((V_p - V_h)/a_h)] + \alpha$, where I_{max} is the maximal peak current, V_h is the voltage for half-inactivation, a_h is the slope factor and α is the non-inactivating component of the curves.

larger depolarization (> +20 mV) partial slow inactivation of RBKV1.5 current was observed; less than 4% inactivation at +20 mV. The activation threshold was about −30 mV and the peak current–voltage (I–V) relationship became virtually linear above that voltage (Fig. 3B). RBKV1.5 current was sensitive to 4-aminopyridine (4-AP) (Fig. 3C) and almost insensitive to external tetraethylammonium (TEA). When suppression of the peak current in the presence of blockers was measured during the 400 ms depolarizing pulses at +20 mV, 4-AP blocked the current with an IC_{50} of 465 μM (Fig. 3D), but TEA blocked the current only by 4% at 10 mM.

Influence of rat Kvβ1 subunit on RBKV1.5 current was investigated by coexpressing them in *Xenopus* oocytes. Coexpressed Kvβ1 caused conversion of RBKV1.5 current from delayed rectifier type to rapidly inactivating, A-type, current (Fig. 4A). When coexpressed with Kvβ1, RBKV1.5 current inactivated rapidly at voltages more positive than 0 mV. The current of RBKV1.5 + Kvβ1 inactivated by 47% within 400 ms pulses at +20 mV, whereas, as described above, less than 4% of RBKV1.5 current inactivated at the same voltage. The inactivating currents were best fitted to a single exponential function (at +20 mV, τ_i = 37.2 ± 4.46 ms; mean ± S.D., n = 5). The time constant for recovery from inactivation was 4.87 ± 0.36 s (n = 7) (Fig. 4B). Recently a third type of K^+ channel β subunit (hKvβ3) for Kv1 subfamily was cloned from human atrium and reported to modulate the kinetics of Kv1.5 channel [12]. Upon coexpression with Kvβ3, Kv1.5 current inactivated at very depolarized potentials, more positive than +30 mV with the faster

time constant of about 3.5 ms which is not voltage-dependent. Since Kvβ3 is coexpressed with human Kv1.5 [12] and Kvβ1 is coexpressed with rabbit Kv1.5 (this study), we may not be able to simply compare the effects of Kvβ1 and Kvβ3 on Kv1.5 current. However, it seems that Kv1.5 current inactivates at less positive voltage and the inactivation is slower when coexpressed with Kvβ1 than with Kvβ3. Voltage dependence of steady-state activation and inactivation of RBKV1.5 current was studied. The activation and inactivation curves were fitted to Boltzmann equations as noted in the legend of Fig. 2. Interestingly, coexpression with Kvβ1 not only produced inactivation of RBKV1.5 current but also affected the steady-state activation curve. Coexpression with Kvβ1 shifted the half-activation potential of RBKV1.5 current to the right (RBKV1.5: -4.87 ± 1.61 mV vs. RBKV1.5 + Kvβ1: 2.79 ± 0.7 mV) and changed the slope factor (RBKV1.5: 10.3 ± 0.4 mV vs. RBKV1.5 + Kvβ1: 13.4 ± 0.2 mV). This result is not the same as that obtained from coexpression of Kvβ1 and Kv1.4 channel, which was described in the first paper on cloning of Kvβ subunits [11]. According to the results, Kvβ1 does not affect the normalized current–voltage relationship when coexpressed with amino-terminal deleted Kv1.4 channel. Thus it seems that Kvβ1 subunit interacts with several members of Kv1 subfamily and its influence on each Kv1 channel is not completely the same. This different effect of Kvβ subunit on different K^+ channels may contribute to the diversity of voltage-dependent K^+ channels. Although any β subunit has not been isolated from rabbit, it is highly possible that RBKV1.5 associates with β subunit(s) contributing to the diversity of voltage-dependent K^+ currents in the native rabbit heart if they are present. Detailed comparison of the properties of RBKV1.5 channel and the native rabbit heart K^+ channels is necessary to reveal the physiological importance of the cloned K^+ channel.

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