Characteristics of Brain Kv1 Channels Tailored to Mimic Native Counterparts by Tandem Linkage of α Subunits

IMPLICATIONS FOR K⁺ CHANNELOPATHIES*

Received for publication, October 8, 2001, and in revised form, February 14, 2002
Published, JBC Papers in Press, February 21, 2002, DOI 10.1074/jbc.M109698200

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Most neuronal Kv1 channels contain Kv1.1, Kv1.2 α, and Kvβ2.1 subunits, and Kvβ2.1 subunits. Accordingly, Kv1.2 (Kv1.1-(1.2)3) constructs produced proteins of 1.2), dimeric (Kv1.1–1.2 or 1.2–1.2), and tetrameric “native-like” hetero-oligomers. The monomeric (Kv1.1 or Kv1.2) constructs produced proteins of Mr ~62,000, 120,000, and 240,000, which assembled into (α)4(β)4 complexes. Each α cRNA yielded a distinct K⁺ current in oocytes, with voltage dependence of activation being shifted negatively as the Kv1.1 content in tetramers was increased. Channels containing 1, 2, or 4 copies of Kv1.1 were blocked by dendrotoxin (k (DTX)k with similarly high potencies, whereas Kv1.2 proved nonsusceptible. Accordingly, Kv1.2/β2.1 expressed in baby hamster kidney cells failed to bind DTXk; in contrast, oligomers containing only one Kv1.1 subunit in a tetramer exhibited high affinity, with additional copies causing modest increases. Thus, one Kv1.1 subunit largely confers high affinity for DTXk, whereas channel electrophysiological properties are tailored by the content of Kv1.1 relative to Kv1.2. This notable advance could explain the diversity of symptoms of human episodic ataxia I, which is often accompanied by myokymia, due to mutated Kv1.1 being assembled in different combinations with wild-type and Kv1.2.

Voltage-gated K⁺ channels are involved in the maintenance of resting membrane potential and control of action potential frequency and threshold of excitation (1). Members of the Shaker-related subfamily (Kv1) are sialoglycoprotein complexes (Mr ~400,000) consisting of four transmembrane channel-forming α subunits (Kv1.1–1.6) and four cytoplasmic regulatory β (Kvβ1–3) proteins (Ref. 2; reviewed in Ref. 3). Heterologously expressed Kv1 members assemble via their N-terminal domain (NAB) (4) into homo- or heteromultimeric channels with distinct electrophysiological and pharmacological properties (3, 5, 6), although the subunit stoichiometries in the plasmalemma used for the recordings have not been determined. Co-expression of Kvβ1 or Kvβ3 with Kv1 α subunits accelerates inactivation of the K⁺ currents (2, 7), whereas Kvβ2 increases surface expression (8–10).

Authentic Kv1 channel proteins were first identified (11) using α and β/dendrotoxin (DTX); αDTX is less discriminating and inhibits Kv1.2 > Kv1.1 > Kv1.6 currents, whereas DTXk is more stringent and specifically blocks Kv1.1 channels (12). Sequential immunoprecipitation, using subunit-specific antibodies, and affinity chromatography on immobilized αDTX and/or DTXk (13–16) unveiled a limited repertoire of subtypes, ranging from Kv1.1 to those containing Kv1.1/1.2, Kv1.1/1.2/1.6, Kv1.2/1.3/1.4/1.6, or Kv1.1/1.2/1.3/1.4. Also, data from immunocytochemistry and immunological analysis established that Kv1.1, Kv1.2, and Kv1.3 are the most abundant subunits found together in channel complexes (14, 15, 17, 18). These subunits are co-localized in the juxta-paranodal region of the nodes of Ranvier, as well as in the axons and terminals of cerebellar basket cells of rat brain (19). Thus far, heterologous expression of Kv1 α and β subunits has failed to mimic the characteristics of neuronal K⁺ currents, highlighting the need to reproduce native K⁺ channels. Additionally, certain neurological conditions are associated with changes in Kv1 channel multimers. For example, an increase in the number of αDTX binding sites occurs in demyelinated brain plaques from patients with multiple sclerosis (20), whereas the content of αDTX and DTXk acceptors is decreased in hippocampus from aging patients or those with Alzheimer’s disease (21). Furthermore, several mutations in Kv1.1 are associated with human disorders (e.g. episodic ataxia I and myokymia) (1, 22, 23), whereas mutations in certain other genes can distort expression and localization of Kv1.1 and 1.2, thereby inducing abnormal phenotypes (e.g. mouse strains Trembler and Shiverer) (24).

Herein, α subunits were tandem-linked to recreate subtypes with predefined stoichiometries for Kv1.1 and 1.2 and to quantitatively relate subunit composition to channel properties. Functional K⁺ channels containing different numbers of Kv1.1 co-assembled with Kv1.2 in the presence of Kvβ2.1 were generated using Semliki Forest virus (SFV), yielding oligomers resembling those prevalent in neurons. Electrophysiological recording of their respective K⁺ currents in oocytes and analysis of the binding of 125S-labeled DTXk and αDTX to transfected mammalian cells revealed that varying proportions of Kv1.1 and 1.2 could subtly influence the biophysical and pharmacological properties of expressed channels. Such systematic profiling should allow identification of K⁺ channel counterparts in neurons and their altered phenotypes in diseases.

* This work was supported by a Wellcome Trust grant (to J. O. D.) and a BBSRC CASE studentship in cooperation with Cambridge Drug Discovery (to F. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: DTX, dendrotoxin; SFV, Semliki Forest virus; BHK, baby hamster kidney.
MATERIALS AND METHODS

Construction of Monomeric, Dimeric, and Tetrameric cDNAs—All constructs were incorporated into pUTp2PA for high expression in *Xenopus* oocytes (9). Monomeric cDNAs were prepared by PCR amplification of rat Kv1.1 and 1.2 subunits, using the respective primer pairs (a) 5'-CCCTCGAGCAACATGGCAGGTGTAAG-3' and 5'-CTGTTGCAATTCTTAAACATCGTGG-3' and (b) 5'-ACTCTCGAGACCATGGCCAG-3' and 5'-CAACTCGAGATCAGTTAACATTTTGTA-3' to introduce Xhol and Sall sites (underlined) with pAKS Kv1.1 or 1.2 cDNA as template (a gift from Prof. O. Pongs). After digestion, the products were ligated into pUTp2PA similarly cleaved. The Kv1.2–1.2 construct was obtained by joining together the two cDNAs via the 5'-untranslated region of the *Xenopus* β-globin gene. For the first position in this tandem, Kv1.2 was amplified from pAKS using primers 5'-CTGCAACTATGTTACTGGTGATGTCAGGGG-3' and 5'-CAACTCGAGATCAGTTAACATTTTGTA-3' to remove the stop codon and introduce SpeI and Xhol sites (underlined). After digestion, the PCR product was ligated into pUTp2PA vector cut with Xhol and Xhol to generate pUTp2PA Kv1.2 (without stop codon). To introduce the second constituent of the dimer, a His6 tag was inserted at the C terminus of Kv1.2, and its initiation codon was removed by PCR to give pUTp2PA Kv1.2 (© His6, © ATG). The latter was amplified using the primer pair 5'-CTGCAACTATGTTACTGGTGATGTCAGGGG-3' (sense) and 5'-CTGTTGCAATTCTTAAACATCGTGG-3' (anti-sense). After digestion with Sall (introduced by primer) and EcoRI (present downstream of coding sequence), the fragment was subcloned into pUTp2PA Kv1.2 (without stop codon) cut with Xhol and EcoRI to generate pUTp2PA Kv1.2–1.2 (© His6, © ATG, 16-amino acid linker, and a stop codon). pUTp2PA Kv1.1–1.2 was designed on the same principle of tandem sequence of cDNAs (4). Amplification of pSFV1 vector cut with SpeI and XhoI (487 or 475 bp downstream of Kv1.1 and 1.2, respectively) and (anti-)sense) after digestion with Sall and EcoRI, pUTp2PA Kv1.1–1.2 was obtained by joining Kv1.2–1.2 downstream of Kv1.1–1.2 after manipulations of the two constructs. A His6 sequence and stop codon were removed from the C terminus of the heterodimer and ligated in-frame to homodimer after deletion of ATG from the first Kv1.2 cDNA to yield pUTp2PA Kv1.1–1.2;.

To prepare a Kv1.2–1.1 chimera, the N-terminal region of Kv1.1 (487 bp) was replaced with the equivalent domain of Kv1.2 (475 bp). pUTp2PA Kv1.1 and 1.2 plasmids were digested with HindIII (outside the coding region) and SalI (487 or 475 bp downstream of Kv1.1 and 1.2, respectively). The isolated larger (©450 bp from pUTp2PA Kv1.1) and smaller (©475 bp from pUTp2PA Kv1.2) fragments were ligated to yield pUTp2PA Kv1.2–1.1; Every construct made above was verified by restriction digestion and dideoxy DNA sequencing. For expression in the SFV system, all Kv1p2PA constructs were subcloned into pSFV vector, employing HindIII and BglII sites to cut out the cDNA fragments that were blunt-ended before ligation with pSFV1 vector (10). cDNAs for each Kv1 construct (in pUTp2PA and pSFV1) and pSFV11 (plasmid encoding viral packaging proteins) were prepared as described previously (10).

Electrophysiological and Biochemical Analysis of Recombinant K⁺ Channels—Oocytes were isolated from mature *Xenopus laevis* females (*Xenopus 1, Blades*) and injected with Kv1.1 or 1.2 cRNA, as described by Main et al. (25). K⁺ currents were recorded after 72 h, using a two-microelectrode voltage clamp amplifier (TEC-03; NPI) as described previously (26) during voltage steps to ±80 mV with a holding potential of ±80 mV. DTX was applied by superfusion (at a rate of 5 ml/min), and its concentration increased cumulatively. Addition of tetraethylammonium chloride (0.1–100 mM) was made with substitution of NaCl to maintain ionic strength. A membrane fraction from the injected and noninjected oocytes was analyzed by SDS-PAGE. Channels were expressed in BHK cells and analyzed in the native state by gel filtration, or by SDS-PAGE and Western blotting as described previously (10). Saturable binding of 125I-labeled DTX and DTX to intact cells expressing the various constructs was measured in triplicate under established conditions (10) by rapid filtration through GF/F glass microfiber filters that had been presoaked in 0.5% (v/v) polyethyleneimine. The radioactivity associated with the washed filters was quantified by radio-counting; data presented (± S.E.) were analyzed using the Graph Pad software (Prism 3.0) based on a one-site model.

RESULTS

Voltage Activation of K⁺ Channel Subtypes Prominent in Neurons Is Varied by Pretermining Their Content of Kv1.1 and Kv1.2 Subunits—a Subunit stoichiometries were pre-defined by linking their cDNAs in an open reading frame (Fig. 1), using a 16-amino acid sequence from 5'-untranslated region of the *Xenopus* β-globin gene. In this way, the expressed Kv1.2–1.2 or Kv1.1–1.2 should assemble into the desired tetrameric proteins, Kv1.2–1.2 or Kv1.1–1.2, respectively, whereas the third construct would yield a channel consisting of one Kv1.1 subunit and three Kv1.2 subunits. Biochemical evidence was obtained for formation of the expected oligomers in oocytes, to validate the data subsequently obtained from electrophysiological analysis of the K⁺ currents. Injection into oocytes of cDNAs for the pUTp2PA Kv1 constructs followed by Western blotting demonstrated that Kv1.1 and Kv1.2 cDNAs were translated into single subunits of Mr ~60,000–64,000, whereas the dimers and tetramer gave proteins with Mr ~120,000 and Mr ~240,000, respectively (Fig. 2A). A two-electrode voltage clamp was used to establish the effects of varying the ratio of Kv1.1 and Kv1.2 in expressed tetramers. Because Kv1β2.1 has no observable effect on activation of Kv1.1 and Kv1.2 channels (26), it was omitted. Oocytes injected with equivalent amounts of cDNAs encoding each of the constructs yielded 1–10 μA K⁺ currents after 72 h, establishing that the expressed proteins were inserted into the plasmalemma as functional channels. Kv1.1, Kv1.2, Kv1.1–1.2, Kv1.2–1.2, and Kv1.1–1.2 gave outward nonactivating K⁺ currents with characteristic voltage

![Fig. 1. Schematic representation of K⁺ channels translated from the different constructed cDNAs](image-url)
Kv1.1 Subunits Influence K⁺ Channel Characteristics

1.2–1.2 (with synaptic membranes) oocytes were injected with the individual cRNAs (lanes 1–3 and 6–8), along with synaptic membranes (lane 4), were subjected to SDS-PAGE (left panel, 9% gel; right panel, 6% gel). After transfer onto polyvinylidene difluoride, the membranes were blocked with 5% (w/v) dried milk before overnight incubation with monoclonal anti-Kv1.2 (lanes 1–5; 1:1000 dilution) or rabbit polyclonal anti-Kv1.1 (lanes 6–8; ~1 μg IgG/ml) and detection with the ECL system. Lane 1, Kv1.2; lane 2, Kv1.1–1.2; lane 3, Kv1.2; lane 6, Kv1.1–1.2; lane 7, Kv1.1; lane 8, Kv1.1-(1.2)₃. Neither Kv1.2 (lane 5) nor Kv1.1 (data not shown) was detectable in the controls injected with water. B, K⁺ currents were recorded from oocytes 72 h after injection with cRNAs encoding Kv1.1 (I), Kv1.2 (II), Kv1.2–1.2 (III), Kv1.1–1.2 (IV), or Kv1.1–1.2 (V). Voltage dependence of activation was measured by voltage pulses of 400 ms in duration, from a holding potential of −80 mV to +40 mV in 10-mV increments (VI). C, activation curves plotted, using a simple Boltzmann function, as the mean conductances (± S.E.) for each construct (Kv1.1 (●), 1.2 (○), 1.2–1.2 (△), 1.1–1.2 (▲), and 1.1(1.2)₃ (▼)) of the peak fractions are shown (inset). The column was calibrated with thyroglobulin, ferritin, catalase, and β-amylase; the arrow indicates the common elution position of the K⁺ channels.

| Table I |
|-----------------------------------------------|
| cRNA injected | n⁰ | V₅₀ ± SD⁰ | k ± SD | IC₅₀ for DTXₖ |
|----------------|-----|------------|------|---------------|
| Kv1.1 | 5 | −30.8 ± 1.59 | 9.02 ± 0.89 | <0.05 (n = 2) |
| Kv1.1–1.2 | 8 | −26.5 ± 1.67 | 8.73 ± 1.14 | 0.1 ± 0.05 (n = 3) |
| Kv1.1–1.2 | 13 | −22.6 ± 1.16 | 10.1 ± 1.19 | 0.45 ± 0.2 (n = 2) |
| Kv1.2–1.2 | 7 | −16.97 ± 0.98 | 8.3 ± 1.13 | ND² |
| Kv1.2 | 10 | −15.1 ± 1.63 | 10.8 ± 0.93 | N.I (n = 3) |

* n⁰, number of oocytes tested.
* All V₅₀ values are significantly different (p < 0.01), except those for Kv1.2 and Kv1.1–1.2.
* ND, not determined; NI, no inhibition at 100 nM toxin.

Recreation of Recombinant K⁺ Channels with α/β Subunit Stoichiometries Mimicking Major Subtypes in Brain—To generate adequate amounts of the recombinant channels for biochemical analysis, pSFV Kv1.1, Kv1.2, Kv1.1–1.2, or Kv1.1–1.2 was expressed in BHK cells to generate four oligomers representing the majority of possible combinations of the most abundant subunits found in central neurons. The expression level was elevated by inclusion of Kvβ2.1, which promotes cell surface targeting; to obtain adequate quantities of the poorly expressed Kv1.1, (10), the N-terminal part was replaced with an analogous moiety of Kv1.2 that regulates the efficiency of assembly (see “Introduction”). This construct gave increased surface expression in BHK cells (−2-fold relative to the unmodified Kv1.1), yielding a subunit of the expected Mₗ, ~60,000–62,000 on immunoblotting (Fig. 3A). The dimer was twice this size and was recognized by both anti-Kv1.1 and anti-Kv1.2...
antibodies (Fig. 3, A–C), confirming the presence of both α subunits. Kv1.1-(1.2)₃ construct gave a protein of the expected molecular weight (240,000) that was also reactive with anti-Kv1.1 and anti-Kv1.2 antibodies (Fig. 3, B and C). Thus, the dimer and tetramer cRNAs were correctly and fully translated, without any detectable proteolytic breakdown products; importantly, the channels were correctly assembled and inserted into the plasmalemma and functional (Table II; detailed later). Direct evidence for the formation of α/β subunit oligomers was provided by the oligomeric sizes of the channels extracted from BHK cells in nonnaturating detergent obtained from gel filtration on Superose 6HR (Fig. 3D); the similar elution position for the monomer, dimer, and tetramer expressed with Kvβ2.1 gave an apparent molecular weight for the oligomer-detergent complex of ~515,000 (Fig. 3D, inset). Because this value is very similar to the size for Kv1.2α(β2.1)₂ (10), it can be concluded that all the Kv1 constructs produced proteins that assembled into tetramers containing four α and four β subunits, as observed for neuronal K⁺ channel complexes (27).

Homomeric Kv1.1 and Kv1.2 Channels Show Different Affinities for DTXk and αDTX; Their K⁺ Currents Exhibit Corresponding Susceptibilities to DTXk—BHK cells expressing Kv(1.2)₄ proved unable to bind ¹²⁵I-DTXk (Table II); accordingly, the K⁺ current generated in oocytes was insensitive to block by 100 nM DTXk (Table I). In contrast, Kv(1.1)₄ displayed high affinity for ¹²⁵I-DTXk (Fig. 4A; Table II), consistent with its K⁺ current in oocytes being inhibited by low concentrations of DTXk (Table I). An avid interaction was reaffirmed for the latter channel by the Kₚ values of DTXk competing for the binding of ¹²⁵I-DTXk (Fig. 4G) and ¹²⁵I-αDTX (Fig. 4I).

Comparison of the binding of both toxins to Kv(1.1)₄ showed that ¹²⁵I-αDTX displayed a 12-fold lower affinity than ¹²⁵I-DTXk (Table II). On the other hand, ¹²⁵I-αDTX exhibited a ~5-fold higher affinity for Kv(1.2)₄ than Kv(1.1)₄ (Table II). Thus, based on both toxin binding and functional blockade, one copy of Kv1.1 is adequate to bestow near maximal affinity for DTXk and susceptibility to inhibition (see “Discussion”).

Moreover, the behavior of the channels in binding ¹²⁵I-αDTX gave a similar trend with no major difference in the Kₚ values upon increasing the number of copies of Kv1.2 in the (α₁α₂β₁)₃ multimers (Table II). Likewise, the similar Kₚ values for αDTX antagonizing ¹²⁵I-DTXk binding to Kv(1.1–1.2)₂ and Kv(1.1–1.2)₃ (Fig. 4H) revealed that two Kv1.2 subunits are sufficient for binding αDTX with high affinity. Because αDTX has a lower affinity for Kv(1.1)₄ than DTXk, it proved significantly less potent in antagonizing ¹²⁵I-DTXk binding, but this was increased substantially (14–34-fold) when Kv1.2 subunits were introduced to the channels (Fig. 4H). As expected, DTXk was less effective in displacing ¹²⁵I-αDTX from Kv1.2-containing multimers than Kv(1.1)₄ (Fig. 4I).

Finally, it is noteworthy that not only can the two toxins discriminate channel subtypes, but the Bₘₐₓ values for αDTX were 2–3-fold higher than for DTXk in the same batch of BHK cells (Table II; see “Discussion”).

**DISCUSSION**

**Successful Recreation of the Most Abundant Kv1 Heteromultimers in Mammalian Neurons**—Kv1.1-, Kv1.2-, and Kvβ2.1-containing oligomers predominate in brain (13, 17); Kv(1.2)₄ is also present, but Kv1.1 always occurs in association with other members (14, 15). Due to their abundance and functional importance (see “Introduction”), we profiled the characteristics of those with several different proportions of Kv1.1 and Kv1.2 to encompass the subtypes found in neurons. All the linked Kv1 subunits were expressed in both amphibian and mammalian cells as single, intact proteins without premature translation or degradation products. Their functionality was documented by the measurements of the K⁺ currents recorded after expression in oocytes, with characteristics matching those expected. When co-expressed with Kvβ2.1, each channel co-assembled into (α₁α₂β₁)₃ complexes and was inserted correctly into the plasma membrane of BHK cells, as established from measurement of high affinity...
binding of DTXₖ and αDTX, which require α subunits to be assembled into tetramers (28).

Increasing the Number of Kv1.1 Subunits in a Tetramer Gave Commensurate Changes in the Voltage Dependence of Activation of the K⁺ Currents—The tetramers containing varying ratios of Kv1.1 and Kv1.2 gave slowly inactivating, outward currents with distinct voltage dependences of activation that differed from either parent. The $V_{1/2}$ values were slightly skewed toward that of Kv1.1, which may be due to the effect of Kv1.1 upon activation (29, 30). However, the $V_{1/2}$ values also clearly reflect the influence of both parental subunits. Establishing the presence of both Kv1.1 and Kv1.2 in this way in the expressed channels constructed by tandem linkage of subunits in the oligomers afforded exploitation of the predetermined stoichiometries for the subsequent toxin block and binding studies.

A Single Kv1.1 Subunit in Tetramers Containing Kv1.2 Creates High Affinity Functional Interaction with DTXₖ—Both saturable binding and competition analysis using intact BHK cells and inhibition of the K⁺ currents in oocytes confirmed that one Kv1.1 subunit in an oligomer is enough to confer a high affinity interaction with DTXₖ and blockade of the currents. This conclusion from measurements on defined populations of native-like subtypes accords with a deduction from functional studies on biochemically uncharacterized channels, namely, that a single toxin-sensitive subunit can give K⁺ currents susceptibility to DTXₖ homologues (6). Mutagenesis of DTXₖ has shown that two domains, 3₁₀ helix and β-turn, that are 14 Å apart contribute to interaction with possibly two adjacent channel subunits (31). Notably, the 3₁₀ helix is essential for both high affinity binding of DTXₖ to Kv1.1 and excluding its interaction with other subunits; in contrast, the β-turn seems less important for recognition of Kv1.1 (31). Hence, it was suggested that the latter region could interact with a different subunit of the K⁺ channels in synaptic membranes; in the present study, this would be another Kv1.1 or Kv1.2. Thus, the slightly lower DTXₖ affinity for Kv(1.1–1.2)₂ compared with Kv(1.1)₄ may be attributed to a reduction in the number of sites for interaction with the 3₁₀ domains and/or a decrease in the affinity of β-turn for the adjacent subunit (i.e. Kv1.2 instead of Kv1.1). With two copies of Kv1.1 and Kv1.2, a significant increase in DTXₖ affinity was not observed, possibly because of its access being restricted due to the two Kv1.1 in some of the oligomer being positioned diagonally rather than adjacently (Fig. 1B).

Notably, a single high affinity site for DTXₖ was observed

![Figure 4](image-url)
regardless of the number of Kv1.1 subunits, whereas synaptic membranes show a high affinity and low affinity binding site (31). The retention of high affinity for DTXk by Kv1.1 channels with up to three insensitive Kv1.2 subunits provides good evidence for the lower affinity site being due to multimerization with other subunits (e.g., Kv1.4, Kv1.3, or Kv1.6) known to be associated with Kv1.1 (13–15, 19) that may result due to steric hindrance. Because Kv1.1, does not exist in normal human brain (14), and a majority of native K+ channels containing Kv1.2 also have Kv1.1 (50%) (13, 17), the latter must represent the bulk of the higher affinity DTXk sites (31) and thus could explain the overlapping location of DTXk and oDTX acceptors in rat brain (32).

Kv1.1 Channels Apparently Have More Sites for oDTX than DTXk—The B_{max} for oDTX binding sites was always 2–3-fold higher than that for DTXk in the same batches of cells. This intriguing finding can possibly be explained by structural elements of DTXk, oDTX (96% homologous to DTXk), and oDTX. Similar residues (e.g., Lys-3/6 and Lys-26) in the two domains (β10 helix and β-turn), equivalent to those important for DTXk recognition of rat Kv1.1-containing channels (31, 33), have also been identified from scanning mutagenesis and thermodynamic mutant cycle analysis in DTXk (34). Mutation of the ShKv1.1 channel revealed that DTXk binds at some distance from the pore, involving Lys-3 and Arg-10 among other residues; these, with Lys-26, form a triangle whose vertices are 20 Å apart. This distance would allow interaction with adjacent subunits through Lys-3, Arg-10 (34), and/or Lys-26 (31). However, mutations in the Shaker chimeric channel did not yield evidence for a contribution of Lys-26 to the strong interaction, possibly because it binds residues not substituted that form part of the DTX acceptor site (35, 36). On the other hand, residues at the N terminal of oDTX are most influential for Kv1 interaction (37) and are equivalent to the essential amino acids in the β10 helix of DTXk, although oDTX lacks such a secondary structural feature; this could underlie its less stringent specificity. Furthermore, the contributions of β-turn residues in oDTX are less pronounced than that for DTXk (33, 37). Based on the collective findings, DTXk appears to interact with two Kv1 subunits, whereas oDTX requires predominantly one interactive domain; hence, twice as many DTXk molecules might be able to bind each tetramer, or perhaps not all the channels are folded perfectly and therefore cannot accommodate the stringent requirements for DTXk binding.

Functional Properties of the Recombinant Channels in Relation to Those of Neuronal K+ Currents in Health and Disease—Because Kv1.1 and 1.2 subunits exhibit distinct properties, it is not surprising that changing their ratios resulted in K+ currents with unique electrophysiological and pharmacological properties. The profiling of their characteristics ought to help molecular entities to be ascribed to the native K+ channels, a feat not feasible to date. Thus, the properties of the recombinant channels were compared with the two types of DTX-sensitive, sustained K+ currents recorded in neuronal cells. Our data for Kv1.1 (1.1–2.1), and Kv1.1(1.2)β indicate that they resemble a DTX-sensitive, low-threshold current (I_{DTX}) in various neurons that activates within −50 to −60 mV and prevents repetitive firing (38, 39). Because Kv1.1 and Kv1.2 are the major subunits known to give sustained outward K+ currents that are highly sensitive to DTX, their combinations must be responsible for such current phenotypes. Gold et al. (40) have described a similar current (I_{K0}) in rat sensory neurons that may relate to a DTXk-susceptible current found in the same preparation (41); as with the currents we observed, I_{K0} activates at low thresholds and is fully activated by +20 mV. From the results herein, it seems that one Kv1.1 subunit in an oligomer with Kv1.2 would be sufficient to give such voltage sensitivity and DTXk susceptibility; moreover, the slowly inactivating nature of the currents excludes the presence of Kv1.4 and thereby implicates either Kv1.1/1.2 or Kv1.1/1.2/1.6 (15).

These properties derived from channels encompassing most combinations of Kv1.1/1.2 give new insights into the molecular basis of the symptoms seen in patients suffering from episodic ataxia/myokymia (22, 23). Because we demonstrated herein that these channels’ biophysical parameters show gradual changes proportional to their content of Kv1.1 subunits, and a variety of human mutations are known to alter the properties of Kv1.1 homomers (23), the diversity of abnormalities in different cases supports the existence of several hetero-oligomeric combinations of mutated and wild-type Kv1.1, together with Kv1.2; note that Kv1.1 does not occur in human neurons (14).

Such subtypes would exist in different locations, neurons, or compartments (e.g. nerve terminals, axons, and so forth) where each normally serves a pivotal role; thus, a spectrum of abnormalities in individual patients is likely to be due to different stoichiometries of Kv1.2, Kv1.1, and a variant that could be mutated at one of several residues (22, 23). Importantly, the major advance accomplished herein will allow elucidation of the modified properties of all these channel forms constructed by tandem linking Kv1.1, its distinct mutants, and Kv1.2 in the various stoichiometries, followed by co-expression with Kvβ2.1. Although this strategy has already been found to be informative for dimers of normal and mutated Kv1.1 (42), creating the authentic α/β combinations should prove much more pertinent.

Acknowledgment—We thank Dr. M. Main at Glaxo-Smith-Kline for help with some recordings from oocytes.

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