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Homology modeling of Kv1.5 channel block by cationic and electroneutral ligands

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ABSTRACT

The inner pore of potassium channels is targeted by many ligands of intriguingly different chemical structures. Previous studies revealed common and diverse characteristics of action of ligands including cooperativity of ligand binding, voltage- and use-dependencies, and patterns of ligand-sensing residues. Not all these data are rationalized in published models of ligand-channel complexes. Here we have used energy calculations with experimentally defined constraints to dock flecainide, ICAGEN-4, benzocaine, vernakalant, and AVE0118 into the inner pore of Kv1.5 channel. We arrived at ligand-binding models that suggest possible explanations for different values of the Hill coefficient, different voltage dependencies of ligands action, and effects of mutations of residues in subunit interfaces. Two concepts were crucial to build the models. First, the inner-pore block of a potassium channel requires a cationic "blocking particle". A ligand, which lacks a positively charged group, blocks the channel in a complex with a permeant ion. Second, hydrophobic moieties of a flexible ligand have a tendency to bind in hydrophobic subunit interfaces.

1. Introduction

The human genome encodes 78 proteins that form homo- and heteromeric potassium channels [1]. Large variations in the gating mechanisms, physiological and pharmacological characteristics of potassium channels underline their key roles in the cell physiology, particularly in regulating excitability of neurons and muscle cells. Many small-molecule ligands of dramatically different chemical structures bind in the inner-pore region of potassium channels [1]. The inner-pore blockers of Kv1.5 channels (Fig. 1) include hydrophobic cations like bupivacaine [2], flecainide and vernakalant [3] as well as uncharged ligands with polar and aromatic moieties, e.g., benzocaine [4], AVE0118 [5], S0100176 [6], ICAGEN-4 [7], and PAP-1 [8]. Mutational analysis of the Kv1.5 channel has revealed pore-facing residues in the inner helices (S6s) and P-loop turns that affect binding of ligands [5–7,9,10].

In the absence of X-ray structures of ligand-bound Kv1.5 channel, homology modeling is the only possibility to suggest structural details of the ligand binding. To rationalize the experimental data on the Kv1.5 channel block, several models have been proposed [3,6,7,9–11]. However, some of the experimental data call for further structural analysis. For example, AVE0118 prevents the channel closure, whereas S0100176 does not demonstrate this effect [5]. The molecular determinants of this difference are unknown. Another problem is the Hill coefficient. While many compounds demonstrate Hill coefficient about 1, some compounds block the Kv1.5 channel with the Hill coefficient greater than one. Examples (Fig. 1) include di-substituted cyclohexyl derivatives like trans-NPCO-DSC [12], catechol derivatives [13], ICAGEN-4 and MSD-D [7]. Notably, all these ligands are uncharged cationophilic molecules, which lack ionizable groups. Furthermore, the voltage dependence of block cannot be explained by a straightforward mechanism. Typically, action of positively charged compounds is voltage dependent, while uncharged blockers are voltage independent in agreement with the classical Woodhull model [14]. However, cationophilic blockers also demonstrate the voltage dependence of action, which is not expected for uncharged ligands. Moreover, sometimes the voltage dependence of cationophilic ligands is opposite to that of cationic blockers of the inner pore [4].

Furthermore, some mutational data are not easily interpretable. In particular, the patterns of ligand-sensing residues identified for charged and uncharged blockers are similar (Table 1). This is not clear because the cationophilic inner pore is expected to discriminate charged and uncharged ligands. Most of ligand-sensing residues face the inner pore in the Kv1.5 homology models, which are based on available X-ray structures (Fig. 2A, B). However, despite isoleucine I502 in the inner helix S6 is exposed into the subunit interface of the pore module rather than into the inner pore, mutations of I502 affect binding of various ligands, although to different extent. Models that suggest ligand binding only in the central pore necessarily consider indirect effects of mutations of this residue on the ligand action. A recent model proposes binding of one Psora-4 molecule in the central pore and four Psora-4 molecules in side pockets between the voltage-sensing helix S4, linker helix L45, and backsides of S5 and S6 [11]. None of these binding models

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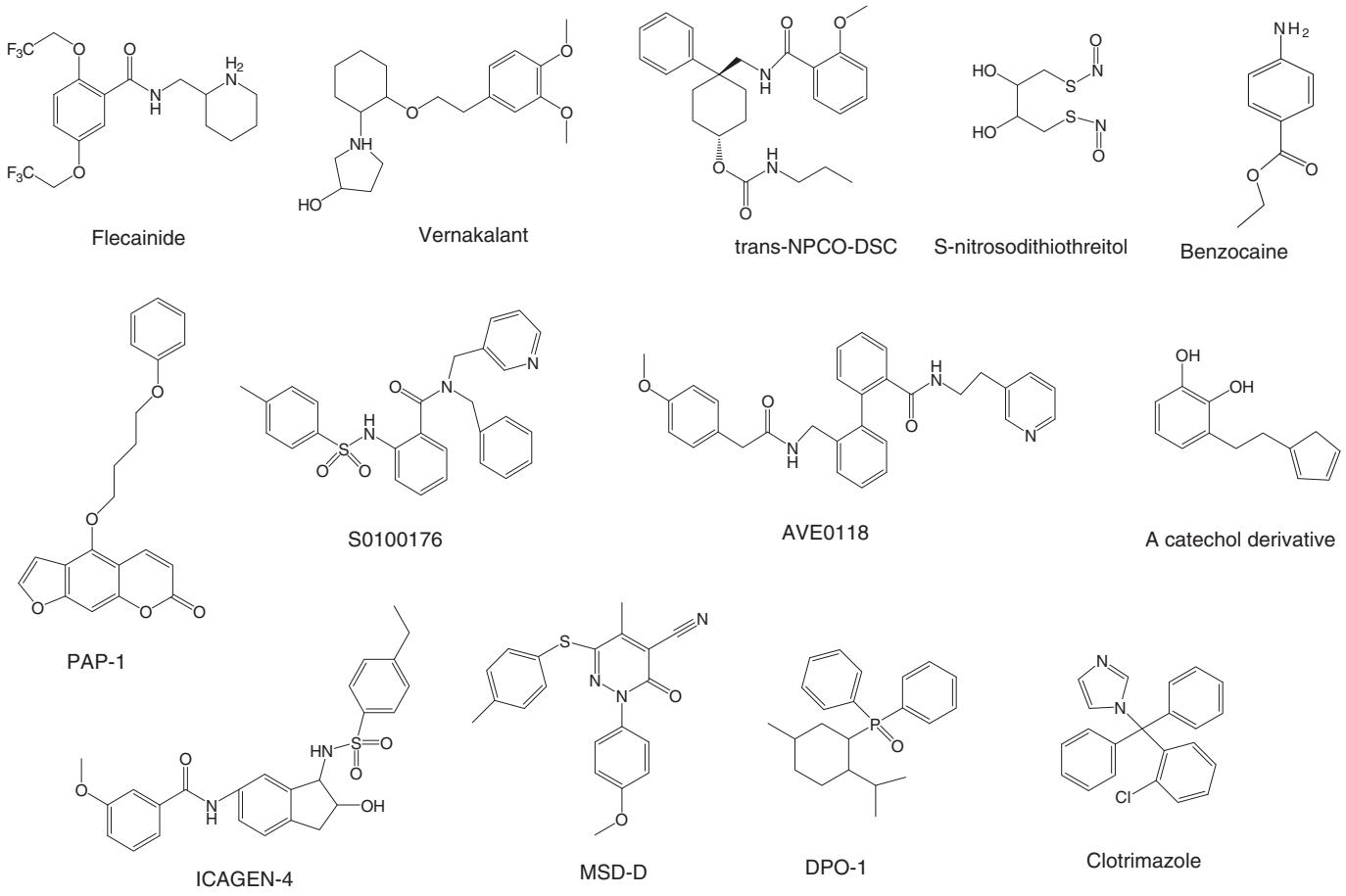

Fig. 1. Chemical structures of some Kv1.5 channel blockers.

demonstrates direct ligand interaction with I502. The authors hypothesize that I502 is important for transition of Psora-4 between the central pore and the side pockets through subunit interfaces. However, I502 affects binding of many structurally diverse ligands, including ligands with the Hill coefficient of one and charged ligands that would hardly pass through the hydrophobic interface. It is unlikely that all these ligands block the channel by the same mechanism as proposed for Psora-4. Therefore, an alternative explanation of the role of I502 in the Kv1.5 ligands action is necessary.

Here, we addressed these unclear issues related to the Kv1.5 channel block using a molecular modeling approach. Limited precision of homology modeling prevents realistic calculation of the binding free energy and therefore unbiased prediction of the drug binding modes. Despite these limitations, homology modeling allowed to elaborate hypotheses on ligand interactions with potassium and sodium channels, which are confirmed by model-directed mutational analysis [8,15–18]. Here we have used previously elaborated models as starting points to explore possibilities of Kv1.5 channel interactions with different ligands. On one hand, energy calculations with a homology model allow us to rule out many hypotheses and ligand-binding modes that are inconsistent with the energetics of ligand–protein interactions. On the other hand, ligand docking in a homology model usually predicts several low-energy complexes. Energetics of these complexes cannot be used as the only criterion of the model correctness.

In view of these limitations of the homology modeling approach, our calculations aimed to find local energy minima where ligand-channel

Table 1
Ligand-sensing residues in P-loops and S6 segments of Kv1.5 and Kv1.3 channels and their involvement in ligand binding in our models[a].

Channel	P-loop	S6	Ligand	Reference
	476	497 501 511 521		
	| **	| | * ** |* * |		
Kv1.5	VVSMTT	VGGKIVGSLC AIAGVLTIAL PVPVIVSNFN Y		
	----↓↓	----------···· ↓↓·-↓↓·↓↓· -↓··-↓---- -	S0100176	[6]
	----↓↓	----------···· ·↓·-↓··↓=↓ -↓↓·-↓---- -	AVE0118	[5]
	----↓↓	----------↓ ↑↓--↓--↓↓-- -↓--------- -	Vernakalant	[3]
	----=↓=	---------- -↓--↓·--↓-- ---------- -	Flecainide	[3]
	---=↓↓	---------- -=---↓↓↑↓↓· -↓-·-↓---- -	ICAGEN-4	[7]
	----·↓↓	--------··↓· ···-↓·↓-↓ -·-↓-·----- -	DPO-1	[35]
Kv1.3	----↓-	---------- -↓--↓·↓↓-↓ -↓--↓↓·--- -	PAP-1	[8]
	------	----·····- ↓-·-↓↓·-↓↓ ↓↓↓·-·-··--- -	Correolide	[36]

↓ Mutation (usually Ala substitution) decreases the channel-blocking potency of the ligand.
↑ Mutation (usually Ala substitution) increases the channel-blocking potency of the ligand.
· Mutation has a weak effect on the channel block by the ligand.
- Not mutated or mutation resulted non-functional channel.
[a] Underlined characters refer to the wild-type Kv1.5 residues that contribute to the binding of respective ligand in our models.

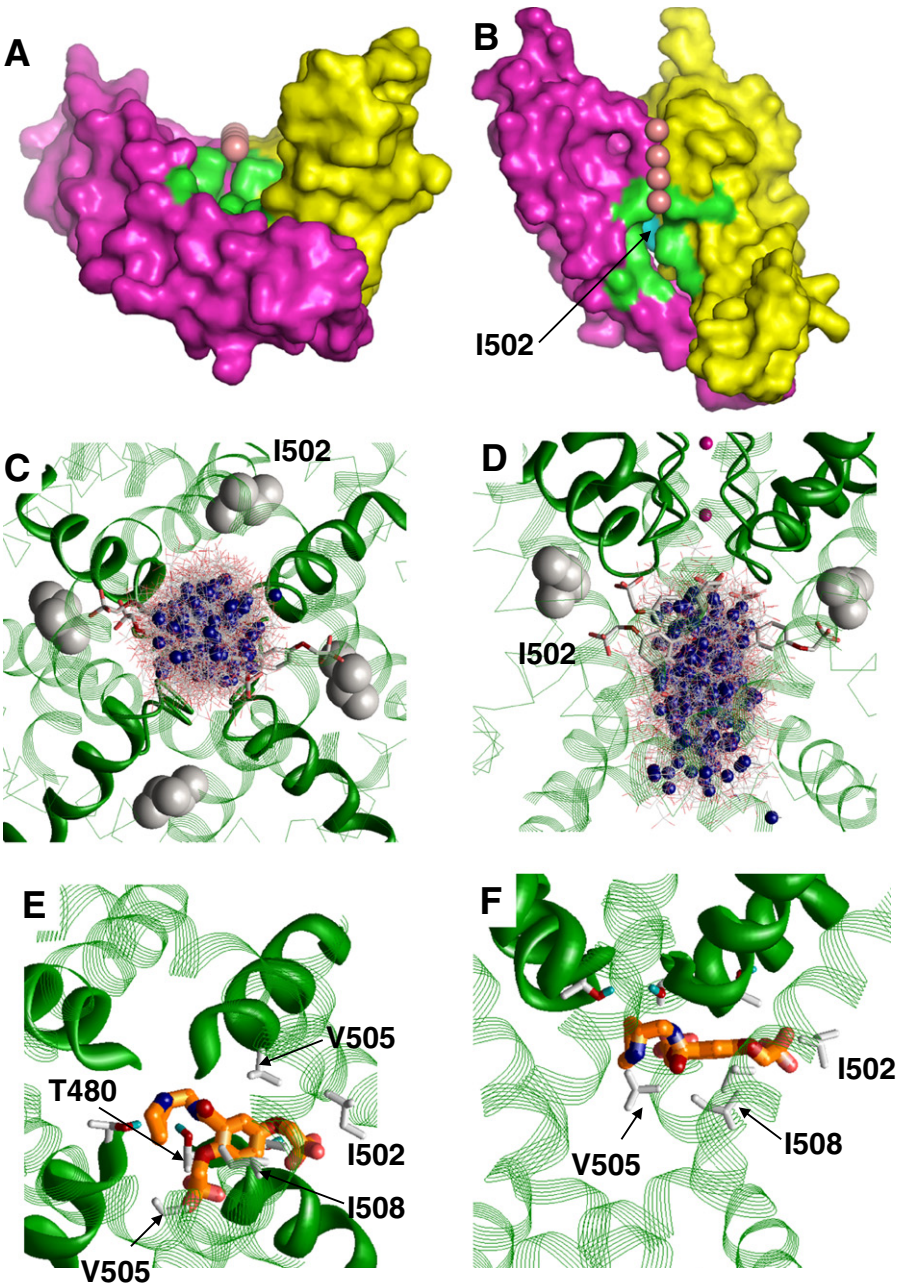

Fig. 2. Ligand-sensing residues and flecainide binding modes. *A* and *B*, A subunit interface viewed from the central cavity at the angles of ~175 and 90° to the pore axis. Purple and yellow surfaces represent two adjacent subunits (inner helices with P-loops). Green color shows ligand-sensing residues T479, T480, V505, I508 and V512 that line the inner pore. Ligand-sensing residue I502 in the subunit interface is cyan. *C* and *D*, Results of hands-free docking of flecainide. Ensemble of 200 lowest-energy complexes of flecainide in the Kv1.5 open channel model obtained from 100,000 randomly generated starting ligand orientations. Blue spheres represent the positively charged amino group. In the majority of obtained binding modes, the entire ligand molecule binds inside the inner pore, but three structures show partial penetration of the ligand moieties (shown as sticks) in the subunit interface where they reach the I502 residues (space filled). The low number of such structures in the ensemble reflects the fact that random seeding most frequently hits the wide inner pore and only rarely hits the narrow subunit interface. *E* and *F*, the proposed binding mode of flecainide. The positively charged ammonium group of each ligand is located in the cation-attractive region in the central cavity. Trifluoromethyl moiety of the ligand protrudes in a subunit interface and reaches the I502 residue.

complexes lack sterical hindrances, unfavorable electrostatic interactions and contacts between hydrophobic and hydrophilic moieties. Among different binding modes that satisfy these criteria we focused on those, which agree with available experimental data and allowed us to suggest structural hypotheses explaining various features of the Kv1.5 channel block.

2. Methods

Our methodology of molecular modeling is described in many studies, e.g. [8,19–21]. Briefly, we use the ZMM program that minimizes energy in the space of internal (generalized) coordinates, the Monte Carlo (MC) energy minimization method [22], the AMBER force field [23,24] with the implicit solvent [25], and atomic charges at ligands, which are calculated by MOPAC [26]. The homology model of Kv1.5 channel was built using the Kv1.2 open-channel structure [27] as a template.

Due to limited precision of the homology modeling approach, we have used "pin" constraints to ensure similarity of the backbone conformation in the model and the template. A pin constraint is a flat-bottom parabolic energy function that imposes an energy penalty if an alpha carbon of the model deviates from the template position by more than

1 Å. For all constraints the energy penalty was calculated using the force constant of 10 kcal mol^{-1} $Å^{-2}$.

Biased ligand docking was performed in few steps. First, the ligand was placed in a specific location manually or by using distance constraints (penalty functions added to the energy expression). The biased position of the ligand was MC-minimized with the distance constraints. Then the distance constraints were removed and the complex was refined by an additional MC-minimization. If during the refining MC-minimization the ligand or the ligand-bound metal ion moved away from the starting geometry, the respective ligand-binding mode was ignored.

The ensemble of low-energy conformations obtained in the refining MC-minimization (up to 10 kcal/mol from the apparent global minimum) was used for statistical analysis of ligand-channel interactions. For each structure the interaction energies between the ligand and all channel residues were calculated and averaged over the ensemble. If the absolute value of this average energy for a particular residue was more than 0.5 kcal/mol, the residue was considered as a significant contributor to the ligand binding.

3. Results

3.1. Ligand-sensing residues in the pore and subunit interfaces

Ligand-sensing residues, which are known from experimental studies, are shown in Table 1. Mutations of the pore-facing residues V505, I508 and V512 affect action of various ligands, suggesting that these ligands bind in the inner pore (Fig. 2A and B). Besides the central cation-attractive cavity, which is lined by ligand-sensing residues, the pore module includes rather hydrophobic subunit interfaces lined by inner helices and the P-helix [1] (Fig. 2A and B). In calcium and sodium channels, the interface between repeat domains III and IV has been proposed to serve as a sidewalk access pathway to the inner pore [28–30]. Some ligand-sensing residues in potassium, sodium and calcium channels line these interfaces rather than the central cavity. An example is I502 in Kv1.5. Mutations of this residue affect block by many ligands, including cationic ligands flecainide and vernakalant (Table 1). Fig. 2B shows that although I502 (colored cyan and marked by arrow) is exposed to the subunit interface, it is potentially accessible from the pore.

To explore this possibility, we performed hands-free docking of flecainide to the Kv1.5 open channel model. 100,000 starting points with random position and orientation of the flecainide molecule in the inner pore were generated and each starting point was optimized in a short MCM trajectory of 10 energy minimizations to remove steric clashes. Fig. 2C and D shows the ensemble of 200 lowest-energy structures. In most of these structures, the entire drug molecule is located within in the inner pore. However, in several structures trifluoromethyl group of flecainide penetrated into a subunit interface and approached I502. The number of such structures is small because during random generation of the starting points the chances of the ligand to hit the narrow interface are much smaller than the chances to occur in the big inner pore.

The binding models in which Kv1.5 ligands are located entirely in the inner pore have been carefully examined in several studies, e.g., [3,6,7,9–11], which explain mutational data on pore-facing residues in P-loops and S6s. However, these models do not explain why mutations of I502 affect drug action. Therefore in this study we focused on the binding modes where ligands interact directly with I502. We used distance constraints to near a ligand and I502. Importantly, stability of the obtained binding modes was checked by unconstrained refining-stage MC-minimizations (see Methods).

3.2. Flecainide and vernakalant

In the recent model of flecainide binding to Kv2.1 [16], which is proposed basing on experimentally determined flecainide-sensing residues, the ligand piperidine ring fits in the central cavity, while the benzamide moiety binds in the subunit interface between the S6 and P helices. In the present study, a homology model of flecainide-Kv1.5 complex was obtained by MC-minimizations (see Methods) using the model [16] as the starting point. In the final lowest-energy structure (Fig. 2C and D) the charged group is located in the cation-attractive region of the inner pore, which corresponds to site s5 for a potassium ion. A trifluoromethyl group entered the subunit interface and interacted directly with I502. The pattern of residues, which contribute to flecainide–channel interactions (Table 1), agrees with mutational data. Fig. 2E and F show a representative complex. Characteristics of the ensemble of low-energy structures are given in Supplementary Fig. S1 and Table S1. The experimentally revealed flecainide-sensing residues in Kv1.5 provided 64% of the total binding energy and 30% of this energy was due to electrostatic interactions of the P-helices with flecainide charged moiety.

Certain similarities between the chemical structures of flecainide and vernakalant allowed us to build a vernakalant-Kv1.5 model (Fig. 3A and B) in which the ligand charged part bound in the inner pore, whereas the uncharged dimethoxyphenyl moiety protruded in the subunit interface and interacted directly with I502. Since vernakalant is larger than flecainide, it occupied more space in the inner pore and also interacted with residues A509 and V512, which do not contribute significantly to flecainide binding (Tables 1 and S2, Fig. S2). The only vernakalant-sensing residues, which did not form direct contacts with the ligand in our model, were A501 and C500. Allosteric effects of these mutations are proposed earlier [3]. Thus, our models agree with most of mutational data on the considered here charged Kv1.5 blockers, including effects of I502 substitutions.

3.3. Model of Kv1.5 with ICAGEN-4

One of the important characteristics of drug action is the Hill coefficient, which reflects cooperativity of biding ligand molecules to a protein. Classical hydrophobic cations, like tetrabutylammonium, block P-loop channels with the 1:1 stoichiometry (the Hill coefficient of 1), but many other blockers demonstrate the Hill coefficient >1 suggesting that at least two ligand molecules block the channel with positive cooperativity. Structurally diverse molecules block Kv1.5 channels (and other members of the Kv1 family) with the Hill coefficient >1. Examples (Fig. 1) are S-nitrosodithiothreitol [31], di-substituted cyclohexyl derivatives [12], catechol derivatives [13], MSD-D and ICAGEN-4 [7].

Among compounds, which demonstrate the Hill coefficient >1, ICAGEN-4 is particularly big and a possibility of binding of more than one ICAGEN-4 molecule in the inner pore is not obvious. To elaborate a homology model of Kv1.5 with ICAGEN-4 we have used the concept of Kv1.3 channel block by a tripartite complex of two electroneutral PAP-1 molecules and a potassium ion [8]. In the respective model, which is supported by extensive mutational and structure-activity experiments [8], the inner pore is blocked by a potassium ion that is chelated by two psoralen moieties, while long flexible 4-phenoxybutoxy moieties of PAP-1 molecules protrude in the subunit interfaces. Following this concept, we docked a tripartite complex containing two neutral ICAGEN-4 molecules and a potassium ion into the central cavity of Kv1.5. Distance constraints were used to near cation-attractive SO_2 groups of two ICAGEN-4 molecules to a potassium ion, while terminal moieties were directed in subunit interfaces.

A representative structure of the MC-minimized complex is shown in Fig. 3C and D, the ensemble of obtained structures is shown in Fig. S3 and its energy characteristics are given in Table S3. The ligands adopted an angular conformation with the methoxyphenyl ends protruding into subunit interfaces and opposite ethylphenyl ends extended along the inner pore. The escape of the methoxyphenyl ends from the inner pore provided a room to accommodate two molecules in the channel. Hydroxyl groups at the vertex of the ligand molecules

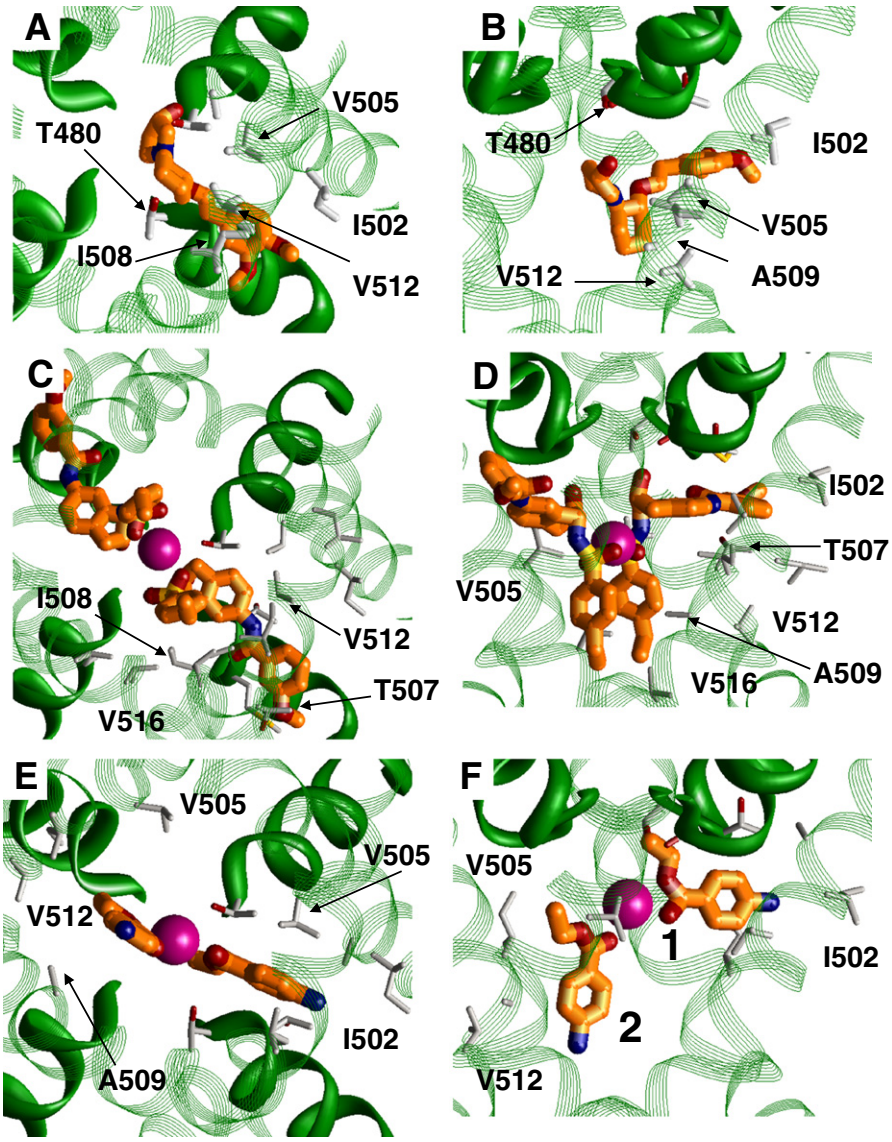

Fig. 3. Proposed binding modes of vernakalant, ICAGEN-4 and benzocaine in the model of Kv1.5. Cytoplasmic (*A*, *C* and *E*) and side (*B*, *D* and *F*) views show the channel with bound ligands. Vernakalant (*A* and *B*), binds like flecainide (see Fig. 2). However, it is bigger than flecainide, occupies more space in the inner pore and interacts with residues, which are not flecainide sensing ones (e.g., V512). ICAGEN-4 (*C* and *D*) and benzocaine (*E* and *F*) form tripartite complexes of two ligand molecules and a potassium ion at site s5. ICAGEN-4 molecules bind in an angular conformation with one end extending in a subunit interface and the opposite end lining the pore. The SO_2 groups chelate a potassium ion, which stabilizes the tripartite complex. Two molecules of benzocaine (*C* and *D*) also chelate a K^+ ion in the inner pore. One molecule binds in the axial orientation, with the aromatic moiety lining the inner pore. Another molecule binds in a horizontal orientation with the aromatic moiety protruding in the subunit interface, thus resembling binding of an "angular" ICAGEN-4 molecule.

appeared at the H-bonding distances from the hydrophilic C-ends of P-helices. A potassium ion was chelated at the cavity center by the SO_2 groups of the two ligands. Such disposition of the SO_2 groups would be electrostatically unfavorable in the absence of a potassium ion. The potassium bridge can explain a positive cooperativity for binding two ligand molecules. Most of the experimentally determined [7] ICAGEN-4-sensing residues (Table 1) directly interacted with the bound ligands, including T507 that does not face the inner pore. Thus, the model allowed us to explain simultaneous binding of two rather big ligands in the Kv1.5 channel. I502 was not mutated in the work [7]. Our model predicts that ICAGEN-4 should interact with this residue.

In our model two ICAGEN-4 molecules chelated the ion by their SO_2 groups. This interaction is not expected to coordinate a potassium ion stronger than eight selectivity filter oxygens do. In our model the block is achieved by hydrophobic ethylphenyl moieties of two ICAGEN-4 molecules that extend along in the inner pore below site s5.

3.4. Model of Kv1.5 with benzocaine

Benzocaine produces dual action on Kv1.5: at nanomolar concentrations it behaves as an agonist by potentiating the current, whereas at micromolar concentrations it blocks the current [4]. Authors of this study demonstrated that both types of action are mediated by benzocaine binding to the inner pore region and that the blocking site overlaps with the binding site for bupivacaine. Surprisingly, blocking action of neutral benzocaine is voltage-dependent and the voltage dependence has opposite sign in comparison with the action of cationic drugs. External tetraethylammonium does not modify the agonistic and blocking

effects of benzocaine, but suppressed the voltage-dependence. Moreover benzocaine and extracellular potassium ions interact to modify the voltage-dependence of channel opening [4].

To explain these data we propose the following mechanism. At low concentrations, benzocaine would bind with high affinity in the "horizontal" orientation in the subunit interface and expose its cation-attractive moiety towards the inner pore, near the selectivity filter (molecule 1 in Fig. 3E and F). Benzocaine molecule in such a binding mode would not block the cannel, but may increase the current by providing additional sites for permeating ions. At higher concentrations, the second benzocaine molecule would bind in the "vertical" orientation to a low-affinity site in the inner pore (molecule 2 Fig. 3C, D). The cation-attractive moiety of the second molecule would also interact with a potassium ion in the inner pore, while the hydrophobic moiety, which extends along the inner pore, would block the permeation. Fig. 3E and F shows only two benzocaine molecules, but up to four molecules could bind in the "horizontal" orientation to the same potassium ion, while their hydrophobic moieties would extend in the subunit interfaces, as proposed for Kv1.3 channel block by PAP-1 [8]. Our model also explains an unusual voltage-dependence of benzocaine action. We propose that neutral ligands coordinate a potassium ion to form a cationic blocking particle. A positive voltage would push the potassium ion out of the ligands, thus decreasing stability of the ligand-potassium blocking particle. The released potassium ion would escape from the inner pore to the extracellular space through the selectivity filter (see also ref. [32]). Non-surprisingly the effect is antagonized by external potassium ions and tetraethylammonium. The description of the two-barrier model of the voltage-dependence is given in Supplementary materials.

It should be noted that available mutational data on benzocaine binding [4] are fragmental and do not allow unambiguous conclusion about its binding site(s). Therefore, the model visualized in Fig. 3E and F is schematic and can be considered only as a preliminary hypothesis.

3.5. Model of Kv1.5 with DPO-1

Kv1.5 is blocked by electroneutral compounds like DPO-1 [33] and clotrimazole [34]. DPO-1 sensing residues, which are found by mutational analysis [35], include T480 and A509 that contribute to binding of various ligands (Table 1), as well as several residues that do not face the pore. Notably, I502 is not among the DPO-1 sensing residues. It should be also noted that mutations of some DPO-1 sensing residues affect activation characteristics of the Kv1.5 channel [35]. Moreover, there is a correlation between the shifts of the activation curve induced by the mutations and their effect on the DPO-1 blocking potency. This correlation was reported for residues L499, L506, L510, and V514, but not for the pore-facing residues T480 and I508. Authors of this study [35] propose that mutations of T480 and I508 decrease the DPO-1 blocking potency by altering the ligand binding site, while other mutations affect the DPO-1 potency by allosteric mechanisms.

A bulky branched DPO-1 molecule contains a highly polarized P = O bond whose oxygen atom would attract a metal ion. Another bulky cationophilic ligand, correolide, is proposed to bind in the inner pore and directly interact by its polar groups with a potassium ion at site s4 between four threonine residues in the TVGYG motifs [20]. The Kv1.3 channel block by correolide is also affected by mutations of some residues that do not line the inner pore [36] and therefore cannot directly interact with the big, bulky ligand. Here we have used a concept of Kv1.3 channel block by correolide to elaborate a model of Kv1.5 block by DPO-1. We have populated the selectivity filter with potassium ions at sites s2 and s4 and imposed an initial distance constraint to maintain proximity between a potassium ion at site s4 and the polar oxygen of DPO-1. The obtained energetically most preferable binding mode is shown in Fig. 4A and B, the ensemble of low-energy structures in Fig. S4, and its energy characteristics are given in Table S4. The central cavity readily accommodated the bulky DPO-1 molecule. The phosphorus atom, which according to MOPAC calculations bears a big positive charge, occurred in the cation-attractive region at the focus of P-helices, while the phosphorous-bound negatively charged oxygen atom retained a close contact with a potassium ion at site s4. This strong attraction counterbalanced repulsion of the DPO-1 oxygen from cationophilic C-ends of the pore helices.

3.6. Models of Kv1.5 with AVE0118 and S0100176

These compounds represent a large group of flexible, uncharged Kv1.5 ligands [37]. Interestingly, patterns of ligand-sensing residues for charged (flecainide and vernakalant) and uncharged (AVE0118 and S0100176) Kv1.5 ligands are similar (Table 1). This is an intriguing problem because the cationophilic inner pore should be attractive for cationic, but not cationophilic ligands. To address this problem, we employed the idea that cationophilic groups of ligands may directly interact with permeant cations and thus form cationic blocking particles [32]. Strutz-Seebohm and coauthors docked uncharged ligands in a homology model of Kv1.5 and predicted hydrophobic interactions of the blockers with pore-lining hydrophobic residues and electrostatic interactions of oxygen atoms of the blockers with a potassium ion at site s4 [7]. This binding mode resembles a model of correolide in Kv1.3 [20] and the binding mode of DPO-1 proposed in the present work.

Since I502 contributes to the binding of AVE0118 (Table 1), we suggested that a part of the ligand binds in the subunit interface like certain moieties of flecainide, vernakalant and ICAGEN-4. We imposed distance constraints to bias interactions of both CO groups of AVE0118 with a potassium ion. The latter occurred at site s5 in the middle of the central cavity and established additional π-cation contacts with the AVE0118 molecule.

In the lowest-energy model (Fig. 4C and D) the hydrophobic methoxyphenyl ring of AVE0118 fitted in the hydrophobic subunit interface and interacted with I502, while bulky pyridine ring remained in the central cavity. The central part of AVE0118 with two aromatic rings bound at the P-loop turns and interacted with residues T479 and T480. The pyridine end of AVE0118 interacted with the pore-facing residues V505, I508, A509, V512 and V516. Two polar carbonyl groups interacted with a potassium ion in the middle of the inner pore, thus forming a cationic blocking particle. The ensemble of low-energy structures is shown in Fig. S5 and its characteristics are given in Table S5.

AVE0118 and S0100176 are uncharged molecules with similar patterns of ligand-sensing residues, although some mutations have quantitatively different effects on the action of these blockers. Importantly, while AVE0118 blocks the channel by the foot-in-the-door mechanism, S0100176 does not prevent the channel closure [5], suggesting a possibility of the trapping block. Our model of AVE0118 binding readily explains the foot-in-the-door mechanism: the large pyridine ring occurred at the level of V516 where it would prevent the activation gate closure. We further docked S0100176 into the Kv1.5 model with a potassium ion (Fig. 4E, F, Fig. S6 and Table S6) using the same methodology that was used to dock AVE0118. Some features of the S0100176 and AVE0118 binding modes are similar. Two polar groups and the aromatic ring of S0100176 coordinated a potassium ion at site s5, near the focus of P-helices. The NH group bound to the backbone carbonyl at the C-end of a P-helix. The hydrophobic toluene moiety avoided the pore and approached I502 in the subunit interface. S0100176 also interacted with V505, I508, and A509. Unlike AVE0118, S0100176 readily adopted a "horizontal" orientation in the channel (Fig. 4E, F) and bound above the activation-gate region where it would not prevent the activation gate closure in agreement with experimental data [5]. It should be noted that S0100176 only weakly interacted with I512 and V516. The toluene moiety of S0100176, which is shorter than methoxyphenyl moiety of AVE0118, did not penetrate deep into subunit interface and only weakly interacted with I502. This may explain much larger sensitivity of AVE0118 to the I502 mutation as compared with S0100176 [5,6]. Our models also explain why two long flexible ligands of similar size demonstrate different mechanisms of block. A terminal moiety at

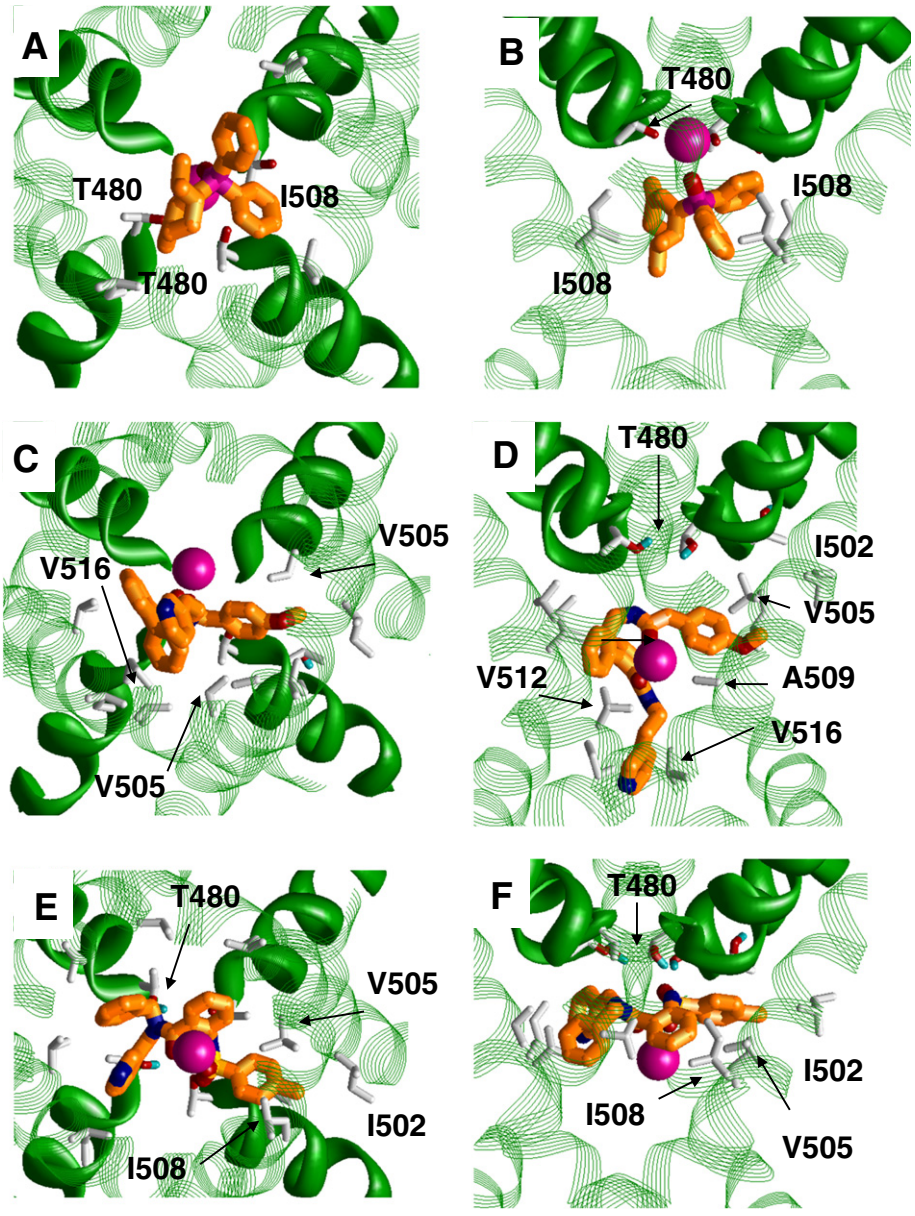

Fig. 4. Proposed binding modes of DPO-1 (*A* and *B*), AVE0118 (*C* and *D*) and S0100176 (*E* and *F*) in the Kv1.5 channel. DPO-1 is stabilized by the attraction of oxygen and phosphorus atoms, respectively, to a potassium ion at site s4 and to the cation-attractive site s5. The DPO-1 molecule interacts directly with T480 in the pore helix and with I505 in the S6 segment. Other residues whose mutation affect DPO-1 action, do not face the pore. AVE0118 and S0100176 provide an aromatic ring and two polar groups to coordinate a potassium ion in the cation-attractive region and thus form "cationic blocking particles". The NH groups approach the backbone oxygens at the C-ends of P-helices. Hydrophobic moieties protrude into subunit interfaces and approach the I502 residue.

one end of a ligand partially penetrates into a subunit interface and several groups in the middle chelate a potassium ion at site s5. This significantly restricts possible orientations of the opposite end of the ligand and its particular orientation becomes dependent on fine details of the ligand structure. In AVE0118, the opposite end extends vertically along the pore, towards the activations gate, whereas in S0100176 it is oriented horizontally (see Fig. 4D and F). Thus, our models are consistent with published data on the action of AVE0118 and S0100176.

4. Discussion

In this study we proposed binding modes for several Kv1.5 channel blockers, which belong to different structural classes. To obtain these models, we applied a homology modeling approach. Some features of previously proposed models have been used to generate starting points for our docking experiments. The aim of these calculations was to explore whether or not the obtained models can rationalize experimental data on the action of diverse Kv1.5 ligands.

4.1. Limitations of modeling

We avoid discussion of structure–activity relationships of Kv1.5 ligands due to limited precision of the homology models. We used a rather simple force field, Coulomb's electrostatics, fixed atomic charges and implicit solvent. The entropy component was not taken into account. As a result, the precision of energy calculations is limited. This approach can be categorized as "coarse grain" one; it can reveal major steric, hydrophobic and electrostatic determinants of drug binding, but does not provide the free energy of binding and thus, the drug affinity. From the other side, simple and fast energy sampling allowed very intensive explorations of the conformational space. Up to 100,000 energy minimizations were performed in each MCM trajectory. Employment of

more comprehensive force fields is not necessary for limited-precision homology models.

We docked ligands only to the open-state Kv1.5 model. Obviously, changes of the channel geometry during activation and inactivation can result in the different binding affinity. Fig. S7 shows three residues, which face the pore and form the entrance to the subunit interface in the closed, open, and open-inactivated potassium channels. Homologues of these residues, T479, V505 and I508, are key ligand-sensing residues in Kv1.5. The state-dependent changes in the mutual disposition of these residues and dimensions of the subunit interface are modest. Such changes are unlikely to explain use-dependency of action. However, big changes in cytoplasmic halves of S6s upon activation gating allow to explain why some ligands prevent channel closure, while others do not. In our models ICAGEN-4 and AVE0118 interact with residues at the cytoplasmic parts of S6, which converge in the closed state. Such ligands should prevent the channel closure. In contrast, other docked ligands interact mainly with the upper half of the inner pore, which does not undergo big rearrangements upon the channel activation gating.

We used constraints to impose certain features of ligand binding modes, particularly penetration of hydrophobic moieties in subunit interfaces and interactions of neutral ligands with potassium ions. Several previously published models of ligand binding in Kv1.5 consider alternative possibilities, in particular binding of the entire ligand molecule in the inner pore and the channel block by electroneutral ligands without involvement of permeating metal ions [3,6,7,9–11]. In the present study we did not attempt to analyze such binding modes: they are already well explored and additional calculations are unlikely to provide novel results. Instead, we have focused on possible binding modes, which were not previously considered.

4.2. Common features of the proposed models

It should be emphasized that we have considered here a very diverse set of ligands. Nevertheless, the proposed ligand-channel models have important common features, which are illustrated in Fig. 5. A long flexible ligand readily adopts an angular conformation. A hydrophobic end protrudes into the hydrophobic subunit interface, while the opposite end binds in the pore and provides the channel block. The central part of both flexible and bulky ligands can contain a positive charge (the ammonium group or a phosphorus atom) or/and coordinate a permeant ion in the cation-attractive region. Combinations of these rather simple features create a large variety of blocking structures with different characteristics (Fig. 5).

Except for permanently charged quaternary compounds like tetraalkylammonium blockers, other cationic blocking particles

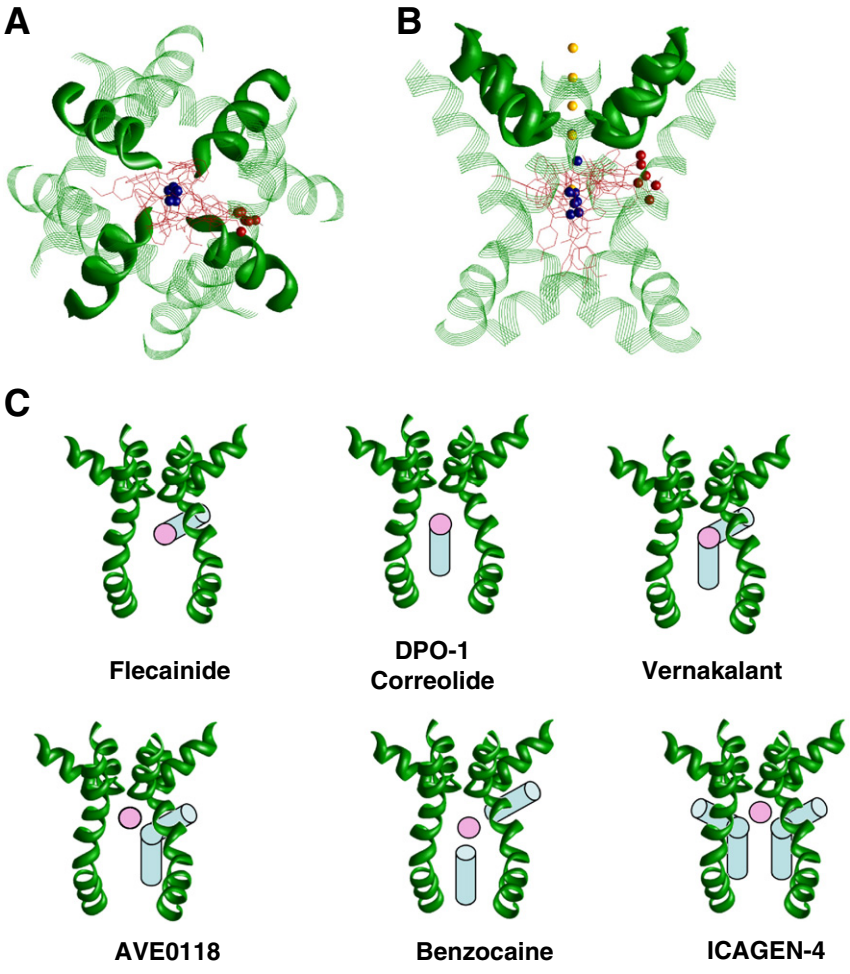

Fig. 5. Common and distinguishing features of the inner-pore block in P-loop channels. *A* and *B*, Cytoplasmic and side views at the superimposed binding modes of all the ligands, which have been docked into the Kv1.5 channel in the present work. Potassium ions in sites s1–s5 are shown at *B* as yellow dots. There are two important common features of the binding modes. First, a cationic moiety of a blocking particle (blue dots), which is either a ligand-bound potassium ion, or a protonated nitrogen, or a phosphorus atom in DPO-1, is located in the cation-attractive central cavity of the inner pore. Second, hydrophobic moieties bind in the hydrophobic subunit interfaces. The heavy atoms of the ligands, which are most distant from the pore axis, are shown as red dots. *C*, The proposed schemes of binding of charged and neutral ligands. The cation-attractive site in the inner pore is occupied by the cation (a pink circle), which can by either a charged group of the ligand or a chelated metal ion. The uncharged moieties of the ligands can either extend along the inner pore or protrude into the subunit interfaces.

are formed due to attachment of either a proton or a metal ion to a ligand. Lining of the inner pore by predominantly hydrophobic residues and local electric field in the focus of P-helices favor such attachment of the positive charge. Parameters of ligand protonation or association with a metal ion, which are obtained in bulk solvent, are hardly applicable for this specific environment of the inner pore. Therefore, relative probabilities of a ligand association with a proton or a metal ion are unknown. In particular, we cannot rule out that some ligands, which are considered neutral, may block the channel in the protonated form. Considering this alternative could affect some structural details of our models, but not their general features.

4.3. Experimental data in view of the models

Various experimental observations, including results of mutagenesis have been rationalized in previously elaborated models [3,6,7,9–11]. Our models are also consistent with these experimental data because, as in the previous models, the bulky parts of the ligand molecules fit the inner pore and interact with the pore-facing residues. Below we discuss only those observations for which our models suggest novel explanations.

4.3.1. I502

Our models suggest direct interaction of ligands with I502, which is accessible from the pore through the subunit interface. Substitutions of I502 decrease potency of flecainide, vernakalant, AVE0118 and S0100176 (the effect on S0100176 is rather small). Sensitivity of the ICAGEN-4 action to this mutation is unknown. Among the considered ligands, only DPO-1 is completely insensitive to mutations of I502 (Table 1). In our model DPO-1 lacks long arms that could enter the subunit interface. This agrees with our proposition that mutations of I502 affect ligand action directly rather than allosterically.

A recent study provides strong evidences that Psora-4 can bind not only in the central pore of Kv1.5, but also in side pockets formed by S4, L45, and backsides of S5 and S6 [11]. The authors suggest that I502 is important for the ligand transition between these sites. We cannot rule out a possibility that additional binding sites exist for other ligands. However, it seems unlikely that all so diverse ligands have high-affinity binding sites and mechanisms of Kv1.5 block similar to those of Psora-4. Noteworthy, PAP-1 [38], a structural analog of Psora-4, has a long hydrophobic tail, which according to model [8] can reach the Kv1.3 analog of I502 in the subunit interface.

4.3.2. Hill coefficient

Cooperative binding of some ligands in Kv1.5 channel, which is manifested as the Hill coefficient >1, calls for structural explanations. One possibility is allosteric mechanism by which binding of the first ligand induces protein changes that facilitate binding of the second ligands (see, e.g., Ref. [11]). In this mechanism the ligand molecules do not interact directly and may bind to sites, which are far from each other. In contrast, our models suggest that the joint chelation of a potassium ion at site s5 is the structural basis for the cooperative ligand binding in the inner pore. This hypothesis can explain cooperative binding in the pore of electroneutral ligands, which have very different chemical structures.

4.3.3. Voltage dependence of ligand action

The Kv1.5-blocking potency of cationic ligands vernakalant and flecainide increases with increase of the positive membrane potential [3]. Electroneutral ligands are not expected to sense the membrane field [39]. However, the inner-pore targeting electroneutral ligands such as benzocaine [4], SNDTT [31], and ICAGEN-4 [7], do demonstrate some voltage-dependency of their action on Kv1 channels. Intriguingly, the channel-blocking potency of some electroneutral ligands does not increase, but decreases with increase of the positive membrane voltages. To explain this we suggest that one or more molecules of a neutral ligand coordinate a potassium ion to form a cationic blocking particle. The electric field breaks such a complex and thus reduces activity. Supplementary Fig. S8 shows prediction of the two-barrier model of the voltage-dependent block [14].

4.3.4. State-dependence of ligand action

Despite we did not model ligand binding in different channel states, a hypothesis on the mechanism of the state-dependent ligand action can be suggested in view of our results. A common feature of our models is the channel block by a cationic particle, which can be either an organic cation or a complex of electroneutral ligand(s) with a permeant metal ion. This common feature suggests a universal role of electrostatic interactions in the inner pore block. Significance of electrostatic repulsion between ions in the selectivity filter and in the inner pore is known [40]. In the open channel this repulsion should be weaker than in the closed channel due to the screening effect of water molecules and the larger room, which allows the blocking particle to adjust and minimize the repulsion. The relation between the channel block and slow inactivation can also have an electrostatic nature: coupling between the ion occupancy of the selectivity filter and slow inactions is known [41]. The electrostatic mechanism is consistent with state-dependent affinity of organic cations. This mechanism can be considered as a general explanation for the state-dependent ligand affinity if electroneutral ligands block the channel not per se, but in a complex with the permeant metal ion.

5. Concluding remarks

Ion channels are among major targets for pharmacological agents. Elaboration of new drugs remains an important and challenging problem. Effective search of new promising structures should be based on understanding of common and distinguishing features of the channel block by different classes of ligands. In this work we demonstrate that molecular models, which are based on rather simple concepts about the mechanisms of channel block, help to rationalize the action of diverse Kv1.5 ligands. We believe that the proposed concepts of the inner pore block may help analyze action of other drugs targeting potassium channels as well as other P-loop channels.

Author contribution

D.B.T. and B.S.Z. contributed equally to the study and should be considered co-senior authors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2013.11.019.

References

[1] H. Wulff, B.S. Zhorov, K+ channel modulators for the treatment of neurological disorders and autoimmune diseases, Chem. Rev. 108 (2008) 1744–1773.
[2] L. Franqueza, M. Longobardo, J. Vicente, E. Delpon, M.M. Tamkun, J. Tamargo, D.J. Snyders, C. Valenzuela, Molecular determinants of stereoselective bupivacaine block of hKv1.5 channels, Circ. Res. 81 (1997) 1053–1064.
[3] J. Eldstrom, Z. Wang, H. Xu, M. Pourrier, A. Ezrin, K. Gibson, D. Fedida, The molecular basis of high-affinity binding of the antiarrhythmic compound vernakalant (RSD1235) to Kv1.5 channels, Mol. Pharmacol. 72 (2007) 1522–1534.
[4] R. Caballero, I. Moreno, T. Gonzalez, C. Valenzuela, J. Tamargo, E. Delpon, Putative binding sites for benzocaine on a human cardiac cloned channel (Kv1.5), Cardiovasc. Res. 56 (2002) 104–117.
[5] N. Decher, P. Kumar, T. Gonzalez, B. Pirard, M.C. Sanguinetti, Binding site of a novel Kv1.5 blocker: a "foot in the door" against atrial fibrillation, Mol. Pharmacol. 70 (2006) 1204–1211.
[6] N. Decher, B. Pirard, F. Bundis, S. Peukert, K.H. Baringhaus, A.E. Busch, K. Steinmeyer, M.C. Sanguinetti, Molecular basis for Kv1.5 channel block: conservation of drug binding sites among voltage-gated K+ channels, J. Biol. Chem. 279 (2004) 394–400.
[7] N. Strutz-Seebohm, I. Gutcher, N. Decher, K. Steinmeyer, F. Lang, G. Seebohm, Comparison of potent Kv1.5 potassium channel inhibitors reveals the molecular basis for blocking kinetics and binding mode, Cell. Physiol. Biochem. 20 (2007) 791–800.
[8] P.I. Zimin, B. Garic, S.B. Bodendiek, C. Mahieux, H. Wulff, B.S. Zhorov, Potassium channel block by a tripartite complex of two cationophilic ligands and a potassium ion, Mol. Pharmacol. 78 (2010) 588–599.
[9] M. Ander, V.B. Luzhkov, J. Aqvist, Ligand binding to the voltage-gated Kv1.5 potassium channel in the open state—docking and computer simulations of a homology model, Biophys. J. 94 (2008) 820–831.
[10] J. Eldstrom, D. Fedida, Modeling of high-affinity binding of the novel atrial anti-arrhythmic agent, vernakalant, to Kv1.5 channels, J. Mol. Graph. Model. 28 (2009) 226–235.
[11] S. Marzian, P.J. Stansfeld, M. Rapedius, S. Rinne, E. Nematian-Ardestani, J.L. Abbruzzese, K. Steinmeyer, M.S. Sansom, M.C. Sanguinetti, T. Baukrowitz, N. Decher, Side pockets provide the basis for a new mechanism of Kv channel-specific inhibition, Nat. Chem. Biol. 9 (2013) 507–513.
[12] W.A. Schmalhofer, R.S. Slaughter, M. Matyskiela, J.P. Felix, Y.S. Tang, K. Rupprecht, G.J. Kaczorowski, M.L. Garcia, Di-substituted cyclohexyl derivatives bind to two identical sites with positive cooperativity on the voltage-gated potassium channel, K(v)1.3, Biochemistry 42 (2003) 4733–4743.
[13] V. Salvador-Recatala, Y. Kim, E. Zaks-Makhina, E.S. Levitan, Voltage-gated k+ channel block by catechol derivatives: defining nonselective and selective pharmacophores, J. Pharmacol. Exp. Ther. 319 (2006) 758–764.
[14] A.M. Woodhull, Ionic blockage of sodium channels in nerve, J. Gen. Physiol. 61 (1973) 687–708.
[15] Y. Du, D.P. Garden, L. Wang, B.S. Zhorov, K. Dong, Identification of new batrachotoxin-sensing residues in segment IIIS6 of the sodium channel, J. Biol. Chem. 286 (2011) 13151–13160.
[16] M. Madeja, W. Steffen, I. Mesic, B. Garic, B.S. Zhorov, Overlapping binding sites of structurally different antiarrhythmics flecainide and propafenone in the subunit interface of potassium channel Kv2.1, J. Biol. Chem. 285 (2010) 33898–33905.
[17] C. Lerche, I. Bruhova, H. Lerche, K. Steinmeyer, A.D. Wei, N. Strutz-Seebohm, F. Lang, A.E. Busch, B.S. Zhorov, G. Seebohm, Chromanol 293B binding in KCNQ1 (Kv7.1) channels involves electrostatic interactions with a potassium ion in the selectivity filter, Mol. Pharmacol. 71 (2007) 1503–1511.
[18] S.Y. Wang, J. Mitchell, D.B. Tikhonov, B.S. Zhorov, G.K. Wang, How batrachotoxin modifies the sodium channel permeation pathway: computer modeling and site-directed mutagenesis, Mol. Pharmacol. 69 (2006) 788–795.
[19] A. Rossokhin, T. Dreker, S. Grissmer, B.S. Zhorov, Why does the inner-helix mutation A413C double the stoichiometry of Kv1.3 channel block by emopamil but not by verapamil? Mol. Pharmacol. 79 (2011) 681–691.
[20] I. Bruhova, B.S. Zhorov, Monte Carlo-energy minimization of correolide in the Kv1.3 channel: possible role of potassium ion in ligand-receptor interactions, BMC Struct. Biol. 7 (2007) 5.
[21] D.P. Garden, B.S. Zhorov, Docking flexible ligands in proteins with a solvent exposure- and distance-dependent dielectric function, J. Comput. Aided Mol. Des. 24 (2010) 91–105.
[22] Z. Li, H.A. Scheraga, Monte Carlo-minimization approach to the multiple-minima problem in protein folding, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 6611–6615.
[23] S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Singh, C. Chio, G. Alagona, S. Profeta, P.K. Weiner, A new force field for molecular mechanical simulation of nucleic acids and proteins, J. Am. Chem. Soc. 106 (1984) 765–784.
[24] S.J. Weiner, P.A. Kollman, D.T. Nguyen, D.A. Case, An all atom force-field for simulations of proteins and nucleic-acids, J. Comput. Chem. 7 (1986) 230–252.
[25] T. Lazaridis, M. Karplus, Effective energy function for proteins in solution, Proteins 35 (1999) 133–152.
[26] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, AM1: a new general purpose quantum mechanical model, J. Am. Chem. Soc. 107 (1985) 3902–3909.
[27] S.B. Long, E.B. Campbell, R. Mackinnon, Crystal structure of a mammalian voltage-dependent Shaker family K+ channel, Science 309 (2005) 897–903.
[28] S. Yamaguchi, B.S. Zhorov, K. Yoshioka, T. Nagao, H. Ichijo, S. Adachi-Akahane, Key roles of Phe1112 and Ser1115 in the pore-forming IIIS5-S6 linker of L-type Ca2+ channel alpha1C subunit (CaV 1.2) in binding of dihydropyridines and action of Ca2+ channel agonists, Mol. Pharmacol. 64 (2003) 235–248.
[29] D.B. Tikhonov, I. Bruhova, B.S. Zhorov, Atomic determinants of state-dependent block of sodium channels by charged local anesthetics and benzocaine, FEBS Lett. 580 (2006) 6027–6032.
[30] J. Payandeh, T. Scheuer, N. Zheng, W.A. Catterall, The crystal structure of a voltage-gated sodium channel, Nature 475 (2011) 353–358.
[31] M.W. Brock, C. Mathes, W.F. Gilly, Selective open-channel block of Shaker (Kv1) potassium channels by s-nitrosodithiothreitol (SNDTT), J. Gen. Physiol. 118 (2001) 113–134.
[32] B.S. Zhorov, D.B. Tikhonov, Ligand action on sodium, potassium, and calcium channels: role of permeant ions, Trends Pharmacol. Sci. 34 (2013) 154–161.
[33] A. Lagrutta, J. Wang, B. Fermini, J.J. Salata, Novel, potent inhibitors of human Kv1.5 K+ channels and ultrarapidly activating delayed rectifier potassium current, J. Pharmacol. Exp. Ther. 317 (2006) 1054–1063.
[34] H. Wulff, M.J. Miller, W. Hansel, S. Grissmer, M.D. Cahalan, K.G. Chandy, Design of a potent and selective inhibitor of the intermediate-conductance Ca2+-activated K+ channel, IKCa1: a potential immunosuppressant, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8151–8156.
[35] Y.M. Du, X.X. Zhang, D.N. Tu, N. Zhao, Y.J. Liu, H. Xiao, M.C. Sanguinetti, A. Zou, Y.H. Liao, Molecular determinants of Kv1.5 channel block by diphenyl phosphine oxide-1, J. Mol. Cell. Cardiol. 48 (2010) 1111–1120.
[36] M. Hanner, B. Green, Y.D. Gao, W.A. Schmalhofer, M. Matyskiela, D.J. Durand, J.P. Felix, A.R. Linde, C. Bordallo, G.J. Kaczorowski, M. Kohler, M.L. Garcia, Binding of correolide to the K(v)1.3 potassium channel: characterization of the binding domain by site-directed mutagenesis, Biochemistry 40 (2001) 11687–11697.
[37] H. Wulff, N.A. Castle, L.A. Pardo, Voltage-gated potassium channels as therapeutic targets, Nat. Rev. Drug Discov. 8 (2009) 982–1001.
[38] A. Schmitz, A. Sankaranarayanan, P. Azam, K. Schmidt-Lassen, D. Homerick, W. Hansel, H. Wulff, Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune diseases, Mol. Pharmacol. 68 (2005) 1254–1270.
[39] W.A. Schmalhofer, J. Bao, O.B. McManus, B. Green, M. Matyskiela, D. Wunderler, R.M. Bugianesi, J.P. Felix, M. Hanner, A.R. Linde-Arias, C.G. Ponte, L. Velasco, G. Koo, M.J. Staruch, S. Miao, W.H. Parsons, K. Rupprecht, R.S. Slaughter, G.J. Kaczorowski, M.L. Garcia, Identification of a new class of inhibitors of the voltage-gated potassium channel, Kv1.3, with immunosuppressant properties, Biochemistry 41 (2002) 7781–7794.
[40] J.D. Faraldo-Gomez, E. Kutluay, V. Jogini, Y. Zhao, L. Heginbotham, B. Roux, Mechanism of intracellular block of the KcsA K+ channel by tetrabutylammonium: insights from X-ray crystallography, electrophysiology and replica-exchange molecular dynamics simulations, J. Mol. Biol. 365 (2007) 649–662.
[41] L.G. Cuello, V. Jogini, D.M. Cortes, E. Perozo, Structural mechanism of C-type inactivation in K(+) channels, Nature 466 (2010) 203–208.