A New K⁺ Channel β Subunit to Specifically Enhance Kv2.2 (CDRK) Expression*

(Received for publication, April 20, 1996, and in revised form, July 26, 1996)

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Cloned K⁺ channel β subunits are hydrophilic proteins which associate to pore-forming α subunits of the Shaker subfamily. The resulting αβ heteromultimers K⁺ channels have inactivation kinetics significantly more rapid than those of the corresponding α homomultimers. This paper reports the cloning and the brain localization of mKvβ4 (m for mouse), a new β subunit. This new β subunit is highly expressed in the nervous system but is also present in other tissues such as kidney. In contrast with other β subunits, coexpression of the mKvβ4 subunit with α subunits of Shaker-type K⁺ channel does not modify the kinetic properties or voltage-dependence of these channels in Xenopus oocytes. Instead, mKvβ4 associates to Kv2.2 (CDRK), a Shab K⁺ channel, to specifically enhance (a factor of up to 6) its expression level without changing its elementary conductance or kinetics. It is without effect on another closely related Shab K⁺ channel Kv2.1 (DRK1). Chimeras between Kv2.1 and Kv2.2 indicate that the COOH-terminal end of the Kv2.2 protein is essential for its mKvβ4 sensitivity. The functional results associated with the observation of the co-localization of mKvβ4 and Kv2.2 transcripts in most brain areas strongly suggest that both subunits interact in vivo to form a slowly-inactivating K⁺ channel. A chaperone-like effect of mKvβ4 seems to permit the integration of a larger number of Kv2.2 channels at the plasma membrane.

Voltage-gated K⁺ channels are involved in a considerable number of physiological functions including neuronal integration, cardiac pacemaking, and hormone secretion. In excitable cells, they contribute to set the membrane resting potential and determine the frequency and duration of action potentials. Electrophysiological studies have identified several subtypes of voltage-gated K⁺ channels with different kinetics, voltage-dependences, and pharmacological properties (1, 2). The purification and functional reconstitution in lipid bilayers of the α-dendrotoxin sensitive K⁺ channels from rat brain has initially revealed that this class of channels is made of the assembly of α subunits (70–80 kDa) associated with smaller β subunits (38–42 kDa) (3–5). On the other hand, molecular biology techniques have now lead to the identification of more than 20 mammalian genes encoding K⁺ channels α-subunits and cloning has also recently led to new structural and functional information concerning hydrophilic K⁺ channel β subunits (Kvβ) (for a review, see Ref. 6). The Kvβ1 family has 3 members: Kvβ1.1, Kvβ1.2, and Kvβ1.3, which are alternative spliced products from the same gene, their calculated molecular masses are 44–47 kDa (7–13). The other β subunits, Kvβ2.1 and Kvβ3.1, have molecular masses of 41 and 45.5 kDa, respectively (13, 14). Sequence alignments indicate that Kvβ subunits have a common conserved core (over 85% amino acids identity) and variable amino termini (40–100 amino acids long). Kvβ1.2 and Kvβ1.3 have been cloned from heart and Kvβ2.1, Kvβ2.2, and Kvβ3.1 from brain. These auxiliary β subunits specifically associate to large pore-forming α-subunits of the Kv1 (Shaker) subfamily to modify their functional properties. For example, the coexpression of Kvβ1.1 or Kvβ3.1 subunits with Kv1.1 or Kv1.4 α subunits results in an increase of their inactivation kinetics. The proposed mechanism is a fast N-type inactivation. The β subunits have an “inactivation ball” in the NH₂-terminal part of their structure which is thought to induce a rapid inactivation by occluding the internal mouth of the pore (6, 13–15). However, Kvβ1.2 and Kvβ3.1 subunits have also been reported to increase the rate of inactivation of Kv1.4 and Kv1.5 channels, also they do not seem to contain an inactivation ball (11, 12). β subunits not only change channel kinetics, they can also change voltage-dependences of α subunits expressed in Xenopus oocytes as recently shown for the co-injection of Kvβ1.3 with Kv1.5 (10).

This paper describes the cloning and localization of a new K⁺ channel β subunit cDNA from mouse brain. This 249-amino acid β subunit is the shortest of all the Kvβ subunits cloned till now. It is highly expressed in the brain but it is also present in different tissues, particularly in kidney. Unlike other β subunits, mKvβ4 is without any effect on members of the Kv1 (Shaker) K⁺ channel family but it specifically enhances the expression of Kv2.2 (CDRK) in Xenopus oocytes, without affecting the inactivation properties. Therefore, its main role appears to be that of a chaperone-like protein to direct more efficiently the Kv2.2 α subunit to its physiological location at the plasma membrane.

EXPERIMENTAL PROCEDURES
cDNA Cloning and in Vitro Traduction—Cloning of cDNA and synthesis of complementary RNA have been described for Kv1.1 (16), Kv1.2 (17), Kv1.3 (18), Kv1.4 (19), Kv1.5 (20), Kv2.1 (21), Kv2.2 (22), Kv2.4 (23), Kv4.1 (24), and hIßK (18) channels. A 1-kb1 DNA fragment encoding the mouse Kvβ4 was amplified by reverse transcriptase-PCR using two primers deduced from the published bovine sequence (Ref. 7, GenBank accession number X70662). This fragment was 32P-labeled and

1 The abbreviations used are: kb, kilobase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

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used to screen a mouse brain cDNA library (Stratagene). Filters were hybridized with the following probes: 5 × sodium phosphate buffer (pH 7.4), 5 mM EDTA, 0.1% SDS. 100 μg of denatured salmon sperm DNA at 42°C for 18 h and washed stepwise to a final stringency of 2 × SSC, 0.3% SDS at 50°C. From 6 × 10⁸ phages screened, 16 positive clones were excised from the λZAP-XR vector into pBluescript SKII (Stratagene) and analyzed by restriction analysis and Southern blotting. Three unrelated cDNA clones, pBS-Kvβ₂, pBS-Kvβ₃, and pBS-Kvβ₄, were sequenced on both strands using the dideoxy nucleotide chain termination method by using Tth DNA polymerase (SW) and subcloned into the pVL1392 vector (PharminGen). The primers were designed to add the T7.Tag epitope (Met-Ala-Glu-Thr) and subcloned into the pVL1393 vector (PharminGen). The three transferr vectors were indentically treated with the COOH-terminal position of mKvβ₂ and the FlagM2 epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) (Eastman Kodak Co.) at the NH₂-terminal position of Kvβ₂. The coding sequence of Kvβ₂ (20) was subcloned into the pVL1393 vector (PharminGen). The three transferr vectors were individually co-transfected with linearized baculovirus DNA (BaculoGold, Pharmingen) into Sf9 cells following the manufacturer’s instructions. Supernatants containing the recombinant viruses (Bac-mKvβ₂(T7), Bac-Kv2.2(M2), and Bac-Kv1.5) were harvested 96h after transfection. The protocols for Sf9 cells culture, viral infection, and protein expression were identical to those described previously (28). Baculovirus-infected Sf9 cells were harvested 72 h after infection. Cells were washed three times with PBS and lysed directly in Laemmli’s sample buffer.

For immunoprecipitation experiments, infected cells were homogenized in 4°C during 1 h in a solubilizing buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 0.1 mM iodoacetamide supplemented with 10 μl of Pan-sorbin (Calbiochem). The insoluble material was removed by centrifugation at 12,000 × g for 15 min at 4°C. Mouse anti-T7.Tag (Novagen), mouse anti-FlagM2 (Eastman Kodak Co.) monoclonal antibodies, or purified rabbit anti-Kv1.5 (Alomone labs, Israel) antibodies were added overnight at 4°C at a 100-fold dilution, then followed by addition of protein A immobilized on Sepharose CL-4B (Sigma) for 1 h at 4°C under slow rocking. Pellets were washed six times in solubilizing buffer. Immunoprecipitated proteins were resolved by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) and transferred onto nitrocellulose membranes (Hybond C-extra, Amersham). Blots were saturated with 5% skim milk supplemented with 0.1% Tween 20 and incubated for 1 h at room temperature then incubated overnight at 4°C with primary antibodies. After several washes with PBS containing 0.1% Tween 20, blots were revealed with purified goat anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1:10,000) (Jackson) for 1 h at room temperature followed by incubation with substrate for enhanced chemiluminescence detection (ECL, Pierce).
RESULTS

Cloning, Sequencing, and Tissue Distribution of Kvβ Subunits—To isolate new members of the K+ channel β subunit family (Kvβ), oligonucleotide primers derived from the coding sequence of bovine Kvβ2 subunit (7) were used. A fragment of around 1 kb was first amplified with reverse transcriptase-PCR from mouse brain which had a high homology with the coding sequence of the bovine brain Kvβ2 cDNA. This fragment was labeled and used to screen a mouse brain cDNA library. Several positives clones were studied by restriction analysis and revealed the existence of three different populations of cDNA. The longest open reading frames derived from a 2.2-kb Kvβ1 and a 3.5-kb Kvβ2 cDNA encode for 401 and 367 amino acid proteins with calculated molecular masses of 44.7 and 41 kDa, respectively, in good agreement with the sizes determined by SDS-PAGE analysis for the corresponding in vitro translated products (46 and 42 kDa) (Fig. 1B). These two β subunits are the equivalents in mouse of those previously cloned from rat brain (13). Our mouse clones were called mKvβ1 and mKvβ2, they had 97 and 90% amino acid identity with ratKvβ1 and ratKvβ2, respectively.

The sequencing of the third cDNA (1.9 kb) revealed a shorter open reading frame of only 747 base pairs. The 5' non-translated region contains 3 termination codons prior to the first initiation codon ATG. The predicted product consists of a 249-amino acid sequence designated as mKvβ1, with a calculated molecular mass of 27.7 kDa which is again in good agreement with the molecular mass of 28 kDa obtained by SDS-PAGE analysis of in vitro translated products (Fig. 1B). Fig. 1A presents a protein alignment of the three cloned Kvβ subunits. mKvβ1 has only 50% similarity with the other cloned β-subunits. The comparison clearly shows that mKvβ1 is the shortest of all, especially in the NH2-terminal portion where the most significant differences are found. In contrast with mKvβ1, mKvβ2 does not contain in its NH2-terminal end the cysteine/serine motif and the cluster of positively charged amino acids, that constitutes the inactivating ball (13). The high homology between mKvβ1, mKvβ2, and mKvβ4 starts at glycine 25 of the mKvβ4 sequence corresponding to glycine 173 in mKvβ1. The global amino acid similarity in that part of the sequence is 83.5 and 85.3% compared to mKvβ1 and mKvβ2, respectively. This high amino acid identity provides evidence that mKvβ4 belongs to the K+ channel β subunit family.

The tissue distribution of the three subunits was investigated by Northern blot analysis (Fig. 2A) using DNA fragments corresponding to the coding sequence of mKvβ1, mKvβ2, and mKvβ4 cDNA clones. The mKvβ1 probe hybridizes with a 3.5-kb transcript in the brain but this subunit is not significantly expressed in other tissues. Conversely mKvβ2 mRNA is highly expressed in brain but is also well detected in heart and lung and is moderately present in kidney and skeletal muscle. mKvβ4 is also highly expressed in brain but is also present in kidney where a 2.5-kb transcript is detected. A longer exposure of the autoradiogram revealed a lower expression of the same transcript in lung, skeletal muscle, and heart. A 4.3-kb transcript is present in kidney, lung, and brain where an additional mRNA of 6.3 kb is also found. In kidney, the mKvβ4 probe also hybridized to a short fragment of 1.3 kb. These various messenger species may correspond to different pre-mRNA transcripts or to isoforms of the mouse mKvβ4 gene.

Fig. 2B shows a characteristic autoradiograph illustrating
the differential distribution of mRNAs encoding mKvβ4 potassium channel subunit in sagittal sections of adult mouse brain obtained with both radiolabeled cRNA and synthetic oligonucleotides of the mouse Kvβ4 homologue. Expression of the mKvβ4 mRNA was ubiquitous throughout the brain, but was clearly concentrated in certain anatomically distinct regions corresponding to gray matter regions. Higher expression levels appeared in the neo- and allocortical regions, hippocampus, olfactory bulb, and cerebellum. In the olfactory bulb, intense labeling was evident in the granule cells. A strong signal was observed throughout the cell layers of the cerebral cortex. A uniformly high hybridization signal was found in all the fields of the hippocampal formation. Transcripts were localized in the CA1-CA4 pyramidal cell layer as well as in the granule cells of the dentate gyrus. Very strong labeling in all lobules of the cerebellum was present and was confined to the granular layer and to the Purkinje cell layer. The molecular layer was very weakly labeled. No labeling was seen in the deep cerebellar nuclei. A diffuse hybridization signal was observed over most other regions including amygdaloid complex, thalamic nuclei, caudate-putamen, and globus pallidus of the basal ganglia, nuclei. A diffuse hybridization signal was observed over most other regions including amygdaloid complex, thalamic nuclei, caudate-putamen, and globus pallidus of the basal ganglia, other regions including amygdaloid complex, thalamic nuclei, caudate-putamen, and globus pallidus of the basal ganglia, thalamus, midbrain, and brainstem (pons and medulla). The microscopic analysis of emulsion-dipped sections performed at higher magnification in the most strongly labeled brain areas (olfactory bulb, hippocampus, and cerebellum, data not shown) shows that the expression of the mKvβ4 potassium channel subunit correspond exactly to the specific expression of the Kv2.2 (CDRK) α subunit in the brain (22, 29, 30).

Functional Expression of mKvβ4, β-Rat Kvβ4, and Kvβ4 have been given a role in the regulation of Shaker type K⁺ channels (Kv1). In order to test for the functional role of mKvβ4, this subunit was first coexpressed in Xenopus oocytes with members of the five most classical structural families of voltage-dependent K⁺ channel (Kv1 to Kv4, and IsK). Neither the peak currents recorded during voltage pulses to +50 mV, nor the activation or inactivation time constants were significantly modified, in the presence of mKvβ4, for Kv1.1 (RCK1), Kv1.2 (RCK5), Kv1.3 (HLK3), Kv1.4 (RCK4), Kv1.5 (RCK7), Kv3.4 (RAW3), or Kv4.1 (data not shown). Similarly no modification has been recorded for the expression of the other member of the Shab subfamily, Kv2.1 (DRK1) (Fig. 3A). In addition, mKvβ4 had no effect on K⁺ channel activity generated in Xenopus oocytes by hIsK (not shown) another type of membrane protein with a single transmembrane segment which generates slow K⁺ channel activity when expressed in oocytes (18). On the other hand, Kv2.2 (CDRK) expression was largely enhanced by co-injection of mKvβ4. Fig. 3B shows the averaged peak currents recorded 4 days after the injection of the Kv2.2 cRNA alone or together with mKvβ4. In these 19 oocyte batches mKvβ4 produced an average increase of more than 5-fold of the Kv2.2 expression (p < 0.0001). Fig. 3C shows the current-potential relationship in the absence or presence of the subunit. The Kv2.2 activation threshold was unchanged in the presence mKvβ4 (21 ± 2 and 21 ± 1 mV (n = 9, respectively). Fig. 3D shows recorded currents 4 days after cRNA injection. The Kv2.2 activation time constant (24.0 ± 0.2 ms, n = 17) was slightly diminished by the coexpression with the new β subunit (21.3 ± 0.3 ms, n = 17).

Fig. 4A shows the Kv2.2 currents recorded in outside-out patch in the presence or absence of the mKvβ4 subunit. The Kv2.2 channel slope conductance (14.7 ± 1.1 pS, n = 3) was unchanged in the presence of mKvβ4 (14.9 ± 0.5 pS, n = 3). However, in this particular series of experiments, the percentage of patches that contained at least one active channel was increased 3-fold in the presence of the β subunit (21.3 ± 0.3 ms, n = 17).

The influence of mKvβ4 on the time course of the functional expression of the Kv2.2 channel in Xenopus oocytes has been studied with the two-microelectrode technique over a period of 8 days after cRNA injection (Fig. 5A). When Kv2.2 was expressed alone the maximum current reached a peak about 2 days after injection and then stabilized. Expression of the Kv2.2 current in the presence of mKvβ4 was of course much higher and reached a steady state about 3 days after injection. In other series of experiments, Kv2.2 and mKvβ4 cRNAs were injected separately. In the particular series of experiments shown in Fig. 5B, a 3-fold increase of the Kv2.2 current level was observed when both cRNAs were injected at the same time, at the same place, with the same pipette. Injection of mKvβ4 1 day before Kv2.2 injection lead to more than a 2-fold increase of the K⁺ current. Conversely, the mean peak current was increased, but only slightly (a factor of 1.5) when Kv2.2 and the β subunit were injected at the same time but with 2 separate pipettes or when mKvβ4 was injected 1 day after Kv2.2 injection.

One first possibility to explain the stimulatory effects of
Fig. 5. **Time course of the functional expression.** A, mean peak current recorded under double microelectrode voltage clamp at +60 mV in oocytes injected with Kv2.2 or Kv2.2 + mKvβ4. Recordings were made on 4 batches of oocytes during 8 days, starting from the day after injection. B, bar graphs showing the mean peak currents elicited by voltage steps to +60 mV with delayed injections of the Kv2.2 α subunit and mKvβ4. The conditions were: 1, oocytes injected with Kv2.2 cRNAs alone; 2, Kv2.2 and mKvβ4 were injected at the same time with the same pipette; 3, the β subunit was injected 1 day before Kv2.2; 4, Kv2.2 and mKvβ4 were injected at the same time (within 15 min) but with two separate pipettes; 5, the β subunit was injected 1 day after Kv2.2. In all cases, injection of Kv2.2 cRNA was performed within the same hour in order to compare the current amplitudes.

mKvβ4 is that the auxiliary subunit could change the regulation of the Kv2.2 channel by second messengers and/or kinases. The Kv2.2 current was found to be insensitive to treatment with the phorbol ester, phorbol 12-myristate 13-acetate (30 nM), forskolin (10 μM), 8-Cl-cAMP (300 μM), 1-oleyl 2-acetyl-glycerol (100 μM), and intracellular variations of Ca2+ added to the coding sequences (FlagM2 for Kv2.2 and T7.TAG for mKvβ4) did not change these properties. Blockers of different types of K+ channel such as dendrotoxin I (10 nM), charybdotoxin (10 nM), glibenclamide (10 μM), tetraethyl ammonium (1 mM), 4-aminopyridine (1 mM), or Ca2+ (1 mM) were without effect on the Kv2.2 channel both in the absence and presence of mKvβ4 (data not shown).

One particularly interesting aspect of the work is that mKvβ4 enhances specifically Kv2.2 (CDRK) expression and not Kv2.1 (DRK1) expression, the second member of the Kv2 potassium channel subfamily. Kv2.1 and Kv2.2 have a global amino acid identity of 61.2%. The NH2-terminal cytoplasmic domain and the hydrophobic core which contains six transmembrane segments have a 84.2% amino acid identity but the remaining COOH-terminal cytoplasmic portion starting at threonine 535 in Kv2.2 displays only 21.0% identity. There-remaining COOH-terminal cytoplasmic portion starting at the COOH-terminal end of Kv2.1 displays only 21.0% identity. There-

![Image](https://example.com/image.png)

**Fig. 6. Expression of the truncated and chimeric constructs.** A, schematic representation of the channels Kv2.2 (CDRK), Kv2.1 (DRK1), the truncated form and the chimerical constructs. The black rectangles represent the six transmembrane domains. For Kv2.1 and Kv2.2 the arrows indicate the place where restriction enzyme cutting was done, at the corresponding positions in cDNAs, to prepare the truncated forms of the channels. The contribution of Kv2.2 (white) and Kv2.1 (gray) sequences in the chimeric constructs are as indicated. Percentages of amino acid identity are indicated.

B, bar graphs showing the relative variations of mean peak current amplitudes (n = 5), elicited by voltage steps to +60 mV, due to the coinjection of mKvβ4. The oocytes were injected with cRNAs coding for Kv2.1, Kv2.2, Kv2.2ΔC248, for the chimeras Kv2.2/Kv2.1 and Kv2.1/Kv2.2, with or without mKvβ4. The inset shows the mean Kv2.2ΔC248 currents in the presence and absence of mKvβ4.

Biochemical Association of mKvβ4 with Kv2.2 and Kv2.1—The functional effect of mKvβ4 on Kv2.2 expression suggested a physical association between these two subunits. To test this possibility, both subunits were expressed in insect Sf9 cells using the baculovirus expression system (Fig. 7A) and their association was shown by co-immunoprecipitation (Fig. 7B). To allow the immunodetection of proteins, “tag” sequences were added to the coding sequences (FlagM2 for Kv2.2 and T7.TAG for mKvβ4). From Bac-mKvβ4-infected cells, a major band was detected by anti-T7.TAG antibodies with the expected molecular mass of 29 kDa. Different bands ranging from 90 to 140 kDa were detected by anti-FlagM2 antibodies from Bac-Kv2.2-infected cells (Fig. 7A). The major band with an apparent molecular mass of 97 kDa corresponds to the Kv2.2 band detected in the brain (30). Other bands could correspond to different glycosylated forms. No signal was obtained from control Sf9 cells infected with Bac-IsK, a baculovirus designed to express the IsK protein (28). From Sf9 cells co-infected with Bac-mKvβ4 and Bac-Kv2.2, mKvβ4 and Kv2.2 were co-precipitated by anti-T7.TAG antibodies (anti-“tagged” mKvβ4), whereas Kv2.2 was not precipitated by anti-T7.TAG from Sf9 cells infected with Bac-Kv2.2 alone (Fig. 7B). Conversely, both proteins were co-immunoprecipitated by anti-FlagM2 antibodies (anti-tagged Kv2.2) from co-infected Sf9 cells (data not shown). These results clearly demonstrate the physical association of mKvβ4 and Kv2.2 subunits.
It was shown recently that Kvβ4 interacts with Kv1 α subunits via its conserved COOH terminus (32). The amino acid similarity between Kvβ1 and mKvβ4 in this particular region being about 85%, it was important to test whether mKvβ4 could interact with α subunits of the Kv1 subfamily. A recombinant baculovirus referred to as Bac-Kv1.5 was constructed to express Kv1.5 in insect cells. From Bac-Kv1.5-infected Sf9 cells, anti-Kv1.5 antibodies detected a band whose apparent molecular mass of 67 kDa was in agreement with the expected size (20) (Fig. 7A). The association of Kv1.5 and mKvβ4 was tested by immunoprecipitation from cells infected with Bac-mKvβ4 and Bac-Kv1.5. Fig. 7C shows that anti-Kv1.5 antibodies co-precipitate Kv1.5 and mKvβ4 subunits and that mKvβ4 cannot be precipitated by anti-Kv1.5 antibodies in the absence of Kv1.5. The formation of mKvβ4-Kv1.5 complexes was confirmed by co-immunoprecipitation reactions using anti-T7-TAG antibodies (data not shown). These results demonstrate that mKvβ4 and Kv1.5 α subunit can associate in spite of the lack of any functional effect.

**DISCUSSION**

Expression studies have shown that many of the cloned α subunits of voltage-dependent ion channels (Na+, Ca2+, and K+) were functional on their own, when expressed in *Xenopus* oocytes, but that they then exhibited pharmacological and biophysical properties sometimes different from native channels. In the last years, many auxiliary subunits have been described (see for reviews, Refs. 33–35). Their physiological role is a regulation of the function of the α subunits to which they associate. So far, auxiliary subunits β and β2 have been cloned for voltage-sensitive Na+ channels (see Ref. 36, for review). One of their primary roles has been shown to stabilize Na+ channels expression at the plasma membrane resulting in a 5–10-fold increase in the number of active voltage-sensitive Na+ channels (36). Not only the functional effects of these β subunits lead to an increased peak current, but it is also associated with a change of activation and inactivation kinetics and a modification of the voltage dependence of the inactivation process (see Ref. 33, for review). Voltage-sensitive Ca2+ channels are formed by a complex of subunits designated as α1, α2, δ, β, and γ. The α1 subunit is the pore-forming protein. The other subunits have a regulatory function, they are essential for a solid expression of the α1 subunit of different types of channels such as cardiac L-type Ca2+ channels, N-type Ca2+ channels, or P-type Ca2+ channels (see Ref. 33). The modulating effects of the β subunits also results in a change of both the biophysical and pharmacological properties of the α1 subunit (36). Auxiliary subunits called β subunits (Kvβ) have also been identified for voltage-sensitive K+ channels (3, 37) as well as for the Ca2+-activated K+ channels (BK) (38).

Coexpression of some of the cloned Kvβ subunits confers rapid A-type inactivation on non-inactivating delayed rectifiers Kv1 channels when expressed in the *Xenopus* oocyte (11–14). K+ channel α and β subunits seem to use inactivating domains that have similar properties. A-type Kv channels have an inactivating domain ("α ball") in their NH2-terminal sequence that can rapidly obturate the internal mouth of the channel upon membrane depolarization (39). A ball and chain mechanism has been proposed to explain the NH2-terminal (N-type) inactivation of A-type α subunits (40). Kvβ subunits also contain a "β ball" in their NH2-terminal end with structural similarities to the α ball. Therefore, both α and β subunits seem to provide a “ball domain” for an inactivating N-type mechanism. The β ball contains a critical cysteine residue which seems to be sensitive to the intracellular redox state as well as a cluster of positively charged amino acids (6). This paper describes a new member of the Kvβ subunit family, mKvβ4. With its 249 amino acids, it is the shortest of all the previously cloned subunits. It has no inactivating ball motif in its amino terminus, suggesting that it cannot modify the inactivation properties of non-inactivating or slowly inactivating or even rapidly inactivating K+ channels. Indeed, mKvβ4 did not modify the electrophysiological expression in *Xenopus* oocytes of a variety of Kv α subunits such as Kv1.4 or Kv1.5 whose properties are altered by other previously cloned β subunits.

It turns out that the sequence of mKvβ4 is very similar to the sequence of α subunits of voltage-dependent ion channels (Na+, Ca2+, and K+). It was of great interest to compare their properties and to know if mKvβ4 could be associated in a similar way to the endogenous counterparts of the rat. Moreover, the difference of expression level between the two β subunits of the NH2-terminal (41) (Fig. 8A). Therefore, it is not very surprising that mKvβ4, unlike mKvβ4, changes the kinetics of a non-inactivating α subunit such as the NH2-terminal deleted form of Kv1.4. Unlike mKvβ4, mKvβ4 is not expressed in kidney or in hippocampus and dentate gyrus (14).

mKvβ4 assayed in the *Xenopus* oocyte expression system had no effect on Kv1.1, Kv1.2, Kv1.3, Kv1.4, and Kv1.5 α subunits or on the other K+ channel α subunits in the other subfamilies such as Kv3.4 or Kv4.1 or on the K+ channel activity produced by Isk. It specifically altered the expression of the Kv2.2 (CDRK) α subunit. The physical association of mKvβ4 with the Kv2.2 α subunit was shown by co-immunoprecipitation from

**Fig. 7. Association of mKvβ4 with Kv2.2 or Kv1.5 in baculovirus-infected Sf9 cells.** A, expression of Kv1.5 and tagged mKvβ4, and Kv2.2 subunits in insect Sf9 cells infected by Bae-Kv1.5, Bac-mKvβ4(T7), and Bac-Kv2.2(M2) virus, respectively. Antibodies used for the detection were anti-T7,TAG for mKvβ4, anti-FlagM2 for Kv2.2, and anti-Kv1.5 for Kv1.5. Control: Bae-IsK infected Sf9 cells. Numbers on left refer to mobility of prestained molecular weight standards (Bio-Rad). B, physical association between mKvβ4 and Kv2.2 proteins was examined by co-immunoprecipitation reactions from Sf9 cells infected by Bac-Kv2.2 alone or in combination with Bac-mKvβ4. Immunoprecipitation was performed by using anti-T7,TAG antibodies (anti-mKvβ4) and co-precipitated Kv2.2 revealed with anti-FlagM2 antibodies. Arrows indicate the position of light and heavy chains of immunoglobulins. The abbreviations used are: IP, immunoprecipitated proteins; SN, supernatant of immunoprecipitation. C, physical association between mKvβ4 and Kv1.5 proteins was examined by co-immunoprecipitation reactions from Sf9 cells infected by Bac-mKvβ4 alone or in combination with Bac-Kv1.5. Immunoprecipitation was performed by using anti-Kv1.5 antibodies and co-precipitated mKvβ4 was revealed with mouse anti-T7,TAG antibodies.

baculovirus-infected insect cells. The function of this β subunit seems to facilitate the trafficking of this particular α subunit toward the plasma membrane. It could even be that Kv2.2 is normally unable to go to the plasma membrane in the absence of mKvβ4 and that its successful expression is due to the presence, in oocyte, of an endogenous β subunit analogous to mKvβ4. This sort of situation has recently been described for GIRKI, one of the major G-protein regulated inward rectifiers which express at the oocyte membrane only because it forms an heterologous assembly with another endogenous Xenopus subunit (41).

That one subunit facilitates the access and integration of another subunit in the plasma membrane is not surprising and there are many other precedents of this situation in the channel field. For example, the amiloride-sensitive Na+ channel is formed of three subunits α, β, and γ. In the colon (42, 43), it is the association with β and γ that brings the functional subunit α to the plasma membrane. The α subunit by itself, hardly integrates into the surface membrane. Another example is channel-inducing factor (44). This protein with a single transmembrane segment associate with K+ channel subunits intrinsic to the oocyte to reveal a slow K+ channel activity which is not detectable in its absence. The amplitudes of the Ca2+ currents of the α, β, and γ subunits of class A, class B N-type, class D L-type, and class E brain Ca2+ channels are also greatly increased by coexpression of αβγ and β subunits (see Ref. 33, for review).

The striking property of mKvβ4 is that it distinguishes between the two clones in the Shab subfamily Kv2.1 (DRK1) and Kv2.2 (CDRK). Not only Kv2.1 and Kv2.2 display very similar delayed rectifier properties, but they also have a very high amino acid similarity in this region (32). In the colon (42, 43), it is the association with β and γ that brings the functional subunit α to the plasma membrane. The α subunit by itself, hardly integrates into the surface membrane. Another example is channel-inducing factor (44). This protein with a single transmembrane segment associate with K+ channel subunits intrinsic to the oocyte to reveal a slow K+ channel activity which is not detectable in its absence. The amplitudes of the Ca2+ currents of the α, β, and γ subunits of class A, class B N-type, class D L-type, and class E brain Ca2+ channels are also greatly increased by coexpression of αβγ and β subunits (see Ref. 33, for review).

The β subunit synthesis and/or stability. While this paper was submitted for publication, Shi et al. (45) reported an increase in Kv1.2 surface expression due to Kvβ2 expression. One chaperone-like effect of Kvβ2 is to increase stability of Kvβ2-Kv1.2 complexes as suggested for mKvβ4-Kv2.2. On the other hand, mKvβ3 is also able to bind Kv1.5 subunit without the alterations observed on Kv2.2 expression. The exact role and physiological consequence of this association remains to be determined in tissues expressing other Kv β subunits.

Acknowledgments—We are grateful to M. Jodar, N. Louradour, G. Jarrett, and F. Aguilà for expert technical assistance and to D. Doume for secretarial assistance. We acknowledge Dr. J-P. Hugnot for fruitful discussion. We gratefully thank Drs. V. Demas and B. L. Tempel, M. A. Tanouye, R. D. Zühlke, and R. H. Joho, X. J. Li, and P. M. Hwang, O. Pongs, and L. Salkoff for the generous gift of Kv1.1, Kv1.4, Kv2.1, Kv2.2, Kv3.4, and Kv4.1 respectively.

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J. Biol. Chem. 1996, 271:26341-26348.
doi: 10.1074/jbc.271.42.26341

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