

# Auxiliary Subunits of Shaker-type Potassium Channels

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*Voltage-gated potassium channels are important determinants of membrane excitability. This family of ion channels is composed of several classes of proteins, including the pore-forming  $Kv\alpha$  subunits and the recently identified auxiliary  $Kv\beta$  subunits. A combination of a large number of genes that encode various  $\alpha$  subunits and  $\beta$  subunits and the selective formation of  $\alpha$ - $\alpha$  and  $\alpha$ - $\beta$  heteromultimeric channels provides rich molecular diversity that allows for regulated functional heterogeneity in both excitable tissues and nonexcitable tissues. Because the  $Kv\beta$  subunits can either upregulate or downregulate potassium currents, depending on the specific subunit combination, it is essential to understand their function at the molecular level. Furthermore, targeted changes of the  $Kv\beta$  expression or disruption of certain  $\alpha$ - $\beta$  interactions could serve as a molecular basis for designing drugs and therapy to regulate excitability clinically. (Trends Cardiovasc Med 1998;8:229-234) © 1998, Elsevier Science Inc.*

In cardiac tissue, the potassium currents regulate resting membrane potential and duration of action potentials [see reviews by Campbell et al. (1992), Deal et al. (1996), and Brown (1997)]. Because the functional requirements in different parts of the heart are different, the heterogeneity of voltage-sensitive potassium currents is essential for proper cardiac function. In addition to the large number of genes encoding the channel subunits and posttranslational modulations of various channel proteins, the diversity of potassium channels is further enhanced by the mix-and-match assembly

of different subunits [see reviews by Jan and Jan (1990) and Salkoff et al. (1992)]. Within the large family of Shaker-like potassium channels, the selective assembly of various subunits includes the heteromultimer formation of distinct pore-forming  $\alpha$  subunits and/or assembly of different kinds of subunits such as the selective association of  $\alpha$  subunits with hydrophilic cytoplasmic  $\beta$  subunit(s). Together, these give rise to the vast heterogeneity of  $K^+$  currents. Changes in expression of a given subunit may alter both composition and stoichiometry of heteromultimers in vivo, which allows incremental tuning of  $K^+$  current system(s) during development and in response to changes in the cellular environment.

## • Primary Structure of $Kv\beta$ Subunits

The modulatory  $Kv\beta$  subunits were first identified biochemically as smaller molecule mass (~40 kD) components that were copurified with the  $\alpha$ -dendrotoxin (isolated from mamba snake venom) acceptor in rat and bovine brain (Rehm and Lazdunski 1988, Parcej and Dolly

1989). The  $\alpha$ -dendrotoxin acceptor with a molecular mass of ~70-80 kD was later found to be the  $Kv1.2$   $\alpha$  subunit (also known as RCK5) (Scott et al. 1990). The presence of small modulatory subunits was suggested by a number of other experiments in various systems (Rudy et al. 1988, Trimmer 1991, Chabala et al. 1993). The gene that encodes the first  $\beta$  subunit,  $Kv\beta2$ , was cloned from bovine brain on the basis of the peptide sequence information (Scott et al. 1994). Independently, the genes encoding  $Kv\beta$  subunits were also isolated from mouse and *Drosophila* by molecular and genetic approaches. First, a cDNA known as F5 was isolated from the interleukin 2 (IL-2) activated murine T lymphocytes by subtractive hybridization. Although the primary sequence was reported earlier (Cohen et al. 1992), the connection of F5 to potassium channels was not known until the cloning of  $Kv\beta2$ . It is now clear that F5 is the mouse homologue of  $Kv\beta2$ . A *Drosophila*  $\beta$  subunit was obtained with a genetic approach, and it is encoded by the Hyperkinetic (*Hk*) locus (Chouinard et al. 1995).

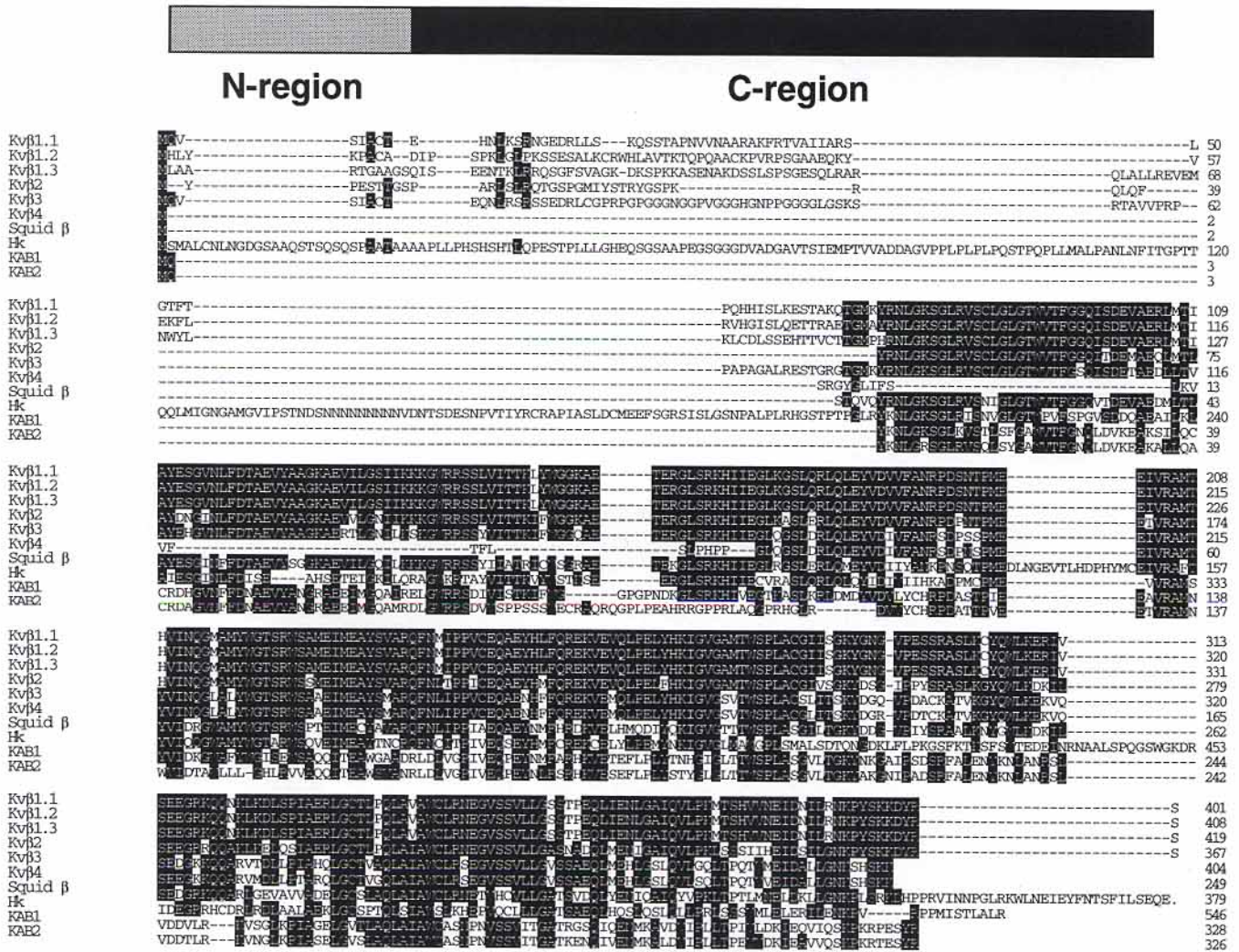
So far, nine homologous genes encoding various  $\beta$  subunits have been isolated, including six from mammals, one from *Drosophila*, and two from plants (Figure 1). Based on primary sequences, there are several interesting features. First, one can separate the primary sequence of the cloned  $\beta$  subunits into two domains: the N-terminal domain (N-region), which varies significantly both in length and in sequence, and the C-terminal core region (C-region), which exhibits high sequence conservation among various  $\beta$  subunits (Figure 1). Second, within the  $Kv\beta1$  group, several splice variants have also been identified, known as  $Kv\beta1.1$ ,  $Kv\beta1.2$ , and  $Kv\beta1.3$  (England et al. 1995a and b, McCormack et al. 1995). These splicing variants have identical C-regions but differ significantly in their N-regions. Because the C-region is involved in specifying interactions with  $\alpha$  subunits (Yu et al. 1996), the different splicing variants that presumably have distinct modulatory activity (see the next section) would interact with the same subset of pore-forming subunits. Third, there are many post-translational modification sites in  $\beta$  subunits. Phosphorylation of  $\beta$  subunits may be directly involved in modulatory ac-

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# Kvβ



**Figure 1.** Primary structure of Kv β subunits. **Upper panel.** A schematic diagram representing the domain arrangement of Kvβ subunits. **Lower panel.** Amino acid sequence alignment of the nine cloned Kvβ subunits. Residues conserved in more than three genes are highlighted. The numbers indicate the corresponding amino acid positions. The sequence were retrieved from Genbank with following accession numbers: human Kvβ1.1 (U33428, S66503); human Kvβ1.2 (I59393, L39833, U16953); human Kvβ1.3 (I55463, I47665); human Kvβ2 (S66502); rat Kvβ3 (X76723, S72562); mouse Kvβ4 (U65593); *Drosophila Hk* (U23545); squid β subunit (kindly provided by Dr. W. Gilly) Arabidopsis KAB1 (L40948); rice KAB2 (U46758).

tivities, including protein half-life of β subunits and association with other known and unknown proteins in the complex (Levin et al. 1996, Jing et al. 1997). Finally, the β subunits appear to be a unique class of channel proteins with no obvious sequence homology to the known auxiliary subunits of other ion channels. Comparison with cloned proteins in a database showed that the C-regions of β subunits share 15% to 30% amino acid identity with the sequences of aldo-keto reductase genes (McCormack and McCormack 1994, Chouinard et al. 1995). Although the

functional significance of this sequence conservation is unknown at the biochemical level, it is likely that these two groups of proteins share a similar structural scaffold (Chothia and Lesk 1987).

### • Modulatory Activity of Kvβ Subunits

The cloned β subunits are a group of homologous proteins expressed in both excitable and nonexcitable tissues. Despite a high degree of sequence homology, they display diverse modulatory effects on the Kv α subunits.

Most α subunits, when expressed individually, form functional tetrameric potassium channels. Depending on the specific subunit, the resultant potassium currents might or might not exhibit fast inactivation that is mediated by a stretch of 20–30 amino acids at the N-terminus, which is also known as an inactivation gate (Hoshi et al. 1990). The first reported modulation by the Kvβ subunit came from coexpression of Kvβ1.1 with the Kv1.1 and Kv1.4 α subunits, where Kvβ1.1 induces fast inactivation in Kv1.1 and accelerates the existing fast inactivation of Kv1.4 (Rettig et al. 1994). Mu-

tation analyses suggested that the N-terminus of the Kv $\beta$ 1 subunit acts similarly as an inactivation gate of inactivating  $\alpha$  subunits. In addition to the N-type inactivation induced by Kv $\beta$ 1.1, the N-region of Kv $\beta$ 1.2 was found to be involved in increasing the rate of C-type inactivation as well (Morales et al. 1996).

Interestingly, not all  $\beta$  subunits induce fast inactivation. The Kv $\beta$ 2 subunit was particularly puzzling because it is expressed abundantly in brain, heart, and a number of other nonexcitable tissues; but coexpression of Kv $\beta$ 2 with  $\alpha$  subunits does not have obvious modulatory activity, such as the fast inactivation induced by Kv $\beta$ 1s. Early work in other channel systems have shown that the auxiliary subunits often facilitate assembly and increase channel expression, presumably because the resultant channel complexes are more similar to those found in vivo and therefore assembly becomes more efficient [see review by Isom et al. (1994)]. Recent studies suggested that Kv $\beta$  subunits are also capable of inducing a moderate increase in channel surface expression (Chouinard et al. 1995, Fink et al. 1996, Shi et al. 1996). However, the Kv $\beta$ -mediated expression increase appears to be more complicated than was previously thought. First, Kv $\beta$ 2 does not display such activity to all interacting  $\alpha$  subunits. When Kv $\beta$ 2 was coexpressed with ShB, no significant changes in current amplitude were observed, suggesting that the physical association itself is not sufficient to facilitate the cell surface expression of  $\alpha$  subunits (Nagaya and Papazian 1997, Xu and Li 1997). Second, the Kv $\beta$ 4-induced increase of the Kv2.2 expression was achieved through its interaction with the C-terminal portion of Kv2.2 (Fink et al. 1996), contrary to the rest of the data, which show that Kv $\beta$  subunits interact through association with the NH<sub>2</sub>-terminal domains of Kv1 subunits (Sewing et al. 1996, Yu et al. 1996, Xu and Li 1997). Third, Kv $\beta$ 2 does not increase but inhibits the expression of Kv1.5 (Accili et al. 1997). In addition, the interpretations are further complicated by the fact that some heterologous systems have been shown to express  $\beta$  subunits endogenously (Uebele et al. 1996). More detailed studies are necessary to sort out the specificity of modulatory effects of Kv $\beta$  subunits.

The limited knowledge about the in

vivo function of  $\beta$  subunits came from the molecular and genetic studies of the *Drosophila Hk* mutants, where the *Hk* locus encodes a fly  $\beta$  subunit. *Hk* protein is the first  $\beta$  subunit that was found to increase the expression of a Shaker channel native to *Drosophila*. In addition, it induces changes in voltage dependence as well as in kinetics of activation and inactivation (Chouinard et al. 1995). The *Hk* null mutation, *HK<sup>IE18</sup>*, displays significant abnormality of the K<sup>+</sup> current in muscle cells and leads to striking neuromuscular hyperexcitability and behavioral hyperactivity (Wang and Wu 1996), consistent with the modulatory activity of *Hk* detected in the heterologous oocyte expression system (Chouinard et al. 1995).

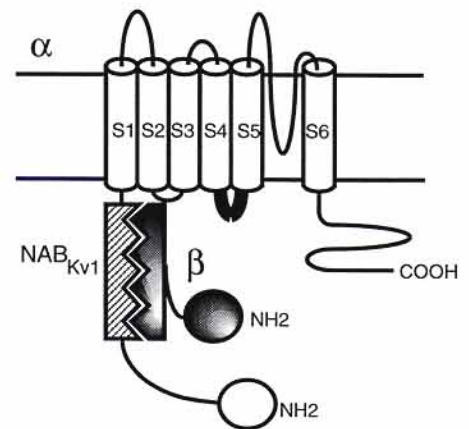
### • The Heteromeric $\alpha$ - $\beta$ and $\beta$ - $\beta$ Interactions

One of the essential questions to understanding the in vivo function of  $\beta$  subunits is the specificity of their modulatory activity. There are four major subfamilies of Kv $\alpha$  subunits: Kv1, Kv2, Kv3, and Kv4. Most biochemical and electrophysiological data confirm that Kv $\beta$ 1 and Kv $\beta$ 2 only interact with the Kv1  $\alpha$  subunits (Sewing et al. 1996, Yu et al. 1996), except in one case that found that Kv $\beta$ 2 can be coimmunoprecipitated with a Kv4  $\alpha$  subunit (Nakahira et al. 1996). More systematic studies using various combinations of eight  $\alpha$  subunit genes (two genes from each of the four subfamilies) indicate that both Kv $\beta$ 1 and Kv $\beta$ 2 interact specifically with the Kv1  $\alpha$  subunits (Yu et al. 1996, Xu and Li 1997). The  $\alpha$ - $\beta$  association is mediated by the interaction of the NAB domain of  $\alpha$  subunits and the conserved core region of  $\beta$  subunits (Sewing et al. 1996, Yu et al. 1996, Xu and Li 1997).

The molecular determinants for establishing the specific  $\alpha$ - $\beta$  interaction are currently unknown. There are some data that correlate the specific amino acid substitutions in  $\alpha$  subunits with the loss of  $\beta$  subunit modulation. The experimental interpretations are primarily based on two point mutations (Y194A and L196A) in Kv1.5, which results in loss of Kv $\beta$ 1.1 sensitivity (Sewing et al. 1996); however, these two positions are in fact highly conserved in all four subfamilies of Kv $\alpha$  subunits (Li et al. 1992, Xu et al. 1995). Because the  $\alpha$ - $\beta$  interac-

tions are subfamily specific, the identified two residues are unlikely to be the primary determinants. Further studies are needed to identify residues that directly mediate the specific  $\alpha$ - $\beta$  interactions.

The ability to form an  $\alpha$ - $\beta$  complex does not necessarily lead to  $\beta$  modulation. This is presumably because multiple compatible interactions are required (Figure 2). For example, Kv $\beta$ 1.2 is capable of binding to the Kv1  $\alpha$  subunits, whereas existing electrophysiological data have clearly shown that Kv $\beta$ 1.2 cannot induce the fast inactivation of Kv1.1 and Kv1.2 as it does to Kv1.4 and Kv1.5, suggesting that the inactivation particle of Kv $\beta$ 1.2 may not be compatible with the receptor in Kv1.1 and Kv1.2 (Isacoff et al. 1991, Morales et al. 1996) or other functional domain that is involved in fast inactivation (Roeper et al. 1998). Such phenomena suggest complex modes by which a  $\beta$  subunit could modulate  $\alpha$  subunits. We know from both in vitro and in vivo data that  $\alpha$  subunits within a given subfamily can form heteromultimers. Given that each  $\alpha$  subunit may exhibit different sensitivity to a given  $\beta$  subunit, the resultant heteromultimers



**Figure 2.** A diagram represents the postulated  $\alpha$ -Kv $\beta$ 1 interaction (Yu et al. 1996). The putative membrane-spanning segments of the  $\alpha$  subunit are designated as S1-S6, and the inactivation gate (present in some Kv1  $\alpha$  subunits) is represented as the open circle. The S4-S5 loop, the putative receptor for the inactivation gate of the  $\alpha$  subunit, is highlighted as a thicker line. The NH<sub>2</sub>-terminal domain of the  $\alpha$  subunit that mediates the  $\alpha$ -Kv $\beta$ 1 association is illustrated as the open box. The shaded model represents a Kv $\beta$ 1 subunit in which the inactivation gate of Kv $\beta$ 1 is designated as a closed circle. The model may not represent the real stoichiometry of the  $\alpha$ -Kv $\beta$ 1 complex.

of  $\alpha$  subunits, depending on their composition and stoichiometry, may show a wide range of heterogeneity.

In addition to the existing complexity of heteromultimeric assembly of  $\alpha$  subunits, biochemical experiments have shown that, at least for Kv $\beta$ 2, the major  $\alpha$ - $\beta$  complexes in brain appear to have a stoichiometry of  $\alpha_4\beta_4$  (Parcej et al. 1992). Because there are more than one  $\beta$  subunit in the complex and the expression patterns of various  $\beta$  subunits, such as Kv $\beta$ 1 and Kv $\beta$ 2, overlap, this raises the question as to whether the  $\beta$  subunits would form heteromultimers. The answer to this question is not yet available; however, recent evidence certainly supports the notion. First, Kv $\beta$ 1 and Kv $\beta$ 2 associate in the absence of  $\alpha$  subunits (Xu and Li 1997). The second set of results supporting this notion came from coexpression studies of Kv $\beta$ 1.1 and Kv $\beta$ 2, which possess distinct modulatory activity; for example, Kv $\beta$ 1.1, but not Kv $\beta$ 2, induces fast inactivation similar to the N-type inactivation intrinsic to some  $\alpha$  subunits. Interestingly, when these two subunits were coexpressed in tissue culture cells, Kv $\beta$ 2 inhibited the fast inactivation induced by Kv $\beta$ 1 (Xu and Li 1997). Third, the same inhibitory effects can be observed with a truncated Kv $\beta$ 2 protein with only the C-region, which mediates the Kv $\beta$ 1-Kv $\beta$ 2 interaction. Because the C-regions are identical within Kv $\beta$ 1s and highly conserved among all  $\beta$  subunits, the heteromultimeric assembly of  $\beta$  subunits may also be found in other Kv $\beta$ s. Fourth, Kv $\beta$ 1.1 and Kv $\beta$ 2 appear to interact with  $\alpha$  subunits via distinct functional stoichiometry (Xu et al. 1998). The interaction of Kv $\beta$ 1.1 subunits with  $\alpha$  subunits is consistent with the  $\alpha_4\beta_n$  model, where  $n$  equals zero, one, two, three, or four, depending on the relative concentration of  $\alpha$  and  $\beta$  subunits. The  $\alpha_4\beta_n$  stoichiometry allows gradual changes of the Kv $\beta$ 1-mediated inactivation. In contrast, Kv $\beta$ 2 subunits self-associate to form oligomers and interact with the  $\alpha$  subunits via  $\alpha_4\beta_4$  stoichiometry, which permits effective multivalent associations with  $\alpha$  subunits. Although it remains unknown whether the  $\beta$  subunits form heteromultimers in vivo, the existing data clearly show that various  $\beta$  subunits are capable of forming heteromultimers. Given the diversity of Kv $\beta$ -mediated modulation, the heteromultimeric assembly of

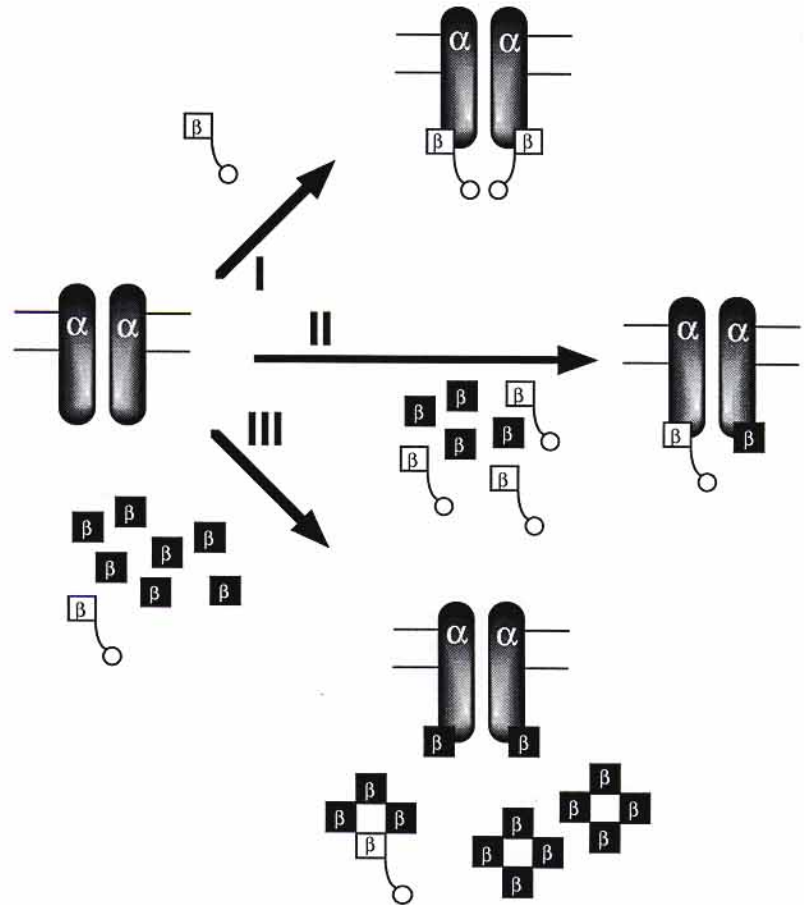
various  $\beta$  subunits could lead to a wider spectrum of modulation, including, but not limited to, inactivation (Figure 3).

### • Kv $\beta$ -Mediated Regulation of Potassium Currents in Cardiac Tissues

The known modulatory targets of Kv $\beta$  subunits are limited to the Kv  $\alpha$  subunits. In cardiac tissue, several Kv  $\alpha$  subunits were found, which include multiple members of different subfamilies (Deal et al. 1996, Brahmajothi et al. 1996, Brown 1997). All Kv1 subfamily  $\alpha$

subunits are compatible to interact with Kv $\beta$ 1s, Kv $\beta$ 2, and Kv $\beta$ 3, some of which have been shown to express in cardiac tissues (England et al. 1995a and b, Majumder et al. 1995, Morales et al. 1995).

Although the first  $\beta$  subunit was cloned from bovine brain, several known Kv $\beta$  subunits were directly cloned from, or found to be expressed in, cardiac tissue. Currently, information concerning their detailed subcellular localization is not available. In heterologous systems, the two types of subunits were expressed in various combinations and found to al-



**Figure 3.** A schematic diagram illustrates the postulated Kv $\beta$ 2-mediated inhibition. In the absence of Kv $\beta$ 2, the expression of Kv $\beta$ 1 will permit it to coassemble with compatible  $\alpha$  subunits. Depending on whether the interacting  $\alpha$  subunits contain an inactivation gate, Kv $\beta$ 1 either accelerates or induces fast inactivation (see 1). In the presence of the high concentration of Kv $\beta$ 2, Kv $\beta$ 2 occupies most of the sites on  $\alpha$  subunits as homomultimeric Kv $\beta$ 2 complexes. As a result, it prevents (or removes) Kv $\beta$ 1 from interacting with  $\alpha$  subunits (III), thereby inhibiting the Kv $\beta$ 1-mediated inactivation. Conceivably, one should observe an intermediate situation where Kv $\beta$ 1 and Kv $\beta$ 2 are present in a comparable concentration (or different concentrations with an appropriate ratio for heteromultimeric interaction, as the Kv $\beta$ 1 and Kv $\beta$ 2 may have considerable difference in affinity for subunit interaction). Under such conditions, most compatible  $\alpha$  subunits should be interacting with heteromultimeric  $\beta$  complexes to produce intermediate effects, i.e. Kv $\beta$ 2 weakens but does not remove the Kv $\beta$ 1-mediated inactivation (II). The model may not represent the actual stoichiometry of  $\beta$ - $\beta$  complex(es). The pathway II has not been confirmed.

ter the channel properties. Therefore, it is conceivable that  $\beta$  subunits are coassembled with various  $\alpha$  subunits, and the formation of  $\alpha$ - $\alpha$ ,  $\alpha$ - $\beta$ , and  $\beta$ - $\beta$  heteromultimers is likely to contribute significantly to the functional diversity of potassium currents in vivo.

Tightly regulated spatial and temporal expression of  $K^+$  channels is essential for determining the cardiac excitability. Pharmacological studies of antiarrhythmic and antihypertensive drugs have shown that modulation of potassium channels could have considerable therapeutic potential [see review by Sanguinetti (1992)]. The ability of  $Kv\beta 1$ s to inactivate  $\alpha$  subunits and the ability of  $Kv\beta 2$  to inhibit the  $Kv\beta 1$ -mediated inactivation suggest several potentially important clinical approaches by which one can manipulate potassium currents in cardiac tissue. First, a disruption of the interactions between  $Kv\alpha$  subunits and  $Kv\beta 1$ s would allow longer channel opening. If there were compounds that could alter the  $Kv\beta 1$  expression or interfere with the interactions between various  $Kv1$   $\alpha$  subunits and  $Kv\beta 1$ s, these compounds might be used to either upregulate or downregulate potassium currents. Alternatively, one can manipulate the expression of  $Kv\beta 2$  by gene delivery techniques, which would reduce the  $Kv\beta 1$ -mediated inactivation. As a result, this would also increase the potassium currents induced by the  $Kv1$   $\alpha$  subunits.

## • Conclusions

The auxiliary  $Kv\beta$  subunits are a unique class of proteins with a rich spectrum of modulatory activities affecting the Shaker-type potassium channels. Although their precise in vivo function requires more detailed investigation, molecular cloning and expression studies have established their important roles in determining the physiological properties of potassium channels in both excitable and nonexcitable tissues. Current understanding of  $\alpha$ - $\beta$  and  $\beta$ - $\beta$  interactions suggests a target for clinical intervention to regulate cardiac excitability.

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**TCM**