Molecular and functional characterization of a rat brain $K_v\beta3$ potassium channel subunit

Stefan H. Heinemann[a,*], Jens Rettig[b,**], Frank Wunder[b], Olaf Pongs[b]

[a]*Max-Planck-Gesellschaft z.F.d.W. e.V., AG Molekulare und zelluläre Biophysik, Drackendorfer Straße 1, D-07747 Jena, Germany*
[b]*Zentrum für Molekulare Neurobiologie Hamburg, Institut für Neurale Signalverarbeitung, D-20246 Hamburg, Germany*

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Abstract A novel potassium channel β-subunit ($K_v\beta3$) was cloned from rat brain being the third member of a $K_v\beta$ subunit gene family. It is a protein of 403 amino acid residues with a 68% amino acid sequence homology to $K_v\beta1.1$. $K_v\beta3$ is primarily expressed in rat brain having a distribution distinct to those of $K_v\beta1.1$ and $K_v\beta2$. This subunit also has a long N-terminal structure and induces inactivation in N-terminal deleted $K_v1.4$ but not in other members of the K_v1 channel family. Similarly to $K_v\beta1.1$, the $K_v\beta3$-induced inactivation is regulated by the intracellular redox potential.

Key words: Potassium channel; Channel inactivation; β Subunit; Gene family; Rat brain

1. Introduction

Voltage-activated potassium channels (K_v channels) play an important role in shaping the electrical signals in excitable cells. One aspect for functional classification of K_v channels is their time course of inactivation. While delayed-rectifier channels inactivate very slowly, A-type channels undergo rapid inactivation. Most of these A-type channels presumably inactivate via the so-called N-type inactivation where the N-terminal end of the channel peptides block the pore from the intracellular side [1]. The pore-forming K_v channel α-subunits constitute a large family of proteins with quite diverse structural and functional properties [2]. Although several α-subunits with A-type characteristics have been cloned from mammalian brain (e.g. $K_v1.4$ [3], $K_v3.4$ [4], $K_v4.1$ [5]), the majority of cloned K_v channels gives rise to currents with a very slow time course of inactivation after heterologous expression and is therefore classified as delayed-rectifiers.

One reason for this bias to delayed-rectifier channels may be the existence of auxiliary K_v channel β-subunits ($K_v\beta$). We recently cloned $K_v\beta1.1$ from rat brain which, upon coexpression in *Xenopus* oocytes, conferred very fast inactivation on non-inactivating delayed-rectifier channels, formed by the α-subunits $K_v1.1$ and $K_v1.4\Delta1$–110 [6]. The homologous β-subunit $K_v\beta2$ did not induce inactivation of delayed-rectifier channels [6] but it accelerated the inactivation of the A-type $K_v1.4$ channels [7]. While the molecular mechanism for the effect of $K_v\beta2$ on $K_v1.4$ channels remains to be elucidated, the potency of $K_v\beta1.1$ to induce inactivation resides in its N-terminal region which is considerably longer than that of $K_v\beta2$ (see Fig. 1). Similarly to the N-terminal structure of A-type *Shaker* channels [1], it serves as an inactivating 'ball' domain which can occlude the channel pore from the cytosolic side [6]. As was shown for rat brain A-type channels [8], a cysteine residue in the N-terminal structure of $K_v\beta1.1$ subunits makes the $K_v\beta1.1$-induced inactivation sensitive to regulation by the intracellular redox potential – i.e. inactivation only occurs in a reducing cytosolic environment [6].

Although $K_v\beta1.1$ is most abundant in rat brain, quite homologue β-subunits with alternatively spliced N-terminal domains were recently cloned from human [9,10] and ferret cardiac tissue [11] indicating that $K_v\beta$ subunits are not only important for neuronal function. The β-subunit from human heart was shown to increase the speed of inactivation in $hK_v1.4$ and $hK_v1.5$ channels and the β-subunit from ferret cardiac tissue accelerated inactivation of a homologue of $K_v1.4$ from ferret. These results showed that several splice variants of $K_v\beta1.1$ subunit proteins exist which have similar functional properties as $K_v\beta1.1$ from rat brain. A $K_v\beta$ subunit, cloned from *Drosophila*, accelerated inactivation of *Shaker* B channels when coexpressed in *Xenopus* oocytes [12].

In this study, we present structural and functional properties of a novel $K_v\beta3$ subunit from rat brain. $K_v\beta1.1$, $K_v\beta2$, and $K_v\beta3$, which are heterogeneously expressed in rat brain, constitute a gene family of potassium channel β-subunits. $K_v\beta3$ imposes inactivation on $K_v1.4\Delta1$–110 channels which, in terms of the speed of inactivation and the regulation by the intracellular redox potential, is similar to the inactivation induced by $K_v\beta1.1$. The specificity of $K_v\beta3$ for $K_v\alpha$ subunits, however, is different as it does not form functional assemblies with $K_v1.5$ and $K_v1.1$.

2. Materials and methods

2.1. cDNA cloning and sequencing

A rat cortex cDNA library in λgt10 was screened at low stringency using a $K_v\beta1.1$ cDNA *Eco*RI fragment (nt. 1–1546, Acc. No. X70662). Plaque DNA transferred to Nylon filters (Stratagene, Heidelberg, Germany) and cross-linked to the membrane by UV irradiation. Filters were equilibrated in 6× SET (1× SET is in mM: 150 NaCl, 1 EDTA, 20 Tris-HCl, pH 7.4), 30% formamide, 1% SDS, 5× Denhardt's solution (1× Denhardt's is 0.2 g/l each of Ficoll, polyosinylpyrrolidone and bovine serum albumin) and 0.1 mg/l denatured DNA from herring testes at 42°C. To this prehybridization solution randomly primed [α-^{32}P]dCTP labeled DNA [13] was added at 1× 10^6 c.p.m./ml for hybridization. The final wash was carried out in 1× SET, 0.1% SDS at 50°C. Filters were exposed to X-ray films (Kodak XAR5, Kodak, New Haven, CT, USA) for 12–48 h with intensifying screens (Cronex Hi$^+$, Du Pont, Bad Homburg, Germany) at −70°C. cDNA of positive λ-clones was subcloned into *Eco*RI cut Bluescript SK$^-$ (Stratagene). The longest open reading frame between nt 388 and 1602 was sequenced (Acc. No. X76723). Both strands of cDNA were sequenced with the

*Corresponding author. Fax: (49) (3641) 304 542.
E-mail: ite@rz.uni-jena.de

**Present address:* Max-Planck-Institut für biophysikalische Chemie, Abt. 140, Am Faßberg, D-37077 Göttingen, Germany.

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dideoxy chain termination method [14]. Sequencing reactions were carried out with Sequenase 2.0 sequencing kit (USB, Cleveland, OH, USA) and either M13-universal, M13-reverse or internal primers, using double stranded plasmid DNA as a template.

2.2. Northern blot analysis

Northern blots of rat tissue poly(A)$^+$ mRNA were obtained from Clontech (Palo Alto, CA, USA). The blots were hybridized according to the manufacturer's protocol. Control hybridizations were done with an actin probe supplied by the manufacturer. cDNA probes were labeled by random priming [13] with [α-^{32}P]dCTP. K$_v\beta$2 cDNA probe corresponded to nt. 1–1701 (Acc. No. X76724). In the case of K$_v\beta$3, the entire cDNA was used as probe. After washes with 0.5× SET, 0.1% SDS at 65°C, Northern blots were autoradiographed. Exposures were to Kodak XAR5 X-ray film with amplifying screens (Cronex Hi$^+$, Du Pont) for 4 h.

2.3. In situ hybridization

Cryostat (15 μm) sections of rat brain were mounted on polylysine-coated slices, dried and fixed for 5 min in 4% paraformaldehyde. Slices were rinsed in 1% PBS for 1 min, transferred to 70% ethanol and subsequently stored in 95% ethanol at 4°C. Hybridization was carried out with [α-^{32}P]dATP-tailed antisense oligonucleotides as described previously [14]. The sequences of the oligonucleotides were CAC CGC CTA CCC ACC AGG ATT TAC TCA TGC AGC TTA AGT GGC TCA TGC AT (nt. 1591–1542 of K$_v\beta$1.1); CGC TAA TAG AGA ATT TCA GAT GTG TGA AAC AGG CAT GGT GGG TTC TGG GA (nt. 65–16 of K$_v\beta$2); GGG ATC CAG AGC TGA TTG CAG GAT GCA GGA AAG TGG TTT TCC AAA GGG T (nt. 1689–1641 of K$_v\beta$3).

2.4. Expression in Xenopus oocytes

The K$_v\beta$3 cDNA Bluescript clone was digested with *Eco*RI. The *Eco*RI restriction fragment was blunt ended and cloned into *Sma*I-linearized pAKS2 [16] to yield pK$_v\beta$3-AKS2. Capped and polyadenylated K$_v\beta$3 mRNA was synthesized in vitro [17] from *Eco*RI-linearized pK$_v\beta$3-AKS2 using SP6 RNA polymerase.

Oocytes were obtained from *Xenopus laevis* and maintained according to ref. [18]. The follicular layer was removed manually after treatment with collagenase. Oocytes were always injected with a total volume of 50 nl. The concentration of the mRNA coding for the α- as well as for the β-subunits were always adjusted to 0.25 μg/μl, i.e. we always

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rKvβ1.1  MQVSIACTEH  NLKSRNGEDR  LLSKQSSTAP  NVVN·····  ·AARAKFRTV  43
rKvβ2                                                   MYPESTTGS   9
rKvβ3    ---------Q  --R--SS---  -CGPRPGPGG  GNGGPVGGGH  GNPPGGGGLG  50

rKvβ1.1  AIIARSLGTF  TPQHHISLKE  STAKQTGMKY  RNLGKSGLRV  SCLGLGTWVT  93
rKvβ2    PARLSLRQ-G  S-GMIY-TRY  GSP-RQLQF-  ----------  ----------  59
rKvβ3    SKSRTAVVPR  P-APAGA-R-  --GRG-----  ----------  ----------  100

rKvβ1.1  FGGQISDEVA  ERLMTIAYES  GVNLFDTAEV  YAAGKAEVIL  GSIIKKKGWR  143
rKvβ2    -----T--M-  -H---L--DN  -I--------  --------V-  -N--------  109
rKvβ3    --S-----T-  -D-L-V---H  ----------  -------RT-  -N-L-S----  150

rKvβ1.1  RSSLVITTKL  YWGGKAETER  GLSRKHIIEG  LKGSLQRLQL  EYVDVVFANR  193
rKvβ2    ---------I  F---------  ----------  --A--E----  ----------  159
rKvβ3    ---Y-----I  F---Q-----  ----------  -Q---D----  ----I-----  200

rKvβ1.1  PDSNTPMEEI  VRAMTHVINQ  GMAMYWGTSR  WSAMEIMEAY  SVARQSNMIP  243
rKvβ2    --P------T  ----------  ----------  --S-------  -------L--  209
rKvβ3    S-PSS-----  -----Y----  -L-L------  ---A------  -M-----L--  250

rKvβ1.1  PVCEQAEYHL  FQREKVEVQL  PELYHKIGVG  AMTWSPLACG  IISGKYGNGV  293
rKvβ2    -I-------M  ----------  ---F------  ----------  -V----DS-I  259
rKvβ3    -------N-F  -------M--  ----------  SV-------S  L-TS--DGQ-  300

rKvβ1.1  PESSRASLKC  YQWLKERIVS  EEGRHQQNKL  KDLSPIAERL  GCTLPQLAVA  343
rKvβ2    -PY------G  -----DK-L-  ----R--A--  -E-QA-----  --------I-  309
rKvβ3    -DACK-TV-G  ------KVQ-  -D-K---ARV  T--L---HQ-  ---VA---I-  350

rKvβ1.1  WCLRNEGVSS  VLLGSSTPEQ  LIENLGAIQV  LPKMTSHVVN  EIDNILRNKP  393
rKvβ2    ----------  ----A-NA--  -M--I-----  ---LS-SI-H  ---S--G---  359
rKvβ3    ----S-----  ----V-SA--  -M-H--SL--  -GQL-PQT-M  ---AL-G--S  400

rKvβ1.1  YSKKDYRS                                                   401
rKvβ2    --------                                                   367
rKvβ3    H---                                                       404
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Fig. 1. Comparison of amino acid sequence of K$_v\beta$1.1, K$_v\beta$2, and K$_v\beta$3 from rat brain. Dashes indicate identical amino acid residues as in rK$_v\beta$1.1, dots indicate gaps which were introduced for a better alignment of the N-terminal sections. The numbers denote the last residues per row. Sequences for rK$_v\beta$1.1 and rK$_v\beta$2 are from Rettig et al. [6]; the cDNA sequence coding for rK$_v\beta$3 has been submitted to the EMBL data library (accession number X76723).

used an identical amount of α-subunit mRNA; β-subunit mRNA of the same mass was coinjected such that there was always a small excess of β over α mRNA molecules due to the smaller molecular weight.

Currents were measured with a two-electrode voltage clamp (Turbo Tec-10CD, npi electronic, Tamm, Germany) or with patch-clamp methods [19]. For two-electrode voltage clamp experiments electrodes were filled with 1 M KCl and had resistances between 0.5 and 0.8 MΩ. The standard bathing medium was normal frog Ringer's solution (NFR) with the composition in mM: 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, 10 HEPES, pH 7.2 (NaOH). Patch-clamp experiments were performed with an EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany). The patch pipettes were fabricated from aluminum-silicate glass and had resistances of between 1–2 MΩ. For minimization of capacitive transients, pipettes were coated with RTV 615 (GE Silicones, Bergen op Zoom, Netherlands). For recordings in the inside-out patch configuration the bath medium contained the solution K-EGTA: 115 KCl, 1.8 EGTA, 10 HEPES, pH 7.2 (KOH). Patch pipettes contained NFR. Oxidizing bath media were obtained by adding about 0.1% H_2O_2 to K-EGTA. A reducing environment was established by addition of 5 mM reduced gluthatione (Fluka Chemie, Buchs, Switzerland). All data recordings were done at room temperature between 18–21°C.

The Pulse + PulseFit software package (HEKA) running on a Macintosh Quadra 650 computer (Apple Computer Inc., Cupertino, CA, USA) was used for data acquisition and analysis. Leak and capacitive currents were corrected on-line using a P/n correction method. Subsequent data analysis and generation of figures were done with IgorPro (WaveMetrics Inc., Lake Oswego, OR, USA).

3. Results and discussion

3.1. Cloning of a $K_v\beta$ Gene Family from Rat Brain

Previously, we have cloned rat $K_v\beta1.1$ and $K_v\beta2$ cDNAs from a λgt10 rat cortex cDNA library by low stringency hybridization with a ^{32}P-labeled probe of bovine $K_v\beta2$ cDNA [6]. When we rescreened the same cDNA library with a ^{32}P-labeled rat $K_v\beta1.1$ cDNA probe, we isolated a new cDNA encoding $K_v\beta3$ (Fig. 1). The longest open reading frame derived from a 1800-base-pair (bp) $K_v\beta3$ cDNA sequence corresponds to a 404-amino acid $K_v\beta3$ protein with calculated M_r 45.2 K. The alignment of the new $K_v\beta3$ sequence with the previously cloned $K_v\beta1.1$ and $K_v\beta2$ sequences shows that the amino-termini of all three β-subunit proteins do not align well, whereas the remaining 337 amino-acid C-terminal sequence of $K_v\beta3$ is 70% identical to the corresponding $K_v\beta1.1$ and $K_v\beta2$ C-terminal sequences. This result supports the notion that the $K_v\beta$ subunit may have a relatively constant core region and variable N-termini. Interestingly, the first 20 amino acids of $K_v\beta1.1$ and $K_v\beta3$ are very similar in sequence. It has been shown that this part of $K_v\beta1.1$ harbors an inactivating domain with a distinct function in $K_v1.1$ and $K_v1.4$ channel inactivation [6]. Therefore, the similarity of $K_v\beta1.1$ and $K_v\beta3$ in the N-terminus suggests that $K_v\beta3$ also contains an N-terminal inactivating domain as shown below.

3.2. Expression patterns of $K_v\beta$ subunits

All three $K_v\beta$ genes are predominantly expressed in brain tissue as revealed by Northern blot analysis. Consistent with our previous report [6], we detected with a $K_v\beta1.1$ probe in Northern blot experiments two mRNA species of 3.6 and

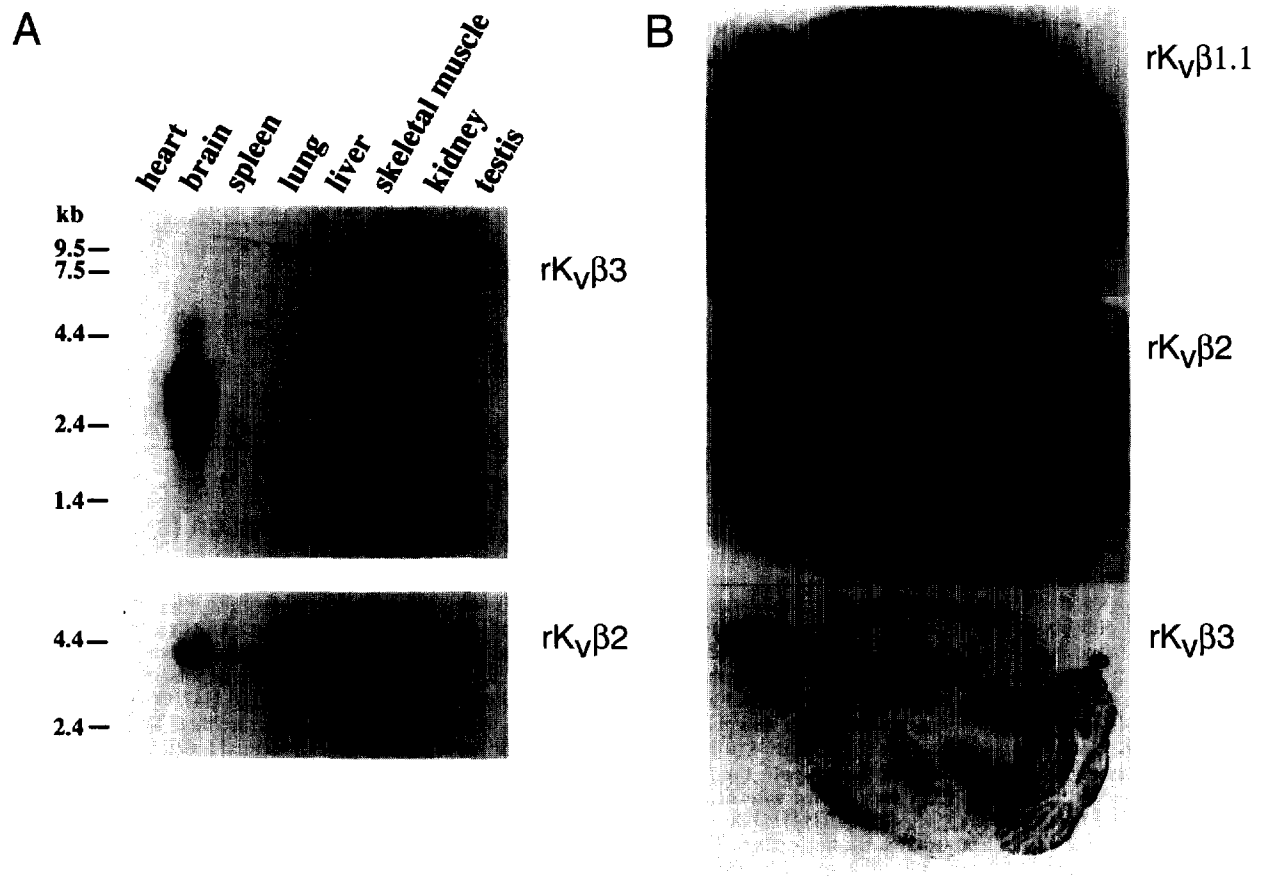

Fig. 2. Tissue-specific localization of $rK_v\beta$ mRNA. (A) Northern blot analysis of mRNAs from the indicated rat tissues with probes specific for $rK_v\beta3$ and $rK_v\beta2$. Transcripts for both β-subunits were only detectable in rat brain with estimated sizes of 4.2 kb ($rK_v\beta2$) and 3.2 kb ($rK_v\beta3$). (B) In situ hybridization of oligonucleotides specific for $rK_v\beta1.1$, $rK_v\beta2$, and $rK_v\beta3$ to horizontal sections of adult rat brain. Exposure times were 14 h.

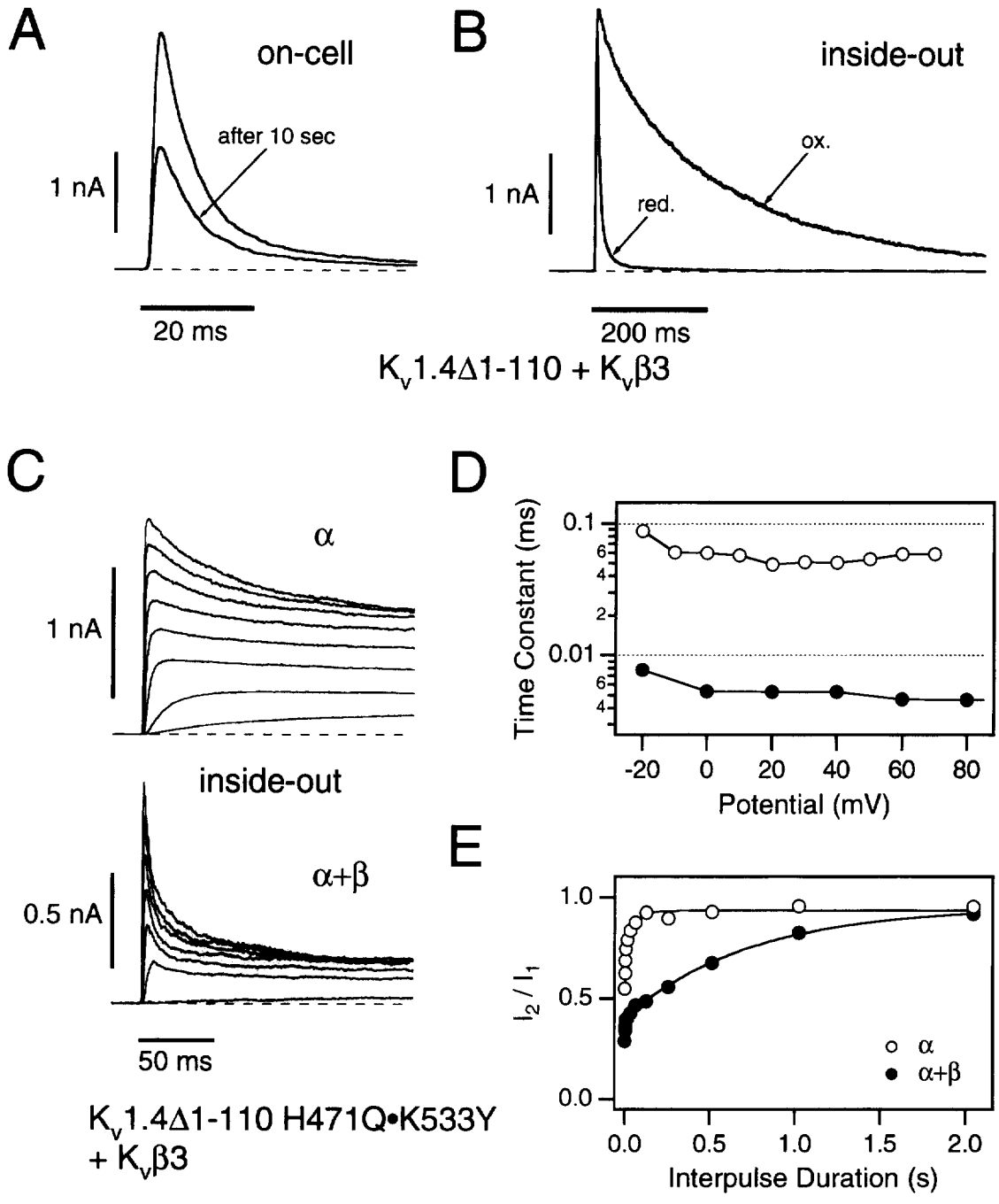

Fig. 3. Functional expression of $K_v\beta3$. (A,B) Patch-clamp current recordings from oocytes expressing K_v1.4Δ1–110 and $K_v\beta3$ in response to step-depolarizations to +50 mV. (A) Two depolarizing pulses were applied in the on-cell configuration with an interpulse interval of 10 s. After this interval at −100 mV, only about 50% of the channels have recovered from inactivation indicating a very slow recovery kinetics. (B) Inside-out patch recordings from the same oocyte as in (A) in a reduced (red.) and oxidized (ox.) environment. (C) Inside-out patch clamp recordings from oocytes expressing the mutant K_v1.4Δ1–110 H471Q·K533Y (α) and the mutant α-subunit plus $K_v\beta3$ (α + β) in response to step depolarizations ranging from -60 to +60 mV in steps of 20 mV. The bath solutions contained K-EGTA plus 5 mM GSH. The mutant K_v1.4Δ1–110 H471Q·K533Y was used in several experiments because it has a faster recovery from slow inactivation and a much weaker dependence on extracellular K^+ than K_v1.4Δ1–110 (see ref. [20]). (D) The inactivation time constant of K_v1.4Δ1–110 H471Q·K533Y currents, based on a single-exponential function with a steady-state component, shows only a weak dependence on the test potential (open circles). For the description of the inactivation time course of current traces obtained from K_v1.4Δ1–110 H471Q·K533Y coexpressed with $K_v\beta3$, two time constants were required. The slow time constant presumably reflects the inactivation intrinsic to the α-subunit K_v1.4Δ1–110 H471Q·K533Y and it was held constant at 62 ms while the fast time constant and all amplitudes were fit parameters. The $K_v\beta3$-induced fast inactivation component (filled circles) also shows only a weak voltage dependence above 0 mV. (E) The fraction of current that recovered from inactivation at −100 mV is plotted as a function of the interpulse interval. Symbols were used as in (D). The pipette solution during recording of all data shown in this figure was NFR.

3.9 kb in rat brain, but not in heart, spleen, lung, liver, skeletal muscle, or kidney RNA (not shown). $K_v\beta2$ and $K_v\beta3$ probes hybridized specifically to rat brain RNA species of 4.2 kb and, respectively, 3.2 kb. Neither $K_v\beta2$ nor $K_v\beta3$ probes yielded signals with RNA species of non-brain tissue (Fig. 2A). The expression of the $K_v\beta$ genes was studied in more detail by in situ hybridization of horizontal sections of adult (P30) rat brain (Fig. 2B). The results show that each $K_v\beta$ gene is distinctly expressed. Notably, high levels of $K_v\beta3$ are expressed in the olfactory bulb and in thalamic nuclei which apparently do not express $K_v\beta1.1$ and $K_v\beta2$ RNAs. On the other hand, $K_v\beta1.1$ and $K_v\beta2$ RNA are detected in hippocampal fields and dentate gyrus, but not $K_v\beta3$. In addition, $K_v\beta1.1$ RNA was detected in corpus striatum, but not $K_v\beta2$ and $K_v\beta3$. Thus, certain areas in rat brain may exclusively express only one of the $K_v\beta1$–3 genes.

3.3. Functional expression of $K_v\beta3$

$K_v\beta3$ was coexpressed with various members of the K_v channel family ($K_v1.1$, $K_v1.4$, $K_v1.5$, $K_v1.6$, $K_v2.1$) but we only found a significant influence on the inactivation time course of $K_v1.4$ channels. The inactivation time constant at +50 mV was for $K_v1.4$ 42 ± 2 ms (n = 5) and for $K_v1.4$ coexpressed with $K_v\beta3$ 34 ± 5 ms (n = 4). Since $K_v1.4$ already inactivates quite rapidly, we performed patch-clamp experiments on oocytes injected with mRNA coding for $K_v1.4\Delta1$–110, a $K_v1.4$ channel mutant with deleted inactivating N-terminal domain, and $K_v\beta3$. In Fig. 3A a superposition of two on-cell current recordings after depolarizations to +50 mV are shown. The second current trace was recorded 10 s after the first one and only about 50% of the current recovered from inactivation during this period where the membrane was clamped to −100 mV. The time constant of inactivation was in this case 7.1 ms. In Fig. 3B recordings obtained in the inside-out configuration are shown on a larger time scale. While the channels undergo very rapid inactivation in a reduced cytoplasmic bathing medium (red., time constant 7.1 ms), the fast inactivation disappears upon replacing the bathing solution by an oxidizing medium (ox., time constant 173 ms). The time course of inactivation in oxidizing environments corresponds to the control where $K_v1.4\Delta1$–110 was injected only (see [6]). A similar regulation of inactivation by oxidation was found for $K_v1.4$ and $K_v3.4$ channels [8] as well as for inactivation induced by the $K_v\beta1.1$ subunit [6]. In those studies, the regulation could be attributed to a cysteine residue in the N-terminal inactivating structure; the mutations C13S in $K_v1.4$ and C7S in $K_v\beta1.1$ removed the regulation by oxidation [6,8]. As can be seen in Fig. 1, the first nine amino acid residues are identical between $K_v\beta1.1$ and $K_v\beta3$. It is therefore very likely that in $K_v\beta3$ the residue C7 serves the same purpose of redox regulation as in $K_v\beta1.1$.

Further coexpression studies with $K_v\beta3$ were carried out with a pore mutant of $K_v1.4\Delta1$–110 where H471 was replaced by Q and K533 by Y. Both mutations eliminate differences between $K_v1.1$ and $K_v1.4$. In particular the mutation K533Y confers channel characteristics of $K_v1.1$ to $K_v1.4$ [20, 21]. For our purpose, we used these mutants in order to eliminate the strong dependence of current amplitude on extracellular K^+ and in order to increase the speed of recovery from inactivation [20]. In Fig. 3C inside-out current recordings at various potentials are shown for $K_v1.4\Delta1$–110 H471Q·K533Y alone (α) and coexpressed with $K_v\beta3$ ($\alpha+\beta$). The residual inactivation of $K_v1.4\Delta1$–110 H471Q·K533Y could be described by a single-exponential component plus a steady-state current. The time constant of inactivation is plotted in panel (D) as a function of the test potential (open circles); above 0 mV it has a nearly voltage-independent value of approximately 60 ms. After coexpression with $K_v\beta3$, inactivation proceeds with two time constants. The slow component was similar to the one for $K_v1.4\Delta1$–110 H471Q·K533Y alone and was held constant at 62 ms for this analysis. The fast component is plotted in (D) as filled circles indicating that the $K_v\beta3$-induced inactivation is about one order of magnitude faster than the intrinsic inactivation of $K_v1.4\Delta1$–110 H471Q·K533Y and that it only has a very small voltage dependence.

Recovery from inactivation was assayed at −100 mV with NFR in the pipette using paired-pulse protocols with a test potential of +60 mV. The fraction of current that recovered in the second pulse is plotted in Fig. 4E as function of the variable

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rKVβ1.1   MQVSIACTEH N·LKS···RN GEDRLLSKQS STAPNVVNAA RAKFRTVAII   46
rKVβ1.2   ??YKP--ADI PSP-LGLPKS SESA-KCRRH L-VTKTQPHG ACWPVKPSGA   48
rKVβ2                                                      MYP    03
rKVβ3     ---------Q ---R-----S S-----·CGPR PGPGGGNGGP VGGGHGNPPG   45

rKVβ1.1   ARSLGTFTPQ HHIS····· ··LKESTA·K QTGMK                    72
rKVβ1.2   TEWKCLERFL RIHGVS---- ---Q-T-KA- ·---A                   76
rKVβ2     ESTT-SPARL SLRQTGSPGM IYSTRYGSP- RQLQF                   38
rKVβ3     GGG--SKSRT AVVPRPPAPA GA-R---G·R G----                   79
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Fig. 4. Alignment of N-terminal rat $K_v\beta$ sequences. The N-terminal sequences of $rK_v\beta1.1$ [6], the partial sequence of $rK_v\beta1.2$ (homologue to $fK_v\beta1.2$ from ferret, [11]), $rK_v\beta2$ [6], and $rK_v\beta3$ are compared. Dashes indicate residues which are identical to those at the homologous position of $rK_v\beta1.1$, dots indicate gaps which had to be introduced for optimal alignment. The first residues of the partial sequence of $rK_v\beta1.2$ have not yet been determined and are denoted here by question marks. The numbers in the right column denote the last residue per row. The first digit in the names of the subunits indicates $K_v\beta$ clones with clearly different core and C-terminal structures. The digit following the period was introduced to distinguish $K_v\beta$ subunits which only differ in their N-terminal structure. Note that the homologues of $rK_v\beta1.2$ from human and ferret heart were in the original publications called $hK_v\beta3$ [9, 10] or $fK_v\beta3$ [11], respectively. According to the notation used here, these subunits should rather be named $hK_v\beta1.2$ and $fK_v\beta1.2$, respectively.

interpulse duration. While the recovery from inactivation of $K_v1.4\Delta1$–110 H471Q·K533Y (open circles) is mainly proceeding with a fast time constant (4.5 ± 1.6 ms), the recovery from inactivation of $K_v1.4\Delta1$–110 H471Q·K533Y coexpressed with $K_v\beta3$ (filled circles) had to be described with two components (4.1 ± 0.8 ms and 730 ± 50 ms) where the slow component reflects the kinetics of recovery from $K_v\beta3$-induced inactivation.

Activation threshold, V_{th}, was determined by fitting first-order Boltzmann functions to the steady-state currents between −80 and −20 mV, obtained in two-electrode clamp experiments. Activation time constant, τ_a, was estimated by fitting a single-exponential function to the rising phases of current traces, obtained at −20 mV. At the same potential, inactivation was described by the ratio of the current after 200 ms and the peak current, I_{end}/I_{peak}. For $K_v1.4\Delta1$–110 H471Q·K533Y we determined $V_{th} = -38.3 \pm 1.3$ mV, $\tau_a = 8.2 \pm 1.0$ ms, and $I_{end}/I_{peak} = 0.987 \pm 0.017$ (mean ± S.D., $n = 16$), while coexpression with $K_v\beta3$ resulted in $V_{th} = -40.0 \pm 2.1$ mV, $\tau_a = 4.6 \pm 1.0$ ms, and $I_{end}/I_{peak} = 0.787 \pm 0.154$ ($n = 25$). Thus, coexpression with $K_v\beta3$ does not change the threshold of activation, while activation time course is accelerated. Since $K_v\beta3$ simultaneously induces inactivation, the effect of accelerating the apparent activation time course is an overestimation.

3.4. Gene family of $K_v\beta$ subunits

Several $K_v\beta$ cDNAs have now been determined encoding an expanding family of K_v channel β-subunits, having molecular weights that vary between 39 and 45 kDa in accordance with the biochemical data. The sequence alignments suggest that β-subunit protein diversity may be generated either by alternatively spliced RNAs or by expressing different genes. In the former case, distinct $K_v\beta1$ subunits may be generated having differing amino-termini, in the latter case distinct $K_v\beta$ subunits are synthesized having both differing amino-terminal and C-terminal core sequences. The highly variable amino-termini are ~40–100 amino acids, the C-terminal core sequences 330 amino acids long. The sequence conservation in the β-core sequences is 70 to 80%. The amino-termini of $K_v\beta1$ and $K_v\beta3$ subunits contain inactivating domains which may function very similarly to the ones found in some $K_v\alpha$ subunits. The $K_v\beta2$ amino-terminus does not contain an inactivating domain. Although there is presently no experimental evidence, the C-terminal core sequences may contain the β-subunit interaction site for binding to α-subunits. Then, the observed sequence differences in the $K_v\beta$ core sequences may be responsible for the differing specificities of $K_v\beta$ subunits to bind to α-subunits, e.g. the lack of $K_v\beta3$ to bind to $K_v1.5$ α-subunits in contrast to $K_v\beta1$.

As shown in Fig. 1, the cDNA clones of $rK_v\beta1$–3 from rat brain show a homologous but non-identical structure of the protein core region and the C-terminal end. Homologues to $K_v\beta1.1$ which show sequence identity to $rK_v\beta1.1$ starting from amino-acid residue Y73 were cloned from human and ferret heart [9–11]; their N-terminal ends, however, are quite different. Morales et al. [11] described PCR cloning of a homologue to the clone from ferret using a rat library. This fragment (U17967) is aligned in Fig. 4 with the N-terminal ends of the full-length clones $rK_v\beta1$–3. Because of the identical core region and C-terminal end, the putative full-length clone of this fragment should rather be considered a close relative (probably generated by alternative splicing of the same gene) of $rK_v\beta1.1$. We therefore propose a nomenclature (similar to the one by Chandy [22] for $K_v\alpha$ subunits) where the first digit indicates $K_v\beta$ clones with clearly different core and C-terminal structures. The digit following the period was introduced to distinguish $K_v\beta$ subunits which only differ in their N-terminal structure. $rK_v\beta1$ from rat brain [6] therefore becomes now $rK_v\beta1.1$, the rat homologue to the β-subunits from human and ferret heart which were in the original publications called $hK_v\beta3$ [9,10] or $fK_v\beta3$ [11] becomes $rK_v\beta1.2$. According to this notation, the subunits from human and ferret heart should therefore be named $hK_v\beta1.2$ and $fK_v\beta1.2$, respectively. However, examination of the genomic organization of the β-subunits will be required for final proof.

The in situ hybridization experiments with rat brain sections have shown that the three $rK_v\beta$ isoforms are distinctly expressed in rat brain areas. The $rK_v\beta1.1$ expression patterns may be compared to the ones of $K_v\alpha$ subunits. The comparison shows that rK_v1 α-subunit expression patterns are distinct from $rK_v\beta$ subunit expression patterns. This result has several implications. Firstly, the assembly of α- and β-subunits in K_v channels is neuron specific and cannot be subunit specific. Secondly, a particular $rK_v\beta$ subunit, e.g. $rK_v\beta1.1$, may assemble in different neurons with different $rK_v1\alpha$ subunit isoforms. Thirdly, a particular rK_v1 α-subunit, e.g. $K_v1.4$, may assemble in different neurons with different $rK_v\beta$ subunits; it may assemble with $rK_v\beta1.1$ in corpus striatum, but with $rK_v\beta3$ in the olfactory bulb. This observation suggests that differing heterooligomerizations between distinct $rK_v1\alpha$ and $rK_v\beta$ subunits may significantly contribute to K_v-channel diversity in the mammalian brain. Somewhat surprisingly, functional heterooligomerization could not yet be shown between $K_v\beta$ subunits and members of the K_v-channel families K_v2, K_v3, and K_v4, suggesting the presence of additional members of the $K_v\beta$ family.

The functional role of $rK_v\beta1.1$ as well as $rK_v\beta3$ clearly seems to be to introduce inactivation in K_v1 potassium channels. Biophysical properties of inactivation induced by $rK_v\beta3$ in $Kv1.4\Delta1$–110 channels are very similar to the one induced by $rK_v\beta1.1$; however, in many cases $rK_v\beta3$-induced inactivation is not complete. The $rK_v\beta1/3$-induced inactivation is quite fast and can be regulated by the intracellular redox potential. This regulation could be attributed to residue C7 in $rK_v\beta1.1$ [6]. Due to the strong conservation of cysteine residues in the N-terminal end of $rK_v\beta1.2$ (C8) and $rK_v\beta3$ (C7) one can assume that they serve the same purpose. Therefore, these β-subunits also provide a means of channel regulation to cellular function.

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