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Molecular Basis of Cardiac Delayed Rectifier K^+ Channel Function and Pharmacology

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Synopsis

Human cardiomyocytes express three distinct types of delayed rectifier potassium channels. hERG1 channels conduct the rapidly activating current I_{Kr}, KCNQ1/KCNE1 channels conduct the slowly activating current I_{Ks}, and Kv1.5 channels conduct an ultrarapid activating current I_{Kur}. Here we provide a general overview of the mechanistic and structural basis of ion selectivity, gating and pharmacology of the three types of cardiac delayed rectifier K^+ channels. Most blockers bind to S6 residues that line the central cavity of the channel, whereas activators interact with the channel at four symmetrical binding sites outside the cavity.

Keywords

gating; hERG; KCNA5; KCNE1; KCNQ1; pharmacology; potassium channel

Introduction

In cardiomyocytes, multiple types of outward delayed rectifier K^+ current (I_K) mediate the late repolarization phase of action potentials [1]. In human cardiomyocytes, three distinct types of I_K are recognized. A rapidly activating current (I_{Kr}) is conducted by channels formed by coassembly of human *ether-a-go-go*-related gene, hERG1 (Kv11.1; gene: *KCNH2*) subunits, both full-length hERG1a α-subunits [2,3] and alternatively spliced hERG1b α-subunits [4]. A slowly activating current (I_{Ks}) is conducted by channels formed by coassembly of KCNQ1 (Kv7.1; gene: *KCNQ1*) α-subunits and auxiliary KCNE1 β-

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subunits [5,6]. In human atrial, but not ventricular, myocytes an ultrarapid activating current (I_{Kur}) is also present and conducted by channels formed by Kv1.5 (gene: *KCN5A*) α-subunits [7,8]. Identification of the molecular basis of I_{Kur}, I_{Kr} and I_{Ks} combined with patch clamp studies of heterologously expressed channels spawned copious biophysical studies designed to probe the molecular mechanisms of channel gating and enabled the screening of small molecule libraries to discover novel compounds that either inhibit or activate these channels. Detailed descriptions of the molecular basis of Kv1.5, hERG1 and KCNQ1 channel gating are reviewed elsewhere [9–12]. Here we provide a general overview of the structural basis of channel gating and feature recent findings regarding modulation of cardiac delayed rectifier K^+ channel by low molecular weight compounds and peptides.

Structural basis of voltage-dependent potassium channel gating

Insights into the molecular mechanisms of K^+ channel gating have been provided by early biophysical studies of *Drosophila* Shaker and other voltage-gated K^+ (Kv) channels combined with structural biology studies of several bacterial channels (KcsA, MthK, KvAP) and vertebrate Kv1.2 channels. Similar to other Kv channel subunits, hERG1, KCNQ1 and Kv1.5 subunits each contain 6 transmembrane α-helical segments (S1–S6) and functional channels are formed by coassembly of four identical or highly similar α-subunits into a tetrameric complex (Fig. 1A). In each subunit, segments S1–S4 form a voltage sensing domain (VSD) and segments S5 and S6 contribute to the central pore-forming domain. The S6 segments line the central cavity of the channel and the cytoplasmic ends of each segment criss-cross one another (the “S6 bundle crossing”) to form a narrow aperture (the activation gate) when the channel is in a closed state. The S4 segment contains multiple basic (positively charged) amino acids and thus serves as the primary voltage sensing structure. In response to membrane depolarization, the S4 segments move in an outward direction and the attached cytoplasmic S4–S5 linker acts as an electromechanical coupler to link the S4 movement to opening of the activation gate (i.e., an outward splaying of the S6 bundle crossing opening) [13]. When the activation gate is in an open configuration, hydrated K^+ ions within the cytoplasm diffuse into the central cavity in response to the outwardly directed electrochemical driving force. As K^+ ions enter the selectivity filter (Fig. 1B), they are stripped of their surrounding water molecules and move stepwise from one high affinity binding site to another until they reach the extracellular vestibule where they are again rehydrated. The selectivity filter of K^+-selective channels is a narrow lumen that is lined by five residues (TVGYG) contributed by each of the four subunits. The hydroxyl group from the Thr residue and the backbone carbonyl oxygen atoms of four following amino acids together form an oxygen network that coordinate dehydrated K^+ ions in a manner that closely resembles the oxygen atoms from 8 water molecules that form the hydration shell surrounding the ion in solution [14].

Activation gating

The amino acid sequence of the Kv1.5 channel is highly homologous to Kv1.2 and so it is reasonable to assume the structural basis of gating described for Kv1.2 also apply to cardiac Kv1.5 channels. Some of the details differ for hERG1 and KCNQ1/KCNE1 channels. Recently it was reported that physical continuity between the S4 segment and pore domain is

not required for normal voltage dependence of activation (and inactivation) gating in hERG1 channels. Channels could still gate relatively normally when subunits were split into two pieces at the S4–S5 linker, a finding inconsistent with the simple idea of electromechanical coupling [15]. For hERG1 channels, specific interactions between Asp540 in the S4–S5 linker and Leu666 in the S6 segment are a key component of activation gating [16,17]. Presumably these interactions can still occur in a split channel. In the heart, the biophysical properties of KCNQ1 channels are modified by KCNE1 β-subunits. Channels formed by coassembly of KCNE1 subunits (2 or more per channel) to KCNQ1 homotetramers exhibit an increased single channel conductance, open at more positive potentials and have a slower rate of activation. Voltage sensor movement associated with KCNQ1 channel activation is divided into two steps with distinct voltage dependences and kinetics [18], corresponding to an intermediate-open or a high permeation activated-open state [19]. KCNE1 subunits inhibit the intermediate-open state and facilitate the activated-open states by altering the interactions between the VSDs and the pore domain [19]. The binding of intracellular phosphatidylinositol 4,5-bisphosphate (PIP2) or ATP promote coupling between voltage sensing at S4 segments and the pore domain in KCNQ1/KCNE1 channels [20,21].

Inactivation gating

Kv1.5 channels exhibit a very slow and time-independent C-type inactivation at depolarized potentials, a process that involves cooperative subunit interactions in related Kv1 channels [22,23]. Auxiliary Kvβ1.2 and Kvβ1.3 subunits interact with the C-terminal domain of Kv1.5 [24] to induce rapid inactivation [25]. It is unclear to what extent Kvβ subunits alter Kv1.5 channel gating in human atrial myocytes. KCNQ1 channels inactivate slightly at positive potentials, but KCNQ1/KCNE1 channels do not inactivate [26]. In hERG1, C-type inactivation is extremely fast and voltage-dependent [27,28] and occurs in sequential steps that culminate in a subtle change in the conformation of the selectivity filter [29]. We employed concatenated tetramers to demonstrate that the extent of subunit cooperativity was dependent on the location of the mutations employed to probe inactivation and that the final step in the gating process was mediated by a concerted, all-or-none cooperative subunit interaction [30].

Deactivation

Return of the transmembrane potential to a negative level (as occurs during repolarization of an action potential) induces channels transition from an open to a closed state, a process called deactivation. The slow rate of deactivation of hERG1 channels is dependent on multiple interactions between the cytoplasmic N-terminus of one subunit with the cytoplasmic C-terminus of an adjacent subunit [31–33]. Slow deactivation is a fully cooperative process (i.e., all-or-none), requiring the N-C interaction between all four subunits. Disruption of a single N-C interaction is sufficient to greatly accelerate deactivation [34]. This has physiological significance as hERG1 channels can be formed by coassembly of hERG1a subunits that contain a full-length N-terminus and hERG1b subunits that have a truncated N-terminus [4,35] that cannot interact with the C-terminus to slow deactivation.

Molecular pharmacology of cardiac delayed rectifier K+ channels

Inhibitors

The majority of Kv1.5 inhibitors are pore blockers. Several potent open channel blockers of Kv1.5, including AVE0118, S0100176, vernakalant, DPO-1, MK-0448, anandamide and acacetin, were developed to selectively block I_{Kur} in atrium as a potential therapy for atrial fibrillation (AF) [36–42]. These Kv1.5 blockers share an overlapping binding site, including 5 critical residues that face towards the lumen of the central cavity: Thr-479, Thr-480, Val-505, Ile-508, and Val-512 [39,43–46]. More recently, Marzian et al. identified Psora-4 as a unique Kv1.5 inhibitor that is both a pore blocker and an allosteric gating modifier [47]. Besides direct pore blockage, Psora-4 also binds to four symmetrical side pockets distant from the central cavity (Fig. 2A). The amino acids that form the pore of Kv1 channels are highly conserved, challenging efforts to develop specific Kv1.5 blockers. Allosteric gating inhibitors such as Psora-4 offer an alternative approach to the design of channel-specific drugs.

The primary motivation driving the discovery of Kv1.5 blockers has been its predicted usefulness for pharmacological cardioversion of recent onset AF or prevention of AF episodes by a mechanism that would not affect the electrophysiological properties of the ventricles. Specifically, because Kv1.5 is highly expressed in human atria but not in the ventricles, block of these channels would be expected to specifically prolong the APD of atrial myocytes (and atrial effective refractory period, AERP) without risk of inducing QT prolongation. The clinical experience to date has not been encouraging for this mechanism. Although MK-0448 was shown in preclinical studies to prolong AERP without effects on the ventricle, the compound was without effect on AERP in healthy human subjects. This finding was predicted by previous mathematical modeling of human atrial action potentials. Simulated inhibition of I_{Kur} was shown to increase plateau height, leading to additional activation of I_{Kr} and no net change in APD [48]. However, as noted by Ravens et al [49] MK-0448 was evaluated by electrophysiological testing over a frequency range lower than what is typically observed during AF. It remains to be demonstrated that Kv1.5 channel inhibition will provide protection against AF in relevant patient populations.

hERG1 channels are blocked by a wide spectrum of compounds, including quinidine, d-sotalol and many other “class III” antiarrhythmic agents such as the highly potent methanesulfonanilides dofetilide and ibutilide. All of these compounds block hERG1 channels by preferentially plugging the central cavity of the channel (Fig. 2B, C). In addition to these antiarrhythmic drugs, several other commonly used medications with diverse chemical structures (e.g., cisapride, terfenadine, astemizole, mexifloxacin, and tamoxifen) were shown to cause high-affinity block on hERG1/I_{Kr} channels and hence, prolong the QT interval [50]. Many hERG1 blockers have an unacceptable risk of inducing arrhythmia. Unintended drug-induced QT prolongation with the potential to cause acquired long QT Syndrome (LQTS) increases the risk of ventricular tachyarrhythmia (most commonly, torsade de pointes) in patients and hence has prompted the withdrawal or restriction of these marketed drugs. The structural basis for high-affinity binding of hERG1/I_{Kr} channel blockers was revealed using a site-directed mutagenesis approach. Two aromatic residues

(i.e., Y652 and F656) located in all four S6 segments that line the central cavity of the channel are the most critical determinants for drug interaction [51,52]. Inactivation-deficient hERG1 mutant channels (e.g., S620T, S631A, G628C/S631C) are much less sensitive to block by these compounds [53–56], so it was commonly assumed that drugs can only bind with high affinity to the inactivated state of the channel. However, a detailed analysis of concatenated hERG1 tetramers containing a variable number of S620T, G628C/S631C or S631A mutation subunits indicate instead that these mutant subunits allosterically interrupt drug binding in a manner that is independent of their ability to disrupt inactivation gating [57]. Peptide toxins isolated from scorpion venoms, such as ErgTx1 and BeKm-1, bind to the external region of the pore to block channels with nanomolar potency [58,59]. Other peptide toxins act as allosteric modifiers of activation gating, including APETx1 from sea anemone and several tarantula toxins. These peptides directly interact with the VSD of hERG1 and reduce current magnitude by shifting the voltage dependence of activation to more positive potentials [60,61].

Chromanol 293B is a relatively selective I_{Ks} blocker which was initially developed as a class III antiarrhythmic drug to prolong action potential duration [62,63]. The benzodiazepine L-7 is a more potent blocker that binds to specific residues in the S6 segments of KCNQ1 channels [64,65]. Other compounds such as indapamide, propofol, and thiopentone exert lower affinity block of KCNQ1 channels [66,67]. KCNE1 subunits displayed allosteric action to facilitate the block of chromanol 293B to KCNQ1 channels with a 6–100 fold increased binding affinity [68].

The amino acids in the S6 segments that line the central cavity of K^+ channels are often highly conserved amongst members of closely related channels (e.g., Kv1.1, 1.2, 1.3, 1.4 and 1.5). This has been a major impediment to the discovery of pore blockers that are highly channel-specific. Another approach to inhibiting K^+ flux is to modulate channel gating processes (e.g., shift the voltage dependence of activation to more positive potentials) with compounds that bind to structural elements (e.g., voltage-sensor domain) that are not as highly conserved as the residues in the S6 segment that form the channel pore. We anticipate that this approach will be greatly facilitated as more K^+ channel structures are elucidated.

Activators

Congenital LQTS is a disorder of cardiac repolarization that predisposes affected individuals to an increased risk of cardiac arrhythmia and sudden cardiac death. The most common cause of LQTSs are loss of function mutations in either hERG1 or KCNQ1 channels, resulting in a reduction of I_{Kr} and I_{Ks}, and a prolongation of ventricular repolarization easily quantified as a longer QTc interval [69–71]. Thus, compounds that activate hERG1 or KCNQ1 channels are an obvious potential approach to restoring normal repolarization for the vast majority of congenital LQTS patients.

In the past 10 years, routine safety screening of compounds for undesirable hERG1 activity led to the serendipitous discovery of several hERG1 activators that act by different mechanisms to allosterically modify channel gating. These agents can either slow deactivation, attenuate C-type inactivation, enhance single channel open probability, induce a hyperpolarizing shift in the voltage dependence of activation, or exert a combination of

two or more of these actions [72]. RPR-260243 was the first reported synthetic hERG1 activator. This compound markedly slows the rate of hERG1 channel deactivation and causes a mild attenuation of inactivation with no effect on the voltage dependence of activation [73,74]. Ginsenoside Rg3, a natural product isolated from ginseng root also dramatically slows hERG1 deactivation rate, but it also causes a modest negative shift in the voltage dependence of activation [75]. ICA-105574 [76,77], ML-T531 [78] and AZSMO-23 [79] profoundly enhance outward hERG1 current by greatly attenuating C-type inactivation, and modestly slow deactivation without affecting the voltage dependence of channel activation. PD-118057 enhances hERG1 current by increasing channel open probability and causing a positive shift in the voltage dependence of inactivation [80]. The putative binding sites for RPR-260243, ICA-105574, and PD-118057 were revealed by the combined approach of alanine-scanning mutagenesis and molecular modeling. Although the specific residues of hERG1 that interact with these agonists vary, all three compounds interact with the channel via a hydrophobic pocket located between two adjacent subunits of the pore domain (Fig. 3) [74,77,80] such that a homotetrameric hERG1 channel would contain four such identical binding sites. Analysis of concatenated hERG1 tetramers revealed that all four binding sites and cooperative subunit interactions are required to achieve maximal effects by ICA-105574 or PD-118057 [81]. Maximal slowing of deactivation by RPR-260243 requires all four hERG1 subunits, whereas only two or more subunits were sufficient to achieve a maximal effect on inactivation [82]. In addition to ginsenoside Rg3 [75], mallotoxin [83] and KB130015 [84] also shift the voltage dependence of hERG1 activation to more negative potentials; however, the binding site for these agents has not yet been defined. NS1643 shifts the voltage dependence of activation to more negative potentials and of inactivation to more positive potentials, but effects vary depending upon the heterologous expression system employed [85–87]. The binding site for NS1643 has eluded definitive identification, but based on detailed molecular modeling and analysis of L529I hERG1 mutant channels, it is likely that NS1643 interacts indirectly with the VSD to facilitate opening of hERG1 channels [88].

R-L3 was the first I_{Ks} activator to be described [89,90] and was shown to slow the rate of deactivation and cause a hyperpolarizing shift in the voltage dependence of channel activation. Overexpression of KCNE1 subunits with KCNQ1 channels abolished the action of R-L3, suggesting that the single transmembrane domain KCNE1 subunit either competes directly, or allosterically, with R-L3 for binding to KCNQ1 channel subunits. The putative binding site for R-L3 between S5 and S6 segments [90] strongly suggests that it too will bind with four-fold symmetry as described for the hERG1 activators. Zinc pyrithione (ZnPy) is a potent activator of KCNQ2 and KCNQ3 channels, but also activates KCNQ1 [91,92]. ZnPy attenuates inactivation, slows the rate of activation and deactivation of KCNQ1 channels and like R-L3 its actions are abolished by over-expression of KCNE1 subunits [91]. Most recently, high-throughput screening identified the compound ML277 as a potent KCNQ1 agonist [93,94] whose efficacy is also reduced by KCNE1 [95]. A combined approach of electrophysiology and molecular dynamic simulation indicated that ML277 binds to the side pockets between adjacent KCNQ1 subunits to allosterically modify channel gating [94]. Nonspecific compounds such as the chloride channel blockers mefenamic acid and DIDS, and the anti-bacterial hexachlorophene can also activate I_{Ks}, but these compounds exert stronger potentiation on KCNQ1/KCNE1 channels than on homomeric KCNQ1 channels [96,97].

Unlike other KCNQ1 channel activators, the negative shift of voltage dependence of channel activation produced by phenylboronic acid is independent of KCNE1 subunits [98]. Polyunsaturated fatty acids (PUFAs) are the most recently identified KCNQ1 agonists [99]. Electrostatic interactions between the negatively charged head group of PUFAs, such as docosahexaenoic acid, and the basic residues in the S4 segment of KCNQ1 shift the voltage dependence of channel activation to more negative potentials (maximal shift of -15 mV) at concentrations similar to total circulating plasma levels of PUFAs. Interestingly, KCNE1 subunits abolish the agonist effect by promoting PUFA protonation, indicating that natural PUFAs do not affect I_{Ks} channels in the heart [99]. If excessive shortening of QT_c can be avoided, hERG1 or KCNQ1/KCNE1 activators may be useful for prevention of arrhythmia associated with prolonged ventricular repolarization in LQTS patients. They might be particularly useful for LQTS patients with particularly aggressive arrhythmia syndromes, who enter periods of arrhythmic storm refractory to conventional management with repeated defibrillation shocks and increased mortality risk.

Summary

Fueled by the molecular cloning of genes encoding the many channels that conduct the wide diversity of known ionic currents, the last two decades have witnessed a great leap forward in our understanding of the structural and mechanistic basis of potassium channel function. Due to their well-recognized importance in cardiac repolarization and the severe medical consequence of inherited gene mutations, the pharmacology of cardiac delayed rectifier K^+ channels has been explored extensively. In the past few decades, high throughput drug screening efforts have focused on the discovery of specific channel blockers to treat atrial fibrillation (Kv1.5 channel blockers) or ventricular arrhythmia (hERG1 and KCNQ1/KCNE1 blockers). More recently, hERG1 and KCNQ1 channel activators were discovered that may prove useful for treatment of ventricular arrhythmia associated with prolonged QT_c intervals. It is anticipated that future antiarrhythmic drug discovery efforts will rely less on screening of large compound libraries and instead capitalize on our increasingly sophisticated understanding of channel structure and gating mechanisms to enable a rational, knowledge-based drug design approach.

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Key Points

- Although the structures of cardiac delayed rectifier K^+ channels, including Kv1.5, Kv7.1 (hERG1) and Kv11.1 (KCNQ1) have not been determined, the molecular basis of their function can be inferred from biophysical studies and known structural features of other K^+ channels.
- The molecular basis of action for several blockers and activators of cardiac delayed rectifier K^+ channels have been determined primarily by functional analysis of mutant channels that alter drug affinity.
- Most blockers inhibit K^+ conductance by binding to the central cavity of the channel. In contrast, hERG1 and KCNQ1 activators bind outside the cavity to four symmetrical sites and alter one or more gating properties.

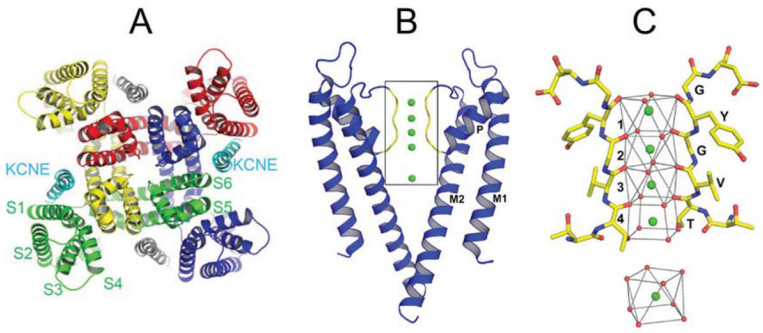

Fig. 1.

Structural features of voltage-gated K^+ channels. **A**, Functional channels are composed of four identical or highly related α-subunits. The S1–S4 transmembrane segments form the VSD. The S5 and S6 segments form the pore domain. In I_{Ks} channels, four KCNQ1 α-subunits are joined by two (or more) KCNE1 β-subunits that are positioned in the cleft as indicated. From Nakajo K. and Kubo Y. KCNQ1 channel modulation by KCNE proteins via the voltage-sensing domain. *J Physiol.* 2015;593:2617–2625. **B**, side-view of the pore domain of the KcsA bacterial K^+ channel in a closed state. Only two of the four subunits are shown; selectivity filter is boxed. **c**, the selectivity filter of a K^+ channel (KcsA) is a narrow lumen where dehydrated K^+ ions (green sphere) transiently bind to sites (1–4) formed by oxygen atoms contributed by TYGYG residues that form the structure. Bottom panel shows a single K^+ ion surrounded by 8 water molecules. B and C: From Alam A. and Youxing J. Structural studies of ion selectivity in tetrameric cation channels. *J Gen Physiol.* 2011;137(5):397–403; with permission.

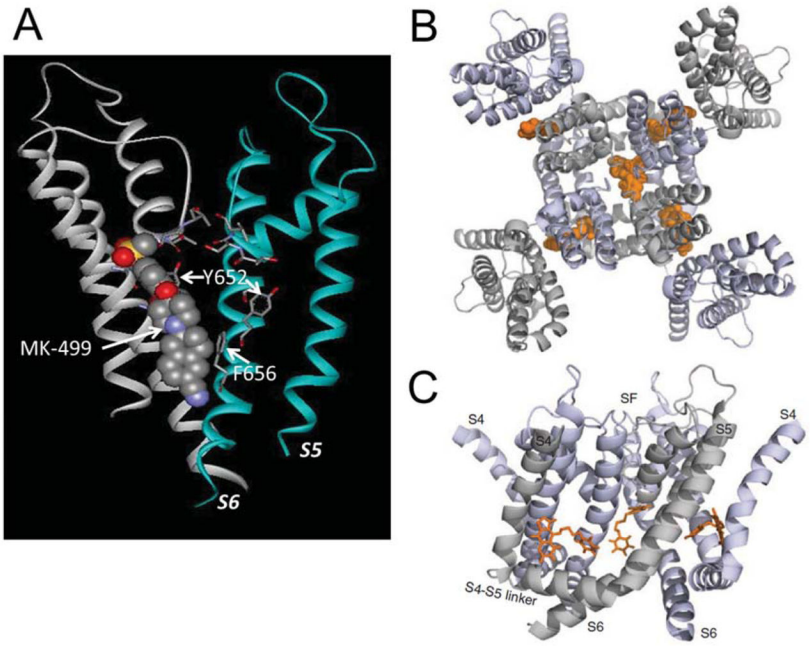

Fig. 2.

K^+ channel inhibitors. **A**, Model of the hERG1 channel pore domain showing block of the central cavity by MK-499, a class III antiarrhythmic agent. The S5 and S6 segments for two of the four subunits are shown. The key binding residues in the S6 segment (Y652 and F656) are indicated. From Mitcheson JS, Chen J, Lin M, Culberson C, and Sanguinetti MC A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci USA*. 2000;97:12329–12333. **B and C**, Psora-4 (orange) binds to multiple sites on the Kv1.5 channel viewed from the extracellular space (B) or from the side (C). From Marzian S, Stansfeld PJ, Rapedius M*, et al.* Side pockets provide the basis for a new mechanism of Kv channel-specific inhibition. *Nat Chem Biol*. 2013;9:507–513; with permission.

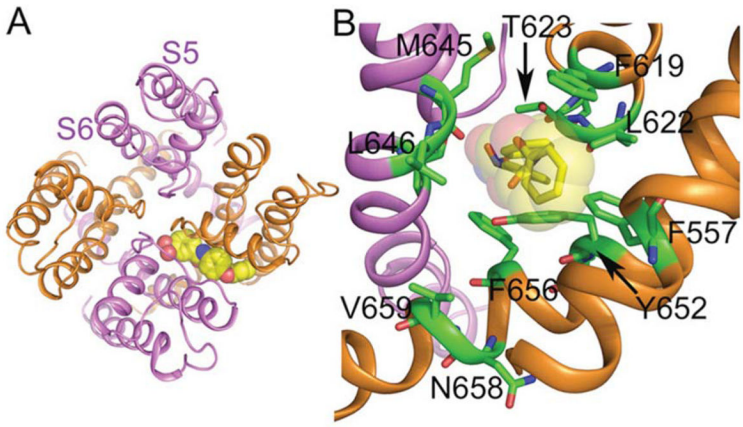

Fig. 3.

Binding site for ICA-105574, a hERG1 channel activator. **A**, open-state pore domain of the channel as viewed from the extracellular space. ICA is shown in space fill occupying one of the four symmetrical sites available. VSDs are not shown. **B,** side view of the binding site with important binding sites residues indicated in single letter code. From Garg V, Stary-Weinzinger A, Sachse F, Sanguinetti, MC. Molecular determinants for activation of human *ether-a-go-go*-related gene 1 potassium channels by 3-nitro-n-(4-phenoxyphenyl) benzamide. *Mol Pharmacol*. 2011;80:630–637; with permission.