Kv1.5-Kvβ Interactions: Molecular Determinants and Pharmacological Consequences

Teresa González, Miren David, Cristina Moreno, Álvaro Macías and Carmen Valenzuela*

Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), C/Arturo Duperier 4, 28029 Madrid, Spain

Abstract: Kv1.5 channels are homotetramers of α-pore subunits mainly present in human atrium and pulmonary vasculature. Thus, Kv1.5 is a pharmacological target for cardiovascular diseases. Kvβ1.3 assemblies with Kvα1.5 and modifies its gating and pharmacology. A further knowledge of α-β interactions and pharmacology will lead a better design of new drugs.

Keywords: Ion channels, Kv channels, Beta subunits, Kvβ1.3, Kv1.5, Bupivacaine.

Most of us appreciate the importance of electricity at a young age. It powers many useful and entertaining man-made devices: a light for seeing the world, a computer for searching the internet, or an omnipresent iPod for listening music. The concept that living organisms use electricity as a fundamental mechanism for signalling across membranes and between cells is less widely appreciated, even though scientists have been thinking about the biological roles of electricity since the late eighteenth century…In humans, electrical signals are used to complete a computation within the cerebral cortex, to secrete insulin after a meal, or to signal that a sperm has entered an egg and for embryogenesis to commence [1].

ION CHANNELS AS DRUG TARGETS

Ion channels are integral components of cellular signaling pathways in almost all living cells. In fact, our ability to do exercise, to perceive colors and sounds, to process language, and, more generally, to initiate muscle contraction depends on electrical messages produced as ion channels in cell membranes open and close [2]. Various ion channels mediate sensory transduction, electrical propagation over long distances, and synaptic transmission. In this review, we will focus on voltage-gated K^+ channels (Kv) responsible of nerve and muscle excitability. Our basic understanding of these proteins maintains the framework and rigor established 50 years ago by Hodgkin and Huxley [3], enriched by much new molecular information [4] and by insights gained from patch-clamp methods [5]. Potassium channels are membrane proteins that can be blocked by many different types of drugs, such as local anesthetics, antiarrhythmics, anticonvulsants, antihypertensive drugs, etc. Therefore, they represent the targets for these therapeutic agents, and their binding sites have been analyzed by studying the interactions of specific channel blockers and site-directed mutant ion channels [6-8].

*Address correspondence to this author at the Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC/UAM, C/ Arturo Duperier 4, 28029 Madrid, Spain; Tel: +34.91.585.4493; Fax: +34.91.585.4401;
E-mail: cvalenzuela@iib.uam.es

In the best case, drug targets would have several characteristics: known biological function; robust assay systems for *in vitro* characterization and high-throughput screening; and finally, they have to be specifically modified by and accessible to small molecular weight compounds *in vivo*. Ion channels have many of these attributes and, thus, they can be viewed as suitable targets for small molecule drugs. If modulatory subunits (β) alter the pharmacology of the Kv pore forming subunits (α), the molecular targets of local anesthetics and/or antiarrhythmic drugs will vary greatly depending on β subunit expression. Therefore, knowledge of the effects of regulatory β subunits present in the human myocardium is a key factor required for determining the characteristics of Kv as drug targets. However, target-based drug discovery programs require the identification, characterization and validation of molecular targets. An approach to these considerations is to associate the gene that encodes the expression of such ion channels with a specific cellular process, disease indication, or mammalian model of disease. In fact, involvement of a gene in an inherited disease provides direct evidence that a given protein is functionally important. During the last decades, there have been made enormous progress identifying mutations in ion channel genes that cause human and animal diseases that result from defects in ion channel function (*channelopathies*).

VOLTAGE-GATED POTASSIUM CHANNELS

Potassium channels are tetramers of α pore-forming subunits. Recapitulation of the physiological features of the native K^+ current frequently requires accessory β-subunits. Most Kvβ subunits assemble with α-subunits giving rise to a α4β4 complex. The first K^+ channel to be identified came from the cloning of the *Shaker* gene of *Drosophila* which causes flies to shake when exposed to ether [9]. However, potassium channels are found in virtually all cells and can be divided in three main structural families depending on whether they possess six, four or two transmembrane domains, the latter with two pores (Fig. (**1**)) [4,10-12]. Those belonging to the family of two transmembrane domains include the inwardly rectifying K^+ channels. In the present review, we will examine the voltage-gated K^+ channels that exhibit six transmembrane spanning domains (6Tm-1P).

1389-5575/10 $55.00+.00

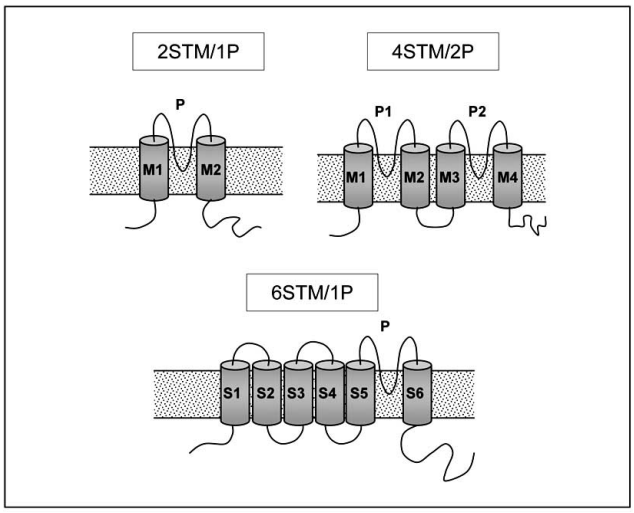

Fig. (1). Schematic Figure representing the three main groups of K^+ channels according to their structure: 2STM/1P, 4STM/2P and 6STM/1P channels.

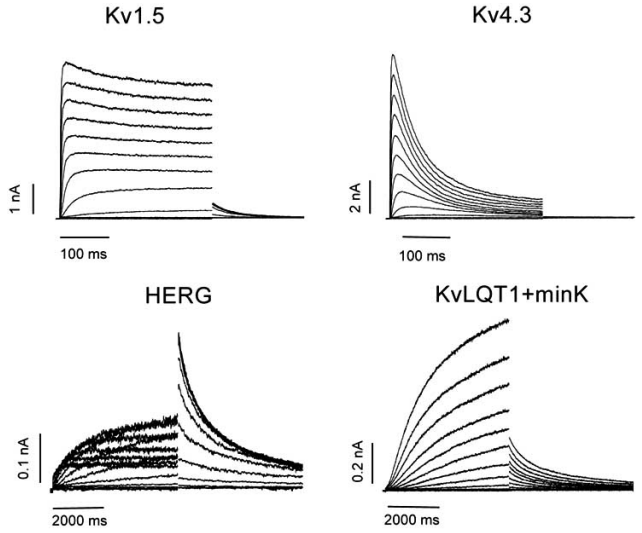

Fig. (2). Original records obtained after transfection of cDNA encoding Kv1.5, Kv4.3, HERG and KvLQT1+minK in *Ltk*$^-$ cells (for Kv1.5 channels) and in CHO cells (for the other three types of channels). Holding potential was maintained in all cases at -80 mV. Cells transfected with cDNA encoding the expression of Kv1.5 or Kv4.3 channels were pulsed every 10 s from -80 and +60 mV. Cells transfected with cDNA encoding the expression of HERG and KvLQT1+minK were pulsed every 30 s from -80 and +60 mV.

Initially, four *Drosophila* subfamilies were described (*Shaker*, *Shab*, *Shal* and *Shaw*), to which the first cloned mammalian K^+ channels were related [13]. In the mammalian KvX.Y nomenclature, Kv reflects voltage-gated K^+ channel, X represents the subfamily and Y the number of the gene within the subfamily. Assembly of four α subunits into a tetrameric structure is needed to create a functional K^+ channel [14-16]. The activation of these channels leads to the genesis of different K^+ currents (Fig. (**2**)). These Kv channels are closed at the resting membrane potential and open upon depolarization. Therefore, they are involved in the repolarization of the action potential, and thus in the electrical excitability of nerve and muscle fibers, including cardiac muscle. Mutation of gene encoding members of these K^+ channel subfamilies lead to a number of human diseases, such as episodic ataxia, long QT syndrome and epilepsy.

6Tm-1P K^+ channels are made up of pore-forming α subunits that may associate with a number of different types of β subunits. To date, twelve different subfamilies of Kv channel α subunits have been described (Kv1-12) [16,17]. The α subunit of the Kv channels corresponds to a single domain of the Na^+ or Ca^{2+} channels and four such subunits form the functional K^+ channel pore. Each α subunit consists of six transmembrane spanning segments (S1 to S6), which are highly conserved, with intracellular N and C termini of variable length. The S4 segment shows high homology with those from voltage-gated Na^+ channel: it is amphipathic, having a positive charged amino acid at every third position. Like in Na^+ channels, the S4 segment is involved in the voltage-dependent activation of Kv channels, and the N-terminus is involved in the voltage-dependent inactivation. The linker between S5 and S6 segments dips back into the membrane and participates in the formation of the channel pore. It is called H5 region or P loop in K^+ channels (a more detailed description of the Kv structure is depicted below). A great variety of Kv channels has been described, far more than was originally anticipated. Part of this diversity is the consequence of the existence of different genes, which encode different Kv channels. However, other factors such as alternative splicing, the formation of heteroligomeric channels and their association with different types of β subunits, also contribute to this diversity.

Activation

Different reports have revealed that the S1-S4 region acts as the voltage sensor of Kv channels [18]. The S4 segment (which presents seven positively charged residues) is the major component of the voltage sensor for gating, although negative charges in S1, S2 and S3 also contribute to this process. S3 is actually formed by two helices referred to as S3a and S3b. In all of the crystal structures determined, S3b forms with S4 a helix-turn-helix structure called the voltage-sensor paddle (Fig. (**3**)) [19]. Depolarization of the membrane causes a physical (outward) movement of S4, which then induces further conformational changes that open the channel pore and permit selective K^+ permeation. This movement has been monitored electrically as the gating current [20], or by means of fluorescence [21]. The available structures of activated voltage sensors represent a remarkable advance and, when considered in the context of other biophysical studies, constrain our thinking about the mechanism of voltage sensing. The voltage sensor is surrounded by a lipid environment and different approaches had led to the hypothesis that membrane phospholipids affect the gating of the channel [22-24]. It has been proposed that Kv channels

may interact with several species of phospholipids and that enzymatic removal of their phospho-head creates an energy barrier for the positively charged voltage sensor to move through the initial gating step and also raises the energy barrier for the downstream step [23].

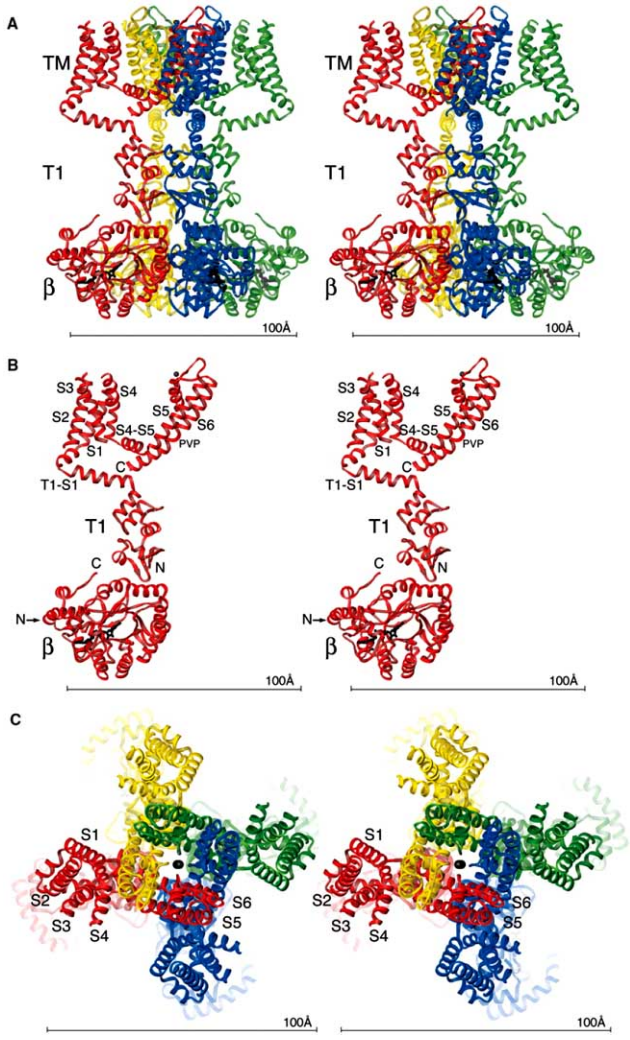

Fig. (3). Views of the Kv1.2–β2 subunit complex. (**A**) Stereoview of a ribbon representation from the side, with the extracellular solution above and the intracellular solution below. Four subunits of the channel (including the T1 domain, voltage sensor, and pore) are colored uniquely. Each subunit of the β subunit tetramer is colored according to the channel subunit it contacts. The $NADP^+$ cofactor bound to each β subunit is drawn as black sticks. TM indicates the integral membrane component of the complex. (**B**) Stereoview of a single subunit of the channel and β subunit viewed from the side. Labels correspond to transmembrane helices (S1 to S6); the Pro-Val-Pro sequence in S6 (PVP); and the N (N) and C (C) termini of the Kv1.2 and β subunits. The position of the N terminus of the β subunit, which is located on the side furthest away from the viewer, is indicated by an arrow. (**C**) Stereoview of a ribbon representation viewed from the extracellular side of the pore. Four subunits are colored uniquely. From Long *et al*. (2007) with permission.

Inactivation

Upon depolarization, Kv channels activate more or less rapidly, rising maximum peak amplitude before the current declines in a slow (C-type) or a fast process (N-type). The N-type inactivation involves the N-terminus of the α or the β subunit. Therefore, upon opening of the channel, the N-terminal domain moves into the internal mouth and occludes the pathway of K^+ ions through the pore [25]. After removal of this region, inactivation can be restored by the corresponding synthetic peptide. Since functional K^+ channels are composed by the assembly of four α subunits, each K^+ channel has four inactivation balls, which in *Shaker* channels correspond to the first 20 amino acids of the N-terminus. Only one of these balls binds to its receptor in the mouth of the ion pore, but the four are needed for a normal inactivation rate of the channel [26]. The *Shaker* inactivation ball binds to a receptor located at the S4-S5 linker, which involves a threonine [27]. On the contrary, C-type inactivation is modified by mutations in the S6 and P regions, and is usually slow and incomplete [28]. This kind of inactivation is present in almost all Kv channels and may reflect a constriction of the pore involving residues on the extracellular surface of the ion pore.

Ion Pore and Selectivity

The ion conduction pathway consists of a sequence of approximately 20 amino acids (P loop) between the S5 and S6 segments with contributions of the S5, S6 and the S4-S5 linker (Fig. (**3**)). The four subunits are oriented in such a way that the S5-P-S6 sections face each other creating the central pore. Within the P region, the amino acid sequence motif (TxTTx)GYG is now considered the K^+-selectivity signature motif [29]. Mutagenesis studies have revealed that the selectivity filter forms an essential part of the permeation pathway. MacKinnon and his colleagues [15] obtained the first crystal structure of a K^+ channel, the bacterial K^+ channel (KcsA) cloned from *Streptomyces lividans* with a 3.2 Å resolution. The KcsA channel is formed by two transmembrane segments (inner and outer helix) equivalent to the S5 and S6 of Kv channels. The X-ray analysis confirms that the channel contains four identical subunits each having two transmembrane α-helices and a P domain. These subunits create an inverted cone or teepee, in which the α-helices resemble the poles of the teepee. The selectivity filter fills the wider base on the extracellular face. The pore has a length of 45 Å; it starts from the inside with a tunnel (18 Å long, ≈ 6 Å wide) which opens into a 10 Å wide cavity followed by the narrow selectivity filter (12 Å). The structure of Kv channels differs from that of the non-voltage-gated bacterial potassium channel KcsA due to a sharp-bend in the S6 helices (equivalent to the inner helices of the KcsA channel). This bend would occur at a Pro-X-Pro sequence that is highly conserved in the Kv channels [30]. Part of the P region of each subunit is α-helical, with their electronegative carboxyl end directed toward the central cavity, which helps to stabilize the potassium ions. The selectivity filter is lined by the carbonyl oxygen atoms of the GYG signature sequence. The tyrosine (Y) points away from the pore and interacts with other conserved aromatic residues of the pore helices. Therefore, a fairly rigid donut of 12 interlocking aromatic amino acids is formed around the selectivity filter that holds the GYG backbone at

the optimal distance from the center to achieve potassium selectivity: the four backbones form a pore with the carbonyl oxygens at the proper distance to compensate the cost of dehydration of a K^+ ion (~ 3 Å). Interestingly, the inner tunnel is largely coated with hydrophobic side chains, explaining why binding of open channel blockers such as tetraethylammonium (TEA) derivatives, antiarrhythmic drugs like quinidine or local anesthetics such as bupivacaine are stabilized by hydrophobic interactions [6,7,31]. MacKinnon and colleagues resolved the X-ray structure of a Kv channel, the Kv1.2 channel [19,32]. This protein was solved to 2.4 Å resolution. In the atomic structure of Kv1.2 and a mutant known as paddle chimera, the S4-S5 linker helices are positioned in such a manner that conformational changes within the voltage sensors can easily be transmitted to the S6 helices in order to facilitate constriction or dilation of the pore. (Fig. (**3**)).

REGULATORY SUBUNITS: BETA SUBUNITS

The diversity of Kv channels structure and function is enhanced by heteromultimerization of different α-subunits and by their association with accessory Kvβ subunits [33], forming complexes of four α-subunits and four β-subunits that modify the electrophysiological characteristics of the α-subunit. Therefore, recapitulation of the physiological features of the native K^+ current frequently requires accessory β-subunits. Most Kvβ subunits assemble with α-subunits giving rise to a α4β4 complex. K^+ channel β-subunits represent a diverse molecular group, which includes cytoplasmic proteins (Kvβ1-3, KChIP and KChAP) that interact with the intracellular domains of Kv channels, single transmembrane spanning proteins, such as minK and minK-related proteins (MiRPs) encoded by the KCNE gene family, and large ATP binding cassette (ABC) transport-related proteins, such as the sulfonylurea receptors (SUR) for the inward rectifiers Kir6.1-6.2 [33]. Moreover, coexpression of β subunits with α-subunits regulates cell surface expression, gating kinetics and drug-sensitivity of K^+ channels. Kv channels play critical roles in the electrical responses throughout the cardiovascular system, being responsible for establishing the resting membrane potential and cellular repolarization in heart and peripheral vascular beds [34-36]. The Kvβ subunits are cytoplasmic proteins that have a mass of ~40 kDa. The proteins β1, β2, and β3 are coded by different genes, and additional variability is produced by alternative splicing on the N-terminal region [7,8]. The Kvβ subunits form a tetrameric structure and are associated in 1:1 ratio with the α-subunit [19,37]. The Kvβ C-termini bind to the T1 domains of the α-subunits, which form a docking platform for the Kvβ subunits (Fig. (**3**)) [38]. In addition to modulating the channel activity at the cell surface, Kvβ subunits control the surface expression of the α-subunit [39]. The interaction of Kv1 α-subunit and Kvβ subunit polypeptides is an early event in Kv1 biosynthesis, occurring in the endoplasmic reticulum [39,40]. Despite dramatic differences in their effects on channel gating, each of the Kvβ subunits displays robust trafficking effects. Kvβ1.1, β1.2, β2, and β3 subunits increase the membrane expression and the mature form of Kv1.2 when they are co-expressed [39,40]. The interaction with Kvβ2 subunits results in increased stability of Kv1.2 α-subunits. There is a dramatic difference in the degradation rates of the free Kv1.2 pool (non-bonded to Kvβ2 subunit, τ~3 h) and the Kv1.2 associated with Kvβ2 (τ ~15 h) [39]. Therefore, although some cytoplasmic Kv1 channel β-subunits affect the inactivation kinetics of the α-subunits, a more general and perhaps more fundamental role is to mediate the biosynthetic maturation and surface expression of Kv channel complexes.

INTERACTION BETWEEN Kv1.5 CHANNELS AND Kvβ1.3 SUBUNITS

In the human myocardium and in blood vessels two β-subunits (Kvβ1.3 and Kvβ2.1) show overlapping expression patterns with Kv1.5 channels. These channels are widely represented in the cardiovascular system [41]. In the heart, the Kv1.5 protein has been located in human atrial and ventricular myocardium explanted from newborn and adult patients [42]. However, electrophysiological studies [43,44] have shown the absence of Kv1.5-like current in human ventricular myocytes. These results suggest that I_{Kur} is the native counterpart to Kv1.5 channels in human atria [17,45-47] and therefore, it contributes to the repolarization process of the human atrial action potential [17,47]. It has been reported that the expression of Kv1.5 protein is reduced in atrial appendages of chronic atrial fibrillation patients [48]; and more recently, a familial form of atrial fibrillation has been attributed to a loss-of-function mutation in Kv1.5 [49]. In the pulmonary vasculature, Kv1.5 plays a critical role in the oxygen-sensitive regulation of arterial tone [50]. Thus, there is significant interest in Kv1.5 as a potential pharmacological target for diseases, such as chronic atrial fibrillation and chronic hypoxic pulmonary arterial hypertension. The two overlapping β subunits exert different effects on the Kv1.5 current. Thus, as shown in Fig. (**4**), the Kvβ2.1 subunit increases the degree of slow inactivation of the current, whereas Kvβ1.3 induces a fast and incomplete inactivation of the current [51-53]. Both, Kvβ2.1 and Kvβ1.3 shift the midpoint of the activation curve towards more negative voltages and slow the deactivation process [51,52]. In addition, Kvβ1.3 subunits modify the pharmacology of the Kv1.5 α-subunits, decreasing their sensitivity to bupivacaine, quinidine and S0100176 [54,55]. The interaction between Kvβ1.3 subunits and Kv1.5 channels has been extensively studied during the past few years [54,56,57]. Zhou *et al.* proposed in 2001 that the inactivation induced by Kvβ1 subunits occurs through the interaction between a fully extended N-terminal peptide. The hydrophobic region of the peptide would extend from the cavity to the intracellular entryway, while the hydrophilic peptide region would emerge from the pore and interact with the aqueous protein surfaces lining the cage formed above the T1 domain. This configuration makes good chemical sense as the cavity and inner pore are lined by hydrophobic amino acids and the T1-S1 linkers outside the pore contain many acidic amino acids that would interact favourably with the multiple basic residues of the inactivation peptide [58]. These authors also conclude that blockers of Kv channels such as TEA and its derivatives as well as Kvβ1 subunits bind to the central ion pore cavity. In 2005, the putative receptor site for the N-terminal inactivating particle of Kvβ1.3 in the Kv1.5 pore was determined, involving residues V505, I508, L510, V512 and V516 (Fig. (**5**)) [54]. Interestingly, two residues located close to the selectivity

filter of the Kv1.5 channel (T480 and A501) greatly increased the degree of fast inactivation produced by the Kvβ1.3 subunit [54]; suggesting that these residues are also involved in the interaction between Kvβ1.3 and Kv1.5. In 2008, the same group analyzed the Kvβ1.3 N-terminal residues involved in the interaction with Kv1.5. This study shows that residues L2 and A3 from the Kvβ1.3 subunit are necessary to induce inactivation. They also show the Kvβ1.3 subunit, in contrast to Kvβ1.1, is Ca^{2+} insensitive and that PIPs antagonize the Kvβ1.3 induced inactivation through interaction with residue R5. Double-mutant cycle analysis indicates that R5 interacts with T480 and A501 of Kv1.5, residues located deep within the pore of the channel. These results can only be explained if the Kvβ1.3 N-terminus assumes a hairpin structure, which was supported by an energy-optimized model of the interaction between the first 11 residues of the Kvβ1.3 subunit and the pore of the Kv1.5 channel (Fig. (**6**)). They propose that Kvβ1.3 is bound, through the R5 residue, to membrane PIP_2 in a pre-blocking state, when dissociates assumes a hairpin structure that enter the central cavity of the open Kv1.5 channel to induce N-type inactivation [56].

PHARMACOLOGICAL CONSEQUENCES OF THE INTERACTION BETWEEN Kv1.5 AND Kvβ1.3

Several studies have demonstrated that Kvβ1.3, but not Kvβ2.1, decreases drug affinity of Kv1.5 for local anesthetics and antiarrhythmic drugs (Fig. (**7**)) [6,54,55,57]. Gonzalez *et al.* demonstrated in 2002 that Kvβ1.3 decreased block induced by bupivacaine and quinidine by 4- and 8-fold, respectively. However, Kvβ2.1 did not affect the sensitivity of the channel to block induced by these drugs [6,55]. In 2005, Decher *et al.* demonstrated that this effect was due to a competition between the drugs and the inactivating particle of the Kvβ1.3 subunit for their binding site. Indeed, mutagenesis analysis demonstrated that the binding site for S0100176 (a new antiarrhythmic drug) overlaps but is not identical to the Kvβ1.3 binding site (Fig. (**6**)) [54,59].

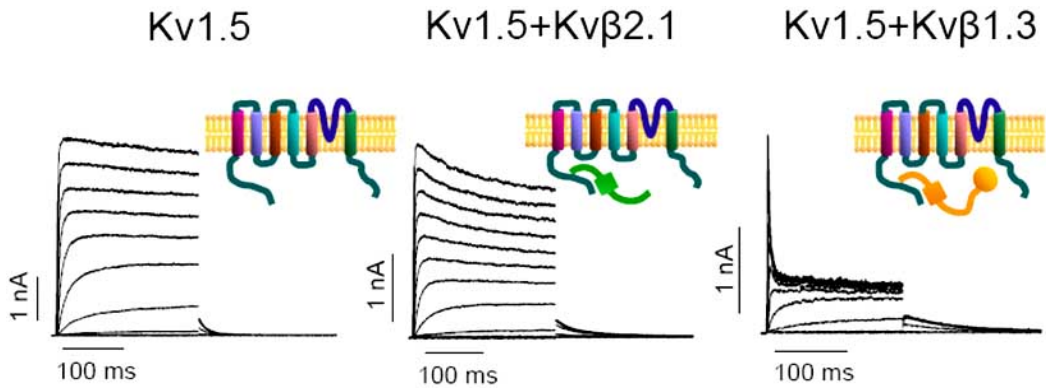

Fig. (4). Original traces recorded from HEK293 cells transfected with Kv1.5 (left), Kv1.5+Kvβ2.1 (middle) and Kv1.5+Kvβ1.3 (right). Note the effects produced by both Kvβ2.1 and Kvβ1.3 on the slow inactivation of the current and on kinetics of the deactivation. Note that the Kvβ1.3 subunit converts the delayed current generated by the activation of Kv1.5 channels in a fast inactivating current.

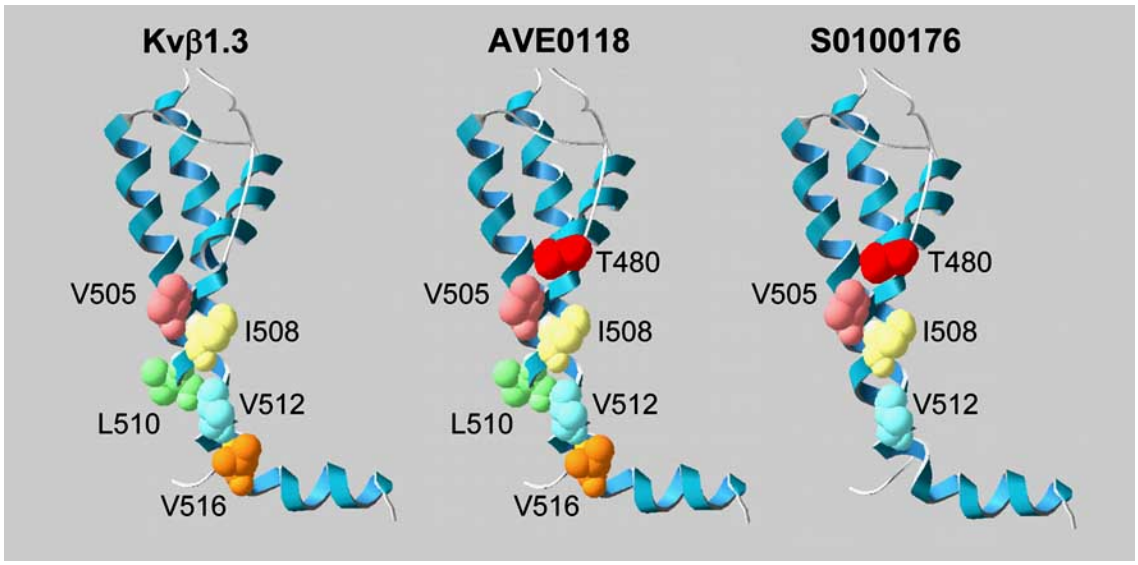

Fig. (5). Partial overlap of drug and Kvβ1.3 binding sites on the S6 domain of Kv1.5 channel. The S5-S6 domains of a single Kv1.5 subunit are depicted with important residues for interaction with Kvβ1.3 (Left panel), AVE0118 (Middle panel) and S0100176 (Right panel). Interacting residues are defined by a mutation-induced decrease in the extent of inactivation caused by Kvβ1.3 or a decrease in block by drug. In addition, mutation of Thr479 or Thr480 enhanced the ability of Kvβ1.3 to inactivate Kv1.5 channels. Homology models are based on the crystal structure of the KcsA channel [15], incorporating a predicted bending of the S6 domain at the PVP motif [54,59,63].

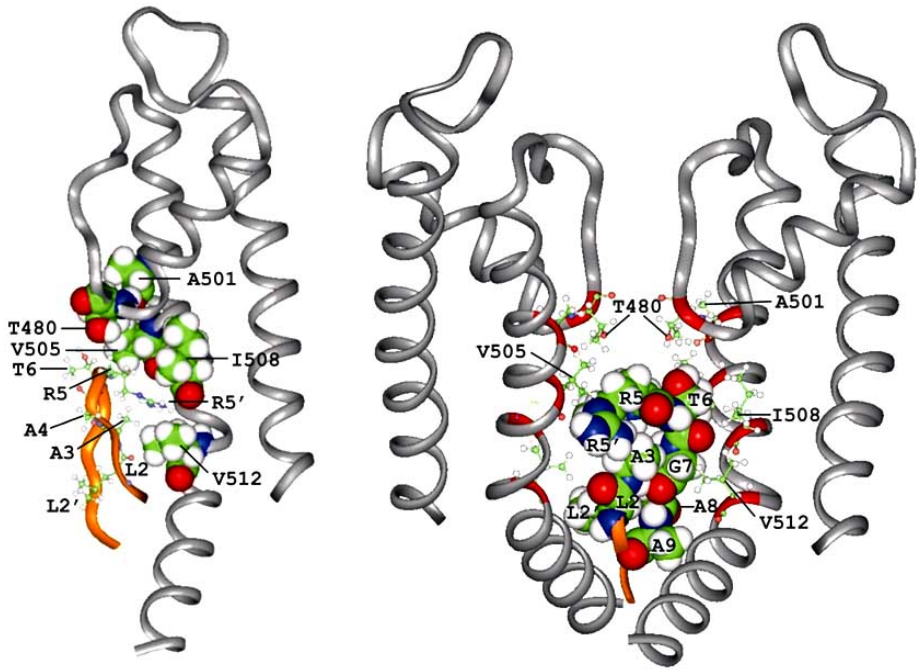

Fig. (6). Energy optimized structural model of Kvβ1.3 bound to the pore of Kv1.5 channels. (Left Panel) Kvβ1.3 N-terminus (residues 1-11) docked into the Kv1.5 pore homology model showing a single subunit. Kvβ1.3 side chains are shown as ball and stick models and residues of the Kvβ1.3-binding site in Kv1.5 are depicted with van der Waals surfaces. The symbol ′ indicates the end of long side chains. (Right Panel) Kvβ1.3 docked into the Kv1.5 pore homology model showing two subunits.

Stereoselective bupivacaine interactions with Kv1.5 channels have been shown to involve at least three amino acids located at the S6 segment (T507, L510 and V514) [7]. Mutation of L510 was previously reported to decrease channel block induced by quinidine, bupivacaine and TEA [6,7,60]. Although leucine at position 510 is predicted to face away from the central cavity, it has been recently demonstrated that mutation of this residue to alanine prevents the interaction with Kvβ1.3, and, thus, its ability to induce N-type inactivation and to alter Kv1.5 channel gating [54]. If L510 determines bupivacaine stereoselectivity and it is also required for Kvβ1.3-induced inactivation, then it could be hypothesized that the inactivating ball of the β subunit and bupivacaine may compete for the same internal receptor site, as it has been suggested for quaternary ammonium derivatives [58]. Stereoselective interactions are very specific and suggest direct and specific three-dimensional relationships in the receptor site. Thus, possible changes in the degree of bupivacaine stereoselectivity on Kv1.5 and Kv1.5+Kvβ1.3 channels were analyzed by Arias *et al*. [57]. In this study, the authors demonstrated that the L510A mutant preserves stereoselectivity of bupivacaine block while diminishes inhibition by Kvβ1.3. As L510 is part of the Kvβ1.3 binding site [54], mutant L510A channels are not blocked by Kvβ1.3. Accordingly, Kvβ1.3 is not able to eliminate the stereoselectivity of bupivacaine block. In contrast, the stereoselectivity of mutant V514A channels, which have an intact Kvβ1.3 binding site [54], remains modulated by Kvβ1.3. The residue L510 is particularly important for the modulation of bupivacaine block, as from our current knowledge L510 is the only residue of the S6 segment that is binding to both bupivacaine [6,7] and Kvβ1.3 [54,57]. These findings support previous reports of an overlapping binding site for drugs and Kvβ1.3 [54,55] and pinpoint the stereoselective effects of Kvβ1.3 to the S6 segment residue L510. These results demonstrate that bupivacaine and Kvβ1.3 compete for an overlapping binding site at the inner cavity of the Kv1.5 channel.

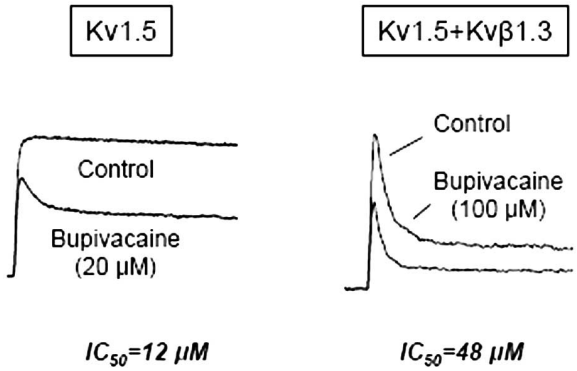

Fig. (7). Pharmacological consequences of the interaction between Kv1.5 and Kvβ1.3. Left panel shows original records obtained during the activation of Kv1.5 channels expressed in HEK293 cells in the absence and in the presence of bupivacaine (20 μM). Right panel shows original records obtained during the activation of Kv1.5+Kvβ1.3 channels expressed in HEK293 cells in the absence and in the presence of bupivacaine (100 μM). The IC_{50} values of bupivacaine block of Kv1.5 and Kv1.5+Kvβ1.3 channels are shown below. Note that Kvβ1.3 decreases the sensitivity of Kv1.5 to bupivacaine block.

This unique feature observed in Kv1.5+Kvβ1.3 seems to be the consequence of their interaction, since the N-terminus of the β subunit (like bupivacaine and other antiarrhythmic

drugs) blocks the open state of Kv1.5 channels; whereas KChIP2.2 assembly to the N-terminal tetramerization (T1) domain of Kv4.3 without an interaction with the ion pore of the channel [61].

Therefore, sensitivity to Kv1.5 channel blocking drugs will vary depending on the regional distribution of β regulatory subunits. The expression of Kvβ1.3 subunits in the myocardium is not homogeneous, for this subunit is expressed to a higher degree in the ventricle than in atria [62]. Within various vascular beds there are marked differences in β subunit expression while Kv1.5 levels change little [53]. Thus, the differential assembly between the Kvα and Kvβ subunits present in the cardiovascular system is another variable to be accounted for in the development of new ion channel modifying agents.

In summary, Kv channels are components of large protein complexes in the plasma membrane. It is very important to understand the changes in Kv channel function induced by partners sharing the same protein complex. Identification of these partners and determination of their influence in channel properties will not only provide us with new insights about channel function but can also lead us to unravel new disguises of these molecular machines in cell physiology and pathophysiology. Moreover, beta subunits modify the sensitivity of Kv to drugs. Therefore, the exact mechanism of these interactions will lead to a more rationale design of new useful drugs.

ACKNOWLEDGEMENTS

This work has been supported by CICYT SAF2007-65868, and Red Temática de Enfermedades Cardiovasculares RECAVA RD06/0014/0006 Grants. TG holds a JAE-Doc contract from the Consejo Superior de Investigaciones Científicas. MD holds a RECAVA contract, CM holds a FPI Grant and AM a JAE-Pre Grant.

REFERENCES

[1] Swartz, K. J. Sensing voltage across lipid membranes. *Nature,* **2008**, *456*, 891-7.

[2] Armstrong, C. M.; Hille, B. Voltage-gated ion channels and electrical excitability. *Neuron,* **1998**, *20*, 371-80.

[3] Hodgkin, A. L.; Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond),* **1952**, *117*, 500-44.

[4] Catterall, W. A. Structure and function of voltage-sensitive ion channels. *Science*, **1988**, *242*, 50-61.

[5] Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.,* **1981**, *391*, 85-100.

[6] Yeola, S. W.; Rich, T. C.; Uebele, V. N.; Tamkun, M. M.; Snyders, D. J. Molecular analysis of a binding site for quinidine in a human cardiac delayed rectifier K^+ channel. Role of S6 in antiarrhythmic drug binding. *Circ. Res.,* **1996**, *78*, 1105-14.

[7] Franqueza, L.; Longobardo, M.; Vicente, J.; Delpon, E.; Tamkun, M. M.; Tamargo, J.; Snyders, D. J.; Valenzuela, C. Molecular determinants of stereoselective bupivacaine block of hKv1.5 channels. *Circ. Res.,* **1997**, *81*, 1053-64.

[8] Nau, C.; Wang, S. Y.; Strichartz, G. R.; Wang, G. K. Point mutations at N434 in D1-S6 of μ1 Na^+ channels modulate binding affinity and stereoselectivity of local anesthetic enantiomers. *Mol. Pharmacol.,* **1999**, *56*, 404-13.

[9] Papazian, D. M.; Schwarz, T. L.; Tempel, B. L.; Jan, Y. N.; Jan, L. Y. Cloning of genomic and complementary DNA from *Shaker*, a putative potassium channels gene from Drosophila. *Science,* **1987**, *237*, 749-53.

[10] Snyders, D. J. Structure and function of cardiac potassium channels. *Cardiovasc. Res.,* **1999**, *42*, 377-90.

[11] Swartz, K. J. Towards a structural view of gating in potassium channels. *Nat. Rev. Neurosci.,* **2004**, *5*, 905-16.

[12] Roden, D. M.; George, A. L., Jr. Structure and function of cardiac sodium and potassium channels. *Am. J. Physiol.*, **1997**, *273*, H511-H525.

[13] Dolly, J. O.; Parcej, D. N. Molecular properties of voltage-gated K^+ channels. *J. Bioenerg. Biomembr.,* **1996**, *28*, 231-53.

[14] MacKinnon, R. Determination of the subunit stoichiometry of a voltage- activated K channel. *Nature,* **1991**, *350*, 232-5.

[15] Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science,* **1998**, *280*, 69-77.

[16] Gutman, G. A.; Chandy, K. G.; Grissmer, S.; Lazdunski, M.; McKinnon, D.; Pardo, L. A.; Robertson, G. A.; Rudy, B.; Sanguinetti, M. C.; Stuhmer, W.; Wang, X. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. *Pharmacol. Rev.,* **2005**, *57*, 473-508.

[17] Deal, K. K.; England, S. K.; Tamkun, M. M. Molecular physiology of cardiac potassium channels. *Physiol. Rev.,* **1996**, *76*, 49-76.

[18] Papazian, D. M.; Timpe, L. C.; Jan, Y. N.; Jan, L. Y. Alteration of voltage-dependence of Shaker potassium channel by mutations in the S4 sequence. *Nature,* **1991**, *349*, 305-10.

[19] Long, S. B.; Campbell, E. B.; MacKinnon, R. Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science,* **2005**, *309*, 897-903.

[20] Bezanilla, F.; Stefani, E. Gating currents. *Methods Enzymol.,* **1998**, *293*, 331-52.

[21] Mannuzzu, L. M.; Moronne, M. M.; Isacoff, E. Y. Direct physical measure of conformational rearrangement underlying potassium channel gating. *Science,* **1996**, *271*, 213-6.

[22] Schmidt, D.; MacKinnon, R. Voltage-dependent K+ channel gating and voltage sensor toxin sensitivity depend on the mechanical state of the lipid membrane. *Proc. Natl. Acad. Sci. U. S. A,* **2008**, *105*, 19276-81.

[23] Xu, Y.; Ramu, Y.; Lu, Z. Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K+ channels. *Nature,* **2008**, *451*, 826-9.

[24] Ramu, Y.; Xu, Y.; Lu, Z. Enzymatic activation of voltage-gated potassium channels. *Nature,* **2006**, *442*, 696-9.

[25] Hoshi, T.; Zagotta, W. N.; Aldrich, R. W. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science,* **1990**, *250*, 533-8.

[26] Gomez Lagunas, F.; Armstrong, C. M. Inactivation in ShakerB K+ channels: a test for the number of inactivating particles on each channel. *Biophys. J.,* **1995**, *68*, 89-95.

[27] Isacoff, E. Y.; Jan, Y. N.; Jan, L. Y. Putative receptor for the cytoplasmic inactivation gate in the *Shaker* K^+ channel. *Nature,* **1991**, *353*, 86-90.

[28] Hoshi, T.; Zagotta, W. N.; Aldrich, R. W. Two types of inactivation in *Shaker* K^+ channels: effects of alterations in the carboxy-terminal region. *Neuron*, **1991**, *7*, 547-56.

[29] Heginbotham, L.; Abramson, T.; MacKinnon, R. A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels. *Science*, **1992**, *258*, 1152-5.

[30] del Camino, D.; Holmgren, M.; Liu, Y.; Yellen, G. Blocker protection in the pore of a voltage-gated K+ channel and its structural implications. *Nature,* **2000**, *403*, 321-5.

[31] Choi, K. L.; Mossman, C.; Aube, J.; Yellen, G. The internal quaternary ammonium receptor site of *Shaker* potassium channels. *Neuron*, **1993**, *10*, 533-41.

[32] Long, S. B.; Tao, X.; Campbell, E. B.; MacKinnon, R. Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. *Nature,* **2007**, *450*, 376-82.

[33] Tamargo, J.; Caballero, R.; Gomez, R.; Valenzuela, C.; Delpon, E. Pharmacology of cardiac potassium channels. *Cardiovasc. Res.,* **2004**, *62*, 9-33.

[34] Nerbonne, J. M.; Kass, R. S. Molecular physiology of cardiac repolarization. *Physiol. Rev.,* **2005**, *85*, 1205-53.

[35] Nelson, M. T.; Quayle, J. M. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.*, **1995**, *268*, C799-822.

[36] Jackson, W. F. Potassium channels in the peripheral microcirculation. *Microcirculation*, **2005**, *12*, 113-27.

[37] Parcej, D. N.; Scott, V. E.; Dolly, J. O. Oligomeric properties of alpha-dendrotoxin-sensitive potassium ion channels purified from bovine brain. *Biochemistry*, **1992**, *31*, 11084-8.

[38] Gulbis, J. M.; Zhou, M.; Mann, S.; MacKinnon, R. Structure of the cytoplasmic beta subunit-T1 assembly of voltage- dependent K+ channels. *Science*, **2000**, *289*, 123-7.

[39] Shi, G.; Nakahira, K.; Hammond, S.; Rhodes, K. J.; Schechter, L. E.; Trimmer, J. S. Beta subunits promote K+ channel surface expression through effects early in biosynthesis. *Neuron*, **1996**, *16*, 843-52.

[40] Nagaya, N.; Papazian, D. M. Potassium channel alpha and beta subunits assemble in the endoplasmic reticulum. *J. Biol. Chem*, **1997**, *272*, 3022-7.

[41] Overturf, K. E.; Russell, S. N.; Carl, A.; Vogalis, F.; Hart, P. J.; Hume, J. R.; Sanders, K. M.; Horowitz, B. Cloning and characterization of a Kv1.5 delayed rectifier K^+ channel from vascular and visceral smooth muscles. *Am. J. Physiol.*, **1994**, *267*, C1231-C1238.

[42] Mays, D. J.; Foose, J. M.; Philipson, L. H.; Tamkun, M. M. Localization of the Kv1.5 K^+ channel protein in explanted cardiac tissue. *J. Clin. Invest.*, **1995**, *96*, 282-92.

[43] Konarzewska, H.; Peeters, G. A.; Sanguinetti, M. C. Repolarizing K+ currents in nonfailing human hearts. Similarities between right septal subendocardial and left subepicardial ventricular myocytes. *Circulation*, **1995**, *92*, 1179-87.

[44] Li, G. R.; Feng, J.; Wang, Z.; Fermini, B.; Nattel, S. Adrenergic modulation of ultrarapid delayed rectifier K+ current in human atrial myocytes. *Circ. Res.*, **1996**, *78*, 903-15.

[45] Tamkun, M. M.; Knoth, K. M.; Walbridge, J. A.; Kroemer, H.; Roden, D. M.; Glover, D. M. Molecular cloning and characterization of two voltage-gated K+ channel cDNAs from human ventricle. *FASEB J*, **1991**, *5*, 331-7.

[46] Snyders, D. J.; Tamkun, M. M.; Bennett, P. B. A rapidly activating and slowly inactivating potassium channel cloned from human heart. Functional analysis after stable mammalian cell culture expression. *J. Gen. Physiol.*, **1993**, *101*, 513-43.

[47] Wang, Z.; Fermini, B.; Nattel, S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K^+ current similar to Kv1.5 cloned channel currents. *Circ. Res.*, **1993**, *73*, 1061-76.

[48] Van Wagoner, D. R.; Pond, A. L.; McCarthy, P. M.; Trimmer, J. S.; Nerbonne, J. M. Outward K+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. *Circ. Res.*, **1997**, *80*, 772-81.

[49] Olson, T. M.; Alekseev, A. E.; Liu, X. K.; Park, S.; Zingman, L. V.; Bienengraeber, M.; Sattiraju, S.; Ballew, J. D.; Jahangir, A.; Terzic, A. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. *Hum. Mol. Genet.*, **2006**, *15*, 2185-91.

[50] Weir, E. K.; Lopez-Barneo, J.; Buckler, K. J.; Archer, S. L. Acute oxygen-sensing mechanisms. *N. Engl. J Med.*, **2005**, *353*, 2042-55.

[51] England, S. K.; Uebele, V. N.; Shear, H.; Kodali, J.; Bennett, P. B.; Tamkun, M. M. Characterization of a voltage-gated K^+ channel β subunit expressed in human heart. *Proc. Natl. Acad. Sci. U. S. A*, **1995**, *92*, 6309-13.

[52] Uebele, V. N.; England, S. K.; Chaudhary, A.; Tamkun, M. M.; Snyders, D. J. Functional differences in Kv1.5 currents expressed in mammalian cell lines are due to the presence of endogenous Kvβ2.1 subunits. *J. Biol. Chem.*, **1996**, *271*, 2406-12.

[53] Coppock, E. A.; Tamkun, M. M. Differential expression of K_V channel α- and β-subunits in the bovine pulmonary arterial circulation. *Am. J. Physiol.*, **2001**, *281*, L1350-L1360.

[54] Decher, N.; Kumar, P.; Gonzalez, T.; Renigunta, V.; Sanguinetti, M. C. Structural basis for competition between drug binding and Kvbeta1.3 accessory subunit-induced N-type inactivation of Kv1.5 channels. *Mol. Pharmacol.*, **2005**, *68*, 995-1005.

[55] Gonzalez, T.; Navarro-Polanco, R.; Arias, C.; Caballero, R.; Moreno, I.; Delpon, E.; Tamargo, J.; Tamkun, M. M.; Valenzuela, C. Assembly with the Kvβ1.3 subunit modulates drug block of hKv1.5 channels. *Mol. Pharmacol.*, **2002**, *62*, 1456-63.

[56] Decher, N.; Gonzalez, T.; Streit, A. K.; Sachse, F. B.; Renigunta, V.; Soom, M.; Heinemann, S. H.; Daut, J.; Sanguinetti, M. C. Structural determinants of Kvbeta1.3-induced channel inactivation: a hairpin modulated by PIP2. *EMBO J*, **2008**, *27*, 3164-74.

[57] Arias, C.; Guizy, M.; David, M.; Marzian, S.; Gonzalez, T.; Decher, N.; Valenzuela, C. Kvbeta1.3 reduces the degree of stereoselective bupivacaine block of Kv1.5 channels. *Anesthesiology*, **2007**, *107*, 641-51.

[58] Zhou, M.; Morais-Cabral, J. H.; Mann, S.; MacKinnon, R. Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. *Nature*, **2001**, *411*, 657-61.

[59] Decher, N.; Pirard, B.; Bundis, F.; Peukert, S.; Baringhaus, K. H.; Busch, A. E.; Steinmeyer, K.; Sanguinetti, M. C. Molecular basis for Kv1.5 channel block: conservation of drug binding sites among voltage-gated K^+ channels. *J. Biol. Chem.*, **2004**, *279*, 394-400.

[60] Caballero, R.; Moreno, I.; Gonzalez, T.; Valenzuela, C.; Tamargo, J.; Delpon, E. Putative binding sites for benzocaine on a human cardiac cloned channel (Kv1.5). *Cardiovasc. Res.*, **2002**, *56*, 104-17.

[61] An, W. F.; Bowlby, M. R.; Betty, M.; Cao, J.; Ling, H. P.; Mendoza, G.; Hinson, J. W.; Mattsson, K. I.; Strassle, B. W.; Trimmer, J. S.; Rhodes, K. J. Modulation of A-type potassium channels by a family of calcium sensors. *Nature*, **2000**, *403*, 553-6.

[62] Wang, Z.; Kiehn, J.; Yang, Q.; Brown, A. M.; Wible, B. A. Comparison of binding and block produced by alternatively spliced Kvbeta1 subunits. *J. Biol. Chem.*, **1996**, *271*, 28311-7.

[63] Decher, N.; Kumar, P.; Gonzalez, T.; Pirard, B.; Sanguinetti, M. C. Binding site of a novel Kv1.5 blocker: a "foot in the door" against atrial fibrillation. *Mol. Pharmacol.*, **2006**, *70*, 1204-11.

Received: December 29, 2009 Revised: February 01, 2010 Accepted: February 09, 2010