The molecular basis of high-affinity binding of the anti-arrhythmic compound, vernakalant (RSD1235), to Kv1.5 channels

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ABSTRACT

Vernakalant (RSD1235) is an investigational drug recently shown to convert atrial fibrillation rapidly and safely in patients (Roy et al., 2004). Here, the molecular mechanisms of interaction of vernakalant with the inner pore of the Kv1.5 channel are compared with the Class IC agent flecainide. Initial experiments showed that vernakalant blocks activated channels and vacates the inner vestibule as the channel closes, and thus mutations were made, targeting residues at the base of the selectivity filter and in S6, by drawing on studies of other Kv1.5-selective blocking agents. Block by vernakalant or flecainide of Kv1.5 wild-type (WT) and mutants was assessed by whole cell patch clamp experiments in transiently transfected HEK293 cells. The mutational scan identified several highly conserved amino acids, T479, T480, I502, V505 and V508, as important residues for affecting block by both compounds. In general, mutations in S6 increased the IC₅₀ for block by vernakalant, with I502A causing an extremely local 25-fold decrease in potency. Specific changes in the voltage-dependence of block with I502A supported the crucial role of this position. A homology model of the pore region of Kv1.5 predicted that, of these residues, only T479, T480, V505 and V508 are potentially accessible for direct interaction, and that mutation at additional sites studied may therefore affect block through allosteric mechanisms. For some of the mutations, the direction of changes in IC₅₀ were opposite for vernakalant and flecainide, highlighting differences in the forces that drive drug-channel interactions.
INTRODUCTION

Atrial fibrillation, the most common sustained cardiac arrhythmia, is associated with ~15% of all strokes (Kannel et al., 1998; Go et al., 2001; Rockson and Albers, 2004) and occurs in about 30% of patients after cardiac surgery (Leung et al., 2004). Most drugs, currently in use for treatment of atrial fibrillation, are indiscriminate, targeting channels in both atrial and ventricular tissue and are associated with life-threatening (ventricular) arrhythmias as a consequence. Vernakalant (RSD1235) is a mixed voltage- and frequency-dependent Na⁺ and atria-preferred K⁺ channel blocker (Roy et al., 2004; Fedida et al., 2005) under development for the acute conversion of atrial fibrillation to sinus rhythm. In recent phase II and III clinical trials vernakalant has shown promise as an intravenous antiarrhythmic agent for rapid conversion of atrial fibrillation to sinus rhythm with an overall rate close to 52% within 90 min of infusion, compared with a placebo conversion rate of just 3.8% (Roy et al., 2005; Pratt et al., 2006; Stiell et al., 2006; Fedida, 2007). Previous studies have shown that one of the actions of vernakalant is block of the atrial specific Ikur current (Fedida et al., 2005), which in human atria is thought to be the result of expression of the KCNA5 gene and Kv1.5 protein (Fedida et al., 1993; Feng et al., 1997). Evidence suggests that the safety of this drug is in part related to the higher sensitivity of atrial-specific Kv1.5 to block by vernakalant over other channels involved in ventricular repolarization, such as hERG (Fedida et al., 2005) and KCNQ1 (unpublished data).

Several antiarrhythmic drugs in development, S0100176 (Decher et al., 2004), AVE0118 (Decher et al., 2006), AZD7009 (Persson et al., 2005), as well as some well-known agents, quinidine (Snyders et al., 1992; Fedida, 1997), and flecainide (Grissmer et al., 1994), and the
local anesthetics bupivacaine (Franqueza et al., 1997) and benzocaine (Caballero et al., 2002), have been shown to block Kv1.5 also. The potency of these agents is affected by introduction of specific mutations in the S6 domain of Kv1.5, that lines the inner vestibule of the channel (V505, T507, I508, L510, V512, V514), or mutations in the deep pore (T479 and T480) near the selectivity filter (Caballero et al., 2002; Decher et al., 2004; Decher et al., 2006; Herrera et al., 2005; Yeola et al., 1996; Franqueza et al., 1997). Many of these same residues are also important sites of interaction for the Kvβ inactivation particle (Decher et al., 2005), for TEA (Choi et al., 1993; Lopez et al., 1994), and for 4-AP block (Kirsch and Drewe, 1993).

Flecainide is also a drug of choice for the acute conversion of atrial fibrillation to sinus rhythm (Fuster et al., 2006), and is known to block Kv1.5 (Grissmer et al., 1994; Herrera et al., 2005), but with much lower potency than vernakalant. In the present study, we have investigated the binding site of vernakalant in the deep pore and S6 of Kv1.5 using both electrophysiology and site-directed mutagenesis. Flecainide has been used as a comparator compound to validate our data against that already present in the literature, and also to extend studies of flecainide block itself. The results demonstrate that I502 in the S6 domain is a key residue in the block of Kv1.5 by vernakalant, but less so for flecainide. We have interpreted our results in the context of the hydrophobicity, size, and potential for cation-π interactions of the different substituted residues, and we have extended our analysis by carrying out homology-modeling and ligand docking of vernakalant on Kv1.5 based on the published crystal structure of Kv1.2 (Long et al., 2005).
MATERIALS AND METHODS

Cell Preparation

Stable lines of HEK293 cells expressing Kv1.5, or transient transfection of mutant Kv1.5 channels were used in all experiments. Primers to generate mutant channels were synthesized by Integrated DNA Technologies, Inc. (IA, USA), and mutants were generated using the Stratagene Quikchange kit (Stratagene, CA, USA). The presence of the mutation was confirmed by DNA sequencing, and because of the size of the WT Kv1.5 clone, only the sequenced region harboring the targeted nucleotides was subcloned as a fragment back into full-length Kv1.5. Transient transfections were performed with HEK 293 cells plated at 20-30 % confluency on sterile coverslips in 25 mm Petri dishes one day prior to transfection. 0.1-4 µg of ion channel DNA was incubated with 1 µg of eGFP DNA (to enable detection of transfected cells) and 2 µl of Lipofectamine 2000 (Invitrogen) in 100 µl of Opti-MEM. This was added to the cells for overnight incubation after washing the cells with 900 µl of MEM with 10 % FBS.

The cells were grown in Minimal Essential Medium (MEM) at 37°C in an air/5 % CO₂ incubator. Media contained 10 % bovine serum and 0.5 mg/ml geneticin for stable lines. On the day before recording, stably-expressing cells were washed with MEM, treated with trypsin/EGTA for one minute and plated on 25 mm² coverslips. All cell culture supplies were obtained from Invitrogen (Mississauga, ON, Canada).

Drugs and Solutions

Control bath solution contained (in mM): 5 KCl, 135 NaCl, 2.8 sodium acetate, 1 MgCl₂, 10 HEPES and 1 CaCl₂, adjusted to pH 7.4 with NaOH. Patch pipettes contained (in mM): 130 KCl, 5 EGTA, 1 MgCl₂, 10 HEPES, 4 Na₂ATP, and 0.1 GTP, adjusted to pH 7.2 with KOH. All

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chemicals used to make solutions were obtained from Sigma-Aldrich (Mississauga, ON, Canada). Vernakalant (formerly known as RSD1235; 3-Pyrrolidinol, 1-[(1R,2R)-2-[2-(3,4-dimethoxyphenyl)ethoxy]cyclohexyl]-,hydrochloride, (3R)), lot numbers DM-155-A and JL-78-12) was synthesized by Cardiome Pharma Corp. (Vancouver, BC, Canada) and prepared as a stock solution (50 mM) in H2O. Flecainide (Sigma-Aldrich, lot number 094K4057) was prepared as a stock solution (100 mM) in 100% DMSO. DMSO concentrations never exceeded 0.1% v/v in the final experimental solutions.

**Whole-Cell Patch-Clamp Recordings**

Coverslips with adherent cells plated on the surface were placed in a superfusion chamber (volume 300 µl) containing the control bath solution at 22°C. Whole-cell current recording and analysis were carried out using an Axopatch 200B amplifier and pClamp10 software (Axon Instruments, CA, USA). Patch electrodes were pulled from thin-walled borosilicate glass (World Precision Instruments, FL, USA) on a horizontal micropipette puller (Sutter Instruments, CA, USA). Electrodes had resistances of 1.0-3.0 MΩ when filled with control filling solution. Analogue capacity compensation and 80% series resistance compensation were used during whole cell measurements. Membrane potentials were not corrected for junction potentials that arise between the pipette and bath solution. For Kv1.5 current recordings, a holding potential of -80 mV was used. Data were sampled at 10-20 kHz and filtered at 5 to 10 kHz. To assess drug block, half-log escalating concentrations of drug were added to the flowing bath solution and current traces were recorded with 400 ms depolarizing pulses to +60 mV at a frequency of 0.1 Hz. Concentrations approximately equal to the 50 % inhibitory value for each drug on the WT
channel were used in the initial investigation of vernakalant and flecainide actions on mutated channels.

**Data Analysis**

Data are represented as mean ± SEM unless otherwise specified. For significance of differences, * represents $p < 0.05$, ** $p < 0.01$; statistical analysis was conducted using Student’s t-test (unpaired). Clampfit software (Axon Instruments, CA, USA) was used to analyze current traces. Data from each test pulse (currents in the presence of drug) was normalized to control test pulse currents obtained before drug exposure. For drug potency studies of the mutant Kv1.5 channels in the presence of vernakalant or flecainide (Figs. 3, 4), currents in the presence of different concentrations of drug were normalized to the current in control and used to generate concentration-response curves for changes in steady state Kv1.5 current. The resulting concentration-response relations were computer-fitted to the Hill equation:

$$i = 1/[1 + (IC_{50}/[D]^n)] \quad [1]$$

where $i$ is the normalized current recorded ($i = I_{drug}/I_{control}$) at drug concentration $[D]$; $IC_{50}$ is the concentration producing half-maximal inhibition; and $n$ is the Hill coefficient.

The voltage dependence of block for vernakalant and flecainide was determined by the calculation of the fractional block ($f = 1 - I_{drug}/I_{control}$) at the potentials in the range of full channel opening (between +10 to +60 mV) and fitting the data to the Woodhull equation:

$$f = [D]/([D]+K_d e^{-\delta z F E/RT}) \quad [2]$$

where $F$, $R$, $z$ and $T$ have their usual meanings, $\delta$ represents the fractional electrical distance, i.e., the fraction of the transmembrane electrical field sensed by a single charge at the receptor site.
Kd* represents the binding affinity at the reference voltage (0 mV). The current amplitude (I_{drug} and I_{control}) was measured at the end of a 400 ms depolarization.

**Homology model**

The Kv1.5 homology model was generated using "First Approach Mode" of SWISS-MODEL (http://swissmodel.expasy.org) and the three-dimensional structure of rKv1.2 (Protein Data file 2A79; (Long et al., 2005)), which is believed to represent the open state of the channel. rKv1.2 and hKv1.5 show 100% identity in S5 and S6 and 88% identity in the pore loop. The figure was generated using DeepView Swiss-PdbViewer (http://swissmodel.expasy.org/spdbv) and Adobe Photoshop software. This homology model was used for docking of vernakalant using ChemBio3D (Cambridge Scientific, MA, USA), AutoDock tools, and AutoDock4 (Scripps research Inst, CA, USA; (Morris et al., 1998)). A lowest-energy conformation of vernakalant, protonated on the nitrogen atom as it would be at physiological pH, was used for docking. The channel macromolecule remained rigid during the docking computation, while vernakalant was flexible.
RESULTS

Delayed closing of Kv1.5 channels in the presence of vernakalant at the inner pore

It was suggested from our previous experiments that block of Kv1.5 by vernakalant was mediated after channel activation, as vernakalant caused a relatively rapid onset of block of channel current upon depolarization (Fedida et al., 2005), but little evidence of resting or ‘tonic’ block of the channel. It is known that the activation gate of Kv channels lies on the intracellular side of the pore (Holmgren et al., 1998), and thus a requirement for channel activation for block to occur suggests intracellular access of the drug to the pore region of the channel. In our initial experiments aimed at localizing the site of binding of vernakalant within Kv1.5 we have examined the ability of the drug to affect activation gate closure upon repolarization, after channel opening (Fig. 1). Control currents in Figure 1A activate rapidly upon depolarization to +60 mV and show only limited inactivation during the 400 ms depolarization shown here. During repolarization to -40 mV a deactivation tail current is observed that reflects channel closure. In the presence of 10 µM vernakalant, rapid block is apparent after channel opening and a steady-state current level is rapidly reached. Upon repolarization, the tail current amplitude is initially less than in control, but decays more slowly and crosses over the control tracing as it declines to the baseline current level (see inset panel). The result suggests that vernakalant delays closing of Kv1.5, and perhaps that the channels cannot close while vernakalant is bound, but require drug expulsion from the inner vestibule before that can happen.

This possibility was tested in the experiment shown in Fig. 1B. Here, after equilibration at 0.1 Hz in control solutions, the cell was exposed to vernakalant as shown. This led to a progressive reduction of steady-state current, and at 200 s, the cell was rested for 3 min at -80 mV and vernakalant was washed from the bath. When the current was activated again by depolarizing
clamp pulses, the current level was immediately restored to the control level. This suggests that vernakalant was not trapped in the closed channels while drug was washed from the bath, but rather that, as channels closed, vernakalant was slowly expelled from the inner pore. This slow expulsion is the cause of the slowed tail current observed in the presence of vernakalant (Fig. 1A).

**Alanine scan of S6: block by vernakalant.**

The data in Figure 1 suggest that the S6 domain that lines the inner vestibule of the channel and the deep pore near the selectivity filter are likely areas where vernakalant could bind to Kv1.5. Extensive studies of some approved and developmental antiarrhythmic agents that target either the cardiac Na+ channel or K+ channels involved in action potential repolarization have suggested that the base of the selectivity filter in the deep pore and the S6 are important areas for antiarrhythmic drug binding within the ion conduction pathway (Decher et al., 2004; Herrera et al., 2005). These regions were targeted for mutational analysis of block by replacement of WT residues with alanines wherever possible, and valines if the WT residue was an alanine, and leucine, valine, or glycine if there was an expression problem with alanine. A summary of the mutants used in the present experiments is shown in Table I, and includes those that we were unable to record current from. Channels transiently or stably (see Methods) expressed in HEK cells were voltage clamped with depolarizing pulses from -80 to +60 mV at 0.1 Hz, and allowed to equilibrate for 5 min before drugs were applied. Control currents in each panel of Figure 2 show some differences between mutants. Most of the examples show the rapidly activating, delayed rectifier current expected from Kv1.5 channels, with a minor degree of slow inactivation
occurring during the 400 ms depolarizations used here. However, a few channels, Kv1.5 T479A, A501V, and V512L showed a definite increase in inactivation rate. Initially, a scan of residues using 10 µM vernakalant was carried out to generally assess the importance of residues in this area. This concentration reduced currents significantly in almost all mutants studied, with an approximately 50% reduction of outward current in WT channels, as has been previously reported (Fedida et al., 2005), and reduced block in most other mutants with the least effect on the I502A mutant (Fig. 2G). Difference currents for some example mutants are shown in Figure 2M, showing the different levels of block, and here, the increased inactivation rate induced by the V512L mutation is apparent as a decaying difference current during the pulse. It was noted that the three mutants with increased inactivation all showed greater block at 10 µM vernakalant than control, and this finding is clearly seen in the bar graph below which summarizes data from the S6 mutations (Fig. 2N). As well, T479 and T480 are located at the inner mouth of the selectivity filter, and any changes that these mutations induce in K⁺ selectivity or permeation might have independent actions on drug block. As a result, the data from these mutants, although of interest in this study, are subject to the caveat above when being compared to the potency data from other mutations in the remaining experiments. Significant reductions in block by 10 µM vernakalant appeared to be centred on I502, with a diminishing effect for mutations moving away from this site, disappearing distally along S6 by residue A509, and proximally by residues V481 and C500 at the base of the selectivity filter.

Full dose-response relationships for vernakalant (Fig. 3) were obtained for the reduced block mutants, I502A, V505A, and I508A, in comparison with a positive control mutation that increased block (T479A), and for a readily available comparative antiarrhythmic agent,
flecainide (Fig. 4), whose block is also reported to be affected by mutations in the lower S6 (Herrera et al., 2005). Vernakalant data in Figure 3 confirmed the 10 µM scan in Figure 2, with increased potency for T479A, and with reduced potency for the other mutants. Original tracings are shown in Figure 3A, and illustrate the actions of vernakalant at concentrations between 1 µM and 1 mM in the different mutants. Steady-state current block was plotted against drug concentration (Fig. 3B) and fitted with a Hill equation to obtain the IC$_{50}$ values for the different mutants, which are also shown in the bar graph below (Fig. 3C).

The most important effect was the reduction in potency for vernakalant centred at I502A which had an IC$_{50}$ of 329 ± 19 µM ($n = 4-10$, see Table I), compared with a control IC$_{50}$ of 13.4 ± 0.9 µM ($n = 5-23$), which is a 25-fold decrease in potency. V505A, I508A, T480A, and C500A showed lesser reductions in potency on Kv1.5 (Table I), of between 3- and 4-fold (Table I).

Similar experiments were carried out for flecainide (Fig. 4A), and in qualitative agreement with previous oocyte data from the nearby residue in the selectivity filter, V481(Herrera et al., 2005), the mutation of T479 to alanine increased potency of flecainide (Fig. 4B, C), in this case 238-fold, from a control IC$_{50}$ of 38.1 ± 1.1 µM ($n = 6-9$, see Table II), to an IC$_{50}$ of 0.19 ± 0.04 µM ($n = 3-8$). I502A showed a diminished potency in the presence of flecainide, with an increase in IC$_{50}$ from 38.1 µM in control to an IC$_{50}$ of 92 ± 5.5 µM ($n = 3-6$), a 2.4-fold reduction in potency (Fig. 4B, C). Clearly, the effect of this mutation on flecainide block is much less than on vernakalant block, and in a related area, insertion of alanines at V505 and I508 increased the potency for flecainide block 3-fold and 5.2-fold respectively (Fig. 4C), in contrast to the reduction in block of 3 to 4-fold observed for vernakalant (Fig. 3C).
Further examination of other amino acid substitutions in S6 on the potency of vernakalant and flecainide action

Given the obvious importance of I502 in the block by vernakalant, a series of other residues were substituted for isoleucine at this site, and close by, in order to alter the hydrophobicity, the residue size and the potential for cation-π interactions in this region. The results for vernakalant are shown in Figure 5, and comparative data for flecainide in Figure 6 (see also Table I). For substitutions at I502, there is a clear modulation of the vernakalant IC50 that is inversely related to the hydrophobicity of the substitution made, with the native isoleucine (most hydrophobic) conferring the most potent block, and alanine (most hydrophilic) the least potent block. Overall, the potency series is I>L>F>A (Fig. 5B). At V505 a similar trend can be seen, albeit less marked, and at I508 no such correlation is apparent (Fig. 5B, 5C). In contrast, cation-π interactions appear unimportant at I502, but become increasingly important moving to 505 and 508 (Fig. 5A, 5C, 5D), such that the potency for vernakalant on I508F was 0.61 µM, the highest obtained in the present series of experiments (Fig. 5A and Table I). This result suggests that π-stacking between aromatic groups of the drug and I508F confers high affinity binding. As well, at I508, a good correlation is apparent between vernakalant IC50 and residue size which suggests that increasing side-chain volume at this location causes some steric hindrance (Fig. 5D).

In the case of flecainide, there is no obvious correlation with the hydrophobicity of the substitution at I502, V505, or I508 (Fig. 5A-D). However, in contrast to vernakalant, residue size appears to correlate linearly with flecainide IC50 values at position 508 (Fig. 6D), and inversely with IC50 at position 505. There is also the suggestion that size is important for flecainide IC50 at position 502, where directional changes in potency match the size change from residue to residue (Fig. 6B). Cation-π interactions appear less important for flecainide than for
 vernakalant, or the geometric needs are more specific ie, the aromatic needs to be a specific distance from other sites of interaction. V505F seems to be an exception to this, where the IC$_{50}$ value is 4.3 µM, compared with 38 µM in the WT when valine is present.

Voltage-dependent block of Kv1.5 by vernakalant and flecainide
Electrophysiological evidence in support of I502 as a critical residue in the interaction of vernakalant with Kv1.5 channels was obtained from studies of the voltage dependence of drug block. As before, comparative experiments were carried out between vernakalant and flecainide (Figs. 7 and 8). In these experiments, cells were exposed to concentrations of vernakalant (Fig. 7) and flecainide (Fig. 8), near their IC$_{50}$s and depolarized to a range of potentials between -80 and +60 mV as illustrated by the protocol at the top of each figure. Current-voltage relationships were determined for WT and mutant channels (T479A, I502A, V505A, I508A) in the presence of either vernakalant or flecainide (Fig. 7B, 8B). The amount of block was measured at the end of the pulse at the time indicated by the arrow (Fig. 7A, 8A) on each set of data records, and, after normalization to the current density, was plotted as a function of the depolarization potential (Fig. 7C, 8C). Data were then fit to a standard model of drug block to obtain the electrical distance of the blocking site into the electric field from the inside of the channel (Woodhull, 1973). In WT channels, the blocking site was located ~17% of the electrical distance across the membrane (Figs. 7C, 8C), and this was only perturbed in the presence of vernakalant in the I502A mutant, where analysis demonstrated a blocking site ~40% of the electrical distance across the membrane. For flecainide, results were somewhat different. While I502A did increase the electrical distance to 32%, a significant increase in distance was also observed in V505A. In general, these results strongly support the inner S6 site for the actions of both
vernakalant and flecainide, but suggest that vernakalant’s site of action is very narrowly aligned with I502, whereas that of flecainide is more distributed over a couple of turns of the S6 helix from I502 to beyond V505.

These differential effects of alanine substitution on the binding in the S6 region of the channel of vernakalant, flecainide, and some other antiarrhythmic agents (including both Na+ channel and K+ channel blockers, data obtained from the literature (Yeola et al., 1996; Herrera et al., 2005; Decher et al., 2004; Decher et al., 2006)), are shown in summary form in Figure 9, with the structures of the molecules in the bottom panel. It can be seen that AVE0118, which has an apparent maximum molecular length of ~18 Å exhibits the most significant changes in IC50 with mutations between I502 and V512, some four turns of the helix, with minor reductions in potency at V516. In contrast, the IC50 for flecainide (molecular length 10 Å) is affected by mutations over a narrower range of residues more located in the deep pore. The atrial K+ channel blocker, S0100176 is affected by mutations in a similar way to AVE0118, but the extent of these effects is more limited, extending from V505 to V512, with only minor effects extending to V516, consistent with the differences in structure between these two drugs (Decher et al., 2006). There are also differential effects of mutations between drugs thought to act primarily on Na+ channels, and those with more dominant K+ channel actions (Fig. 9). Flecainide and quinidine show increases in potency with mutations in the lower part of S6 between position 503 and 516, whereas those drugs that are more potent on K+ channels, including vernakalant, AVE0118, and S0100176, all show decreases in potency with mutations throughout the same region (Fig. 9). However, unlike AVE0118, S0100176 and quinidine,
T479A increased vernakalant and flecainide sensitivity, suggesting a common interaction at this site.

**Structural considerations from homology modeling**

Mutations at C500 and A501 both resulted in significant, though opposite, changes in IC$_{50}$ for vernakalant (Table I), and in contrast no differences were observed for AVE0118 and S0100176 (Decher et al., 2004; Decher et al., 2006) (Fig. 9). A homology model of Kv1.5 based on Kv1.2 (Long et al., 2005) was constructed (Fig. 10), and this puts these two residues at the base of the pore helix with a predicted H-bond between C500 and T477 in the adjacent helix (Fig. 10C). Mutation of the analogous residue in Kv2.1 (C393A) affected the stability of the open state and ion permeation without affecting external or internal TEA block (Liu and Joho, 1998). The A501V mutation had the most dramatic effects on inactivation and was one of the more sensitive mutants to vernakalant (Fig 2, and Table I), and it is possible that disruption of the putative H-bond, with a subsequent change of inactivation gating is responsible, at least in part, for the potency increase and the differential effect of vernakalant on this residue compared with A501.

In the homology model of Kv1.5, the side chains of I502 appear to point away from the internal cavity of the vestibule and towards two highly conserved leucines (L437 and L441; Figs. 10A, 10B) in an adjacent subunit (Fig. 10B), which makes it interesting that mutations at this site have such large effects on drug affinity. How might mutation at this residue affect drug affinity? Given that potential interactions between I502 and the leucines in the adjacent subunit would involve hydrophobic interactions, changes in hydrophobicity at I502 might interfere with subunit packing and thus alter the binding site via an allosteric mechanism. Depending on the number of
other interactions between the drug and the channel and the strength of these interactions, the changes around I502 may have greater or lesser affects. At this site, changes are more dramatic for vernakalant than for AVE0118, which interacts over a greater number of residues within the channel (Fig. 9), consistent with its more extended configuration (~18Å).

**Molecular docking of vernakalant in Kv1.2**

In an attempt to predict the vernakalant binding site in Kv1.5, the crystallized pore region of Kv1.2 (Long et al., 2005) was used with an energy-minimized conformation of vernakalant (Fig. 11). Using Autodock tools, a docking grid was built that encompassed the entire pore region of the channel, for the macromolecule, and a ligand docking grid that included the complete structure of vernakalant. Ten energy minimized docking conformations were obtained from the Autodock program, and the most stable conformation is illustrated in Figure 11. This view of the channel pore shows only those residues predicted to interact with vernakalant, with the exception of I502, which does not interact but is shown in its position facing away from the vestibule. The view is from the cytoplasmic surface and reveals residues in S6, V505, and I508 that are predicted to interact with vernakalant, as well as residues at the base of the pore helix, T480 and M478, that also interact with the compound. T480 in all four subunits, in this view, marks the narrowest point of the deep pore at the selectivity filter. In this docking conformation vernakalant appears to be folded in upon itself with its ether linkage facing the T480 of three subunits (green, magenta and white) at the base of the selectivity filter. The cyclohexane ring on one side of the ether linkage of vernakalant coordinates with V505 of two subunits (green and magenta), and the attached dimethoxy groups lie with V505 and I508 in the same subunit. The phenyl ring of vernakalant interacts closely with V505 and I508, predominantly in the white...
subunit. The pyrrolidine ring (cyclic amine) and its attached hydroxyl group interact with V505 and I508. In the other energy-minimized docking conformations many of the same amino acids as those shown in Figure 11 were modeled to interact with vernakalant.

Overall, this docking conformation of vernakalant in the pore of Kv1.5 gives excellent agreement with the experimental predictions from mutational studies described above, highlighting as it does, the threonine residue at the base of the pore helix (T480), and V505 and I508 in the S6.

**Vernakalant block of other voltage-gated ion channels**

An alignment of a range of voltage-gated potassium channels for the S5, selectivity filter and S6 regions is shown in Figure 12. It can be seen that at the equivalent position of I502, in most of the Kv3 and Kv4 channels, as well as hERG channels, there is a leucine. In our present studies, substitution of isoleucine with leucine decreased the sensitivity of Kv1.5 to 24.8 \(\mu\)M, and this is close to the reported sensitivity of Kv4 and hERG channels for vernakalant (Fedida et al., 2005). Another possible explanation for the difference in the latter case may be the presence of the tyrosine at the equivalent position to I508 in hERG. I508Y in our experiments increased the IC50 for vernakalant on Kv1.5 to 24.7 \(\mu\)M (Table I), again similar to the reported value for hERG. This tyrosine along with a phenylalanine at the equivalent position of V512 in Kv1.5 (Fig. 12) has been implicated in the binding of several classes of drug to hERG, leading to acquired long QT syndrome, as reviewed in (Sanguinetti and Mitcheson, 2005). Thus, the reduced potency for vernakalant on \(K^+\) channels other than Kv1.5 may reflect, at least in part, the 3-dimensional structural differences inferred from the present study.
DISCUSSION

In this study we have examined the binding of the novel atrial antiarrhythmic agent, vernakalant, to the Kv1.5 channel which underlies the rapidly-activating atrial K⁺ current, Iₖur. Previous work had suggested that channel activation was required for drug action (Fedida et al., 2005), and in support of this, as also described for AVE0118 (Decher et al., 2006), vernakalant was shown to slow channel closing by hindering movement of the deactivation gate, like a ‘foot-in-the-door’ (Fig. 1). This initial observation allowed us to narrow the scope of our mutational analysis of the drug binding site to the inner vestibule and deep pore of the channel.

Findings from the alanine scan

An alanine scan was carried out of residues in the deep pore/pore helix (T479-V481) and lower S6 (C500-V512), though the mutations here were limited somewhat based on the crystal structure of Kv1.2 (Long et al., 2005) to those that were predicted to face the inner cavity, except for I502. Where the literature indicated that an alanine was incompatible with expression we made other changes as summarized in Table I. The results of this mutational scan of the Kv1.5 deep pore and S6 domains show a great deal of overlap with key residues shown to be involved in block by other compounds (Caballero et al., 2002; Decher et al., 2004; Decher et al., 2006; Herrera et al., 2005; Yeola et al., 1996). This is not surprising given the limited number of amino acid side chains that will project into the inner cavity and be available for interaction with a candidate blocker. Mutations that resulted in a significant change in the vernakalant IC₅₀ involved residues T479, T480, C500, A501, I502, V505, I508 and P532. Despite the overlap in binding sites, there are apparent differences in both the importance of various residues to block
by the various compounds, (compare changes in IC$_{50}$ for vernakalant and flecainide with mutation at I502, V505 and I508 (Figs. 5 and 6)), and in the distance along S6 involved (Fig. 9). Block by both AVE0118 and S0100176 is affected by mutations at L510, V512 and V516 (Decher $et$ $al.$, 2004; Decher $et$ $al.$, 2006). Although L510 and V516 were not screened in the present study, the lack of significant difference in block by vernakalant beyond I508 (Fig. 2), (with the particular exception of P532L (see below)) suggests a minor role of those residues in vernakalant’s interaction with the channel. We have shown that residue I502 appears to be a special case in that although mutations to this residue had large effects on the potency of vernakalant action, it appears to face away from the cavity and indeed, homology docking (Fig. 11) did not indicate any direct interaction of the residue with vernakalant.

Other amino acid substitutions give further information on vernakalant binding in the S6

Several further mutations were made at the key residues identified by the alanine scan (Fig. 5 and Table I). The more conservative threonine to serine mutations at 479 and 480, result in a similar reduction in side chain volume as alanine but maintains the polar hydroxyl group. Both of these mutations decreased the potency of the drug (Fig 5A) and resulted in WT-like inactivation rates (not shown). The minimized energy docking predicts significant interactions between T480 with the 6-carbon rings of vernakalant (Fig. 11), but T479 is also likely to contribute to K$^+$ coordination (Zhou and MacKinnon, 2004), and so mutations here may be having multiple effects on permeation with downstream effects on inactivation. In oocytes, a V481L mutation also made Kv1.5 more sensitive to flecainide without affecting inactivation (Herrera $et$ $al.$, 2005), despite evidence suggesting that this residue is important for K$^+$ coordination (Long $et$ $al.$, 2005). As shown in Table I, we saw a significant increase in potency
on mutation of T479 to an alanine but not for the V481L mutation. T479A has the additional affect of enhancing C-type inactivation (Fig. 2B) which complicates interpretation of the changes in IC$_{50}$. It is interesting to note that this mutation does not have the same effect on drug block by all compounds studied, with some becoming more potent (Vernakalant and Flecainide, Tables I and II) and others less so (AVE0118, S0100176 and quinidine (Decher et al., 2004; Decher et al., 2006; Yeola et al., 1996); Fig. 9).

**I502, potential hydrophobic interactions in S5?**

At I502, insertion of a phenylalanine residue (F) capable of $\pi$-stacking appeared unable to facilitate vernakalant block. Rather, the IC$_{50}$ at I502 is inversely related to hydrophobicity (Fig. 5A), highlighting the potential hydrophobic interactions with I502 in determining inner vestibule architecture. Since I502 is predicted to point away from the inner cavity of the channel we have hypothesized in our homology modeling that interactions of I502 with leucines 437 and 441 in the adjacent S5 subunit play a significant role in determining such interactions (Figs. 10, 11). Interestingly, the IC$_{50}$ of flecainide is increased more by this mutation than by the alanine (Fig. 6), perhaps indicating some steric effects of this mutation. The importance of I502 in flecainide block has been studied previously (Herrera et al., 2005). In that study, the I502L mutation was adequate for rendering Kv1.5 as sensitive to flecainide as Kv3.1 and Kv4.2. Those studies were carried out using *Xenopus* oocytes and thus the exact IC$_{50}$ values are different but the trend was the same as observed in the HEK cells utilized in our study.

Further evidence that the residue I502 is of importance in regulating the potency of vernakalant action on Kv1.5 was obtained from electrophysiological studies of the voltage-dependence of
block (Figs. 7, 8). Here it was found that the mutation I502A shifted the calculated binding site of vernakalant within the electric field from 0.17 to 0.39 of the way across the electric field from the inside of the pore. This action of I502A was unique for the sites tested in this manner for vernakalant, and suggests that the mutation is able to specifically disrupt high affinity access of the drug to the permeation pathway, as the IC$_{50}$ for block was reduced 25-fold in this mutant (Fig. 3). The increased electrical distance that results from the mutation may reflect a deeper penetration of the drug into the inner vestibule after loss of its coordinated site, and an upper limit of intracellular access of the drug due to the proximity of the narrowing of the selectivity filter. If vernakalant is now moving deeper into the pore the increase in IC$_{50}$ may also reflect competition with K$^+$ for interaction with residues at the base of the filter. In comparison, the similar mutation for flecainide did not change the electrical distance as much (0.32 vs. 0.17), and the potency of block was only altered 2.4-fold (Fig. 4). In flecainide, the V505A mutation was able to induce a similar, if opposite change in potency to I502A, which suggests that flecainide binding to the channel is coordinated along a broader region of S6 than vernakalant and in a less critical manner by the isoleucine at position 502 and the valine at position 505.

Additional mutations at V505 and I508 resulted in further changes in IC$_{50}$ (Figs. 5 and 6) and mostly tracked with changes in size, though flecainide in particular may be capable of cation-$\pi$-stacking interactions with an aromatic amino acid placed at V505 but not at I508. A hydrophobic aromatic amino acid more distant from the pore appears more favorable for block by vernakalant, (compare I508F and I508Y in Fig. 5). These mutations are of particular interest because the equivalent residues in the proteins underlying the ventricular delayed rectifier currents $I_{K_s}$ (KvLQT1), and $I_{Kr}$ (hERG), are phenylalanine and tyrosine respectively (Fig. 12).
While the I508Y mutant has a similar IC\textsubscript{50} to hERG (Fedida \textit{et al.}, 2005), data for I\textsubscript{Ks} show that this channel does not appear to be sensitive to vernakalant (M. Pourrier, unpublished data) indicating that the extensive sequence differences between this channel and Kv1.5 (Fig. 12) make any predictions difficult.

Several other mechanisms exist by which a given mutation could affect drug block, including: 1) loss of a side chain quality needed for direct interaction with the drug (hydrophobic, electrostatic, \pi-stacking); 2) local or global changes in the binding site without the residue making direct contact with the drug itself; 3) a steric effect, with the mutation blocking access to the binding site; 4) a change in the gating state of the channel. A naturally occurring polymorphism in Kv1.5, P532L, appears to have steric effects on quinidine (Drolet \textit{et al.}, 2005) and propafenone access (Simard \textit{et al.}, 2005). Quinidine traversed the same distance across the electric field to block Kv1.5 channels with the P532L mutation, suggesting that the binding site was not affected. However, block was reduced and evidence suggested that in the absence of the proline, a helix might form and impede access to the inner vestibule (Drolet \textit{et al.}, 2005). It appears that this mutation may have a similar effect on access for vernakalant (Figure 2L).
ACKNOWLEDGEMENTS

We thank Fifi Chiu and Kyung Hee Park for help with cell culture and cloning of mutant channels. As well, we thank Dr. David Steele for molecular support, primer design and cloning, and Dr T. Claydon for homology modeling. We thank Dr. Grace Jung for help with the energy minimized form of vernakalant.
REFERENCES


FOOTNOTES

This work was supported by a research grant from Cardiome Pharma Corp, and operating grants from the Heart and Stroke Foundations of British Columbia and Yukon and the CIHR. DF was supported by a Career Investigator Award from the Heart and Stroke Foundation of Canada. The first two authors contributed equally to the execution and interpretation of this study.
LEGENDS FOR FIGURES

Figure 1. Tail current cross-over reveals an inner pore site of access for vernakalant. (A) Voltage protocol for recording of Kv1.5 currents from HEK cells. Cells were held at -80 mV and pulsed to +60 mV for 400 ms before repolarizing to -40 mV to record tail currents. Protocol was repeated at 0.1 Hz intervals. Current traces in the lower panel were obtained in control and after 5 min exposure to 10 µM vernakalant. Inset panel shows tail currents at -40 mV in control and vernakalant on an expanded time base to highlight cross-over. In this figure as in all others, numbers adjacent to tracings refer to drug concentrations in µM unless otherwise stated. (B) Diary plot of steady-state Kv1.5 current at the end of 400 ms depolarizations. Pulse frequency was 0.1 Hz. Between 200 and 380 s the cell was rested at -80 mV while vernakalant was washed out of the bath.

Figure 2. Alanine scan of pore-S6 with 10 µM vernakalant. (A-L) Kv1.5 currents in control and in the presence of 10 µM vernakalant in each case. Cells were pulsed from -80 to +60 mV for 400 ms at 0.1Hz. Channel mutation is identified above each pair of current tracings. See Table I for complete results including a listing of non-expressing mutants. (M) Difference currents showing the vernakalant-sensitive currents for a selection of the mutant in panel A, as labeled. (N) Bar graph of fraction of Kv1.5 current block by 10 µM vernakalant. Numbers in brackets denote number of cells studied, and are shown ± SEM. **, p<0.01 compared with control block.
Figure 3. Dose-response relationships for vernakalant on selected Kv1.5 mutant channels. (A) Selected current tracings from T479A, I502A, and V505A mutant Kv1.5 channels exposed to increasing concentrations of vernakalant. Cells were held at -80 mV and pulsed to +60 mV as indicated in the protocol at top. In this figure as in all others, numbers adjacent to tracings refer to drug concentrations in μM unless otherwise stated. (B) Dose response relationships obtained from data tracings as in panel A were normalized and plotted vs. log [vernakalant], and fitted to a Hill equation (see Methods for details). Data points are shown ± SEM. IC₅₀ values may be found in the text and Table I. (C) Bar graph comparing IC₅₀ values for mutant Kv1.5 channels exposed to vernakalant. Numbers in parentheses refer to the number of cells studied, and are shown ± SEM. **, p<0.01 compared with control IC₅₀ value.

Figure 4. Dose-response relationships for flecainide on selected Kv1.5 mutant channels. (A) Selected current tracings from Kv1.5 WT, T479A, I502A, V505A, and I508A mutant Kv1.5 channels exposed to increasing concentrations of flecainide. Cells were held at -80 mV and pulsed to +60 mV. (B) Dose response relationships obtained from data tracings as in panel A were normalized and plotted vs. log [flecainide], and fitted to a Hill equation (see Methods for details). Data points are shown ± SEM. IC₅₀ values may be found in the text and Table II. (C) Bar graph comparing IC₅₀ values for mutant Kv1.5 channels exposed to flecainide. Numbers in parentheses refer to the number of cells studied, and are shown ± SEM. *, p<0.05 compared with control IC₅₀ value.

Figure 5. Various amino acid substitutions to selected residues in S6, and their block by vernakalant. (A) 3-axis graph of the effect of different amino acid substitutions of the WT
residues (abscissa) on the resultant mutant Kv1.5 channel IC\textsubscript{50} (ordinate) for block by vernakalant. Note color coding of substituted residues, with WT and alanine mutants in pale blue: red = leucine; yellow = phenylalanine; green = tyrosine; dark blue = serine. (B) Correlation graphs comparing vernakalant IC\textsubscript{50} (left ordinate in \(\mu\text{M}\)) vs. hydrophobicity (right ordinate, Kyte-Doolittle units), and size (left ordinate, Å\textsuperscript{3}) for a range of amino acids substituted at I502 (B), V505 (C), and I508 (D). Spearman correlation coefficients: for I502 for size and hydrophobicity were -0.4, and -1.0, respectively; for V505, \(r = 0.0\) and -0.4; for I508, \(r = -0.4,\) and -0.6 (-1.0 without tyr).

**Figure 6. Various amino acid substitutions to selected residues in S6, and their block by flecainide.** (A) 3-axis graph of the effect of different amino acid substitutions of the WT residues (abscissa) on the resultant mutant Kv1.5 channel IC\textsubscript{50} (ordinate) for block by flecainide. Note color coding of substituted residues, with WT and alanine mutants in pale blue: red = leucine; yellow = phenylalanine. (B-D) Correlation graphs comparing flecainide IC\textsubscript{50} (left ordinate in \(\mu\text{M}\)) vs. hydrophobicity (right ordinate, Kyte-Doolittle units), and size (left ordinate, Å\textsuperscript{3}) for a range of amino acids substituted at I502 (B), V505 (C), and I508 (D). Spearman correlation coefficients: for I502 for size and hydrophobicity were 0.4, and -0.6, respectively; for V505, \(r = -0.5,\) and 0.5; for I508, \(r = 1.00,\) and 0.5.

**Figure 7. Voltage-dependent block of selected pore and S6 mutants by vernakalant.** (A) Kv1.5 Current tracings in control (above) and during exposure to 10 \(\mu\text{M}\) vernakalant (below) at a range of membrane potentials from -80 to +60 mV, as shown in the protocol at top, data from the same cell. (B) Current-voltage relationships were obtained from WT and selected mutant Kv1.5
channels at various concentrations. Data points were obtained from experiments carried out as illustrated in panel A. Currents measured at the end of clamp pulses (at arrows in A) are plotted ± SEM. vs. voltage clamp step potential for different concentrations of vernakalant (in µM). For WT data, n = 12, for T479A, n = 6; for I502A, n = 6; for V505A, n = 8; for I508A, n = 8. (C) Normalized voltage-dependent block is plotted vs. clamp potential and fitted with a Woodhull equation to calculate the fractional electrical distance of drug binding (δ, see inset legend for values, and Methods and text for explanation).

Figure 8. Voltage-dependent block of selected pore and S6 mutants by flecainide. (A) Kv1.5 Current tracings in control (above) and during exposure to 30 µM flecainide (below) at a range of membrane potentials from -80 to +60 mV, as shown in the protocol at top, data from the same cell. (B) Current-voltage relationships obtained from WT and selected mutant Kv1.5 channels as labeled on each graph. Data points were obtained from experiments carried out as illustrated in panel A. Currents measured at the end of clamp pulses (at arrows in A) are plotted ± SEM. vs. voltage clamp step potential for different concentrations of flecainide (in µM). For WT data, n = 12, for T479A, n = 6; for I502A, n = 6; for V505A, n = 8; for I508A, n = 8. (C) Normalized voltage-dependent block is plotted vs. clamp potential and fitted with a Woodhull equation to calculate the fractional electrical distance of drug binding (δ, see inset legend for values, and Methods and text for explanation).

Figure 9. Comparison of effects of alanine substitution on block of Kv1.5 by vernakalant, flecainide and other antiarrhythmic agents. Percent changes in IC₅₀ or fractional block were calculated from derived data (vernakalant and flecainide) or from values obtained from available
published work involving alanine mutations (see text for details). Molecular structures of the five compounds are shown in the lower panel. NB; at residue 501 the mutation was an alanine to a valine

**Figure 10.** Kv1.5 pore homology model illustrating residues that when mutated altered sensitivity to block by vernakalant. (A) Kv1.5 homology model was generated for the S5-p-loop-S6 region based on the crystal structure for Kv1.2. (A) The model shows side chains for residues T479, T480, I502, V505 and I508. (B) The side chain of I502 appears to point towards L437 and 441 (shown in space fill representation) in the adjacent subunit. (C) Homology model predicts an H-bond between C500 in S6 and T477 in the pore helix.

**Figure 11. Lowest energy docking conformation of vernakalant displayed with interacting residues of Kv1.5.** This conformation shows the drug-interacting residues on the four channel subunits as predicted by the model. The view is from the cytoplasmic side of the channel, looking up into the open pore and selectivity filter. Residues and conformations were predicted by AutoDock4 using the crystal structure of rKv1.2 (Protein Data file 2A79; Long et al., 2005). The channel structure was kept rigid and vernakalant allowed to be flexible. Different colors were used to label amino acids from each subunit of the channel tetramer. I502 was added to the figure although it was not predicted to be an interacting residue. The large spheres are space-filled representations of specific atoms. Carbons are shown as grey, oxygen as red, hydrogen as blue/green, and nitrogen as dark blue.

**Figure 12.** Alignment of S5, pore and S6 regions from a number of potassium channels.
Alignment of potential pore interaction regions from a variety of K channels. // refers to omitted sequence in the pore turret. Identical residues in different channels are identified by (-). The span of the S5, selectivity filter and S6 regions are identified by the solid black bars above the sequence. Residues, I502, V505 and I508 are identified by highlighting in the top row for Kv1.5, and the I502 equivalent residue in the other channels. Underscored residues in KVLQT1 and hERG channel genes are discussed in the text.
Table I: Summary of Fractional Block and IC_{50} Values for Block by Vernakalant of Wild-type and Mutant Kv1.5 Channels.

<table>
<thead>
<tr>
<th>Fractional Block ± SEM</th>
<th>Significance</th>
<th>n</th>
<th>IC_{50} ± SEM (µM)</th>
<th>Hill coefficient</th>
<th>Fold change</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>WT 0.45 ± 0.03</td>
<td></td>
<td>23</td>
<td>13.35 ± 0.93</td>
<td>1.00</td>
<td>8.31</td>
<td>5-23</td>
</tr>
<tr>
<td>T479A 0.77 ± 0.05 **</td>
<td></td>
<td>9</td>
<td>1.63 ± 0.09</td>
<td>0.6</td>
<td>3.94</td>
<td>3-10</td>
</tr>
<tr>
<td>T479S 0.23 ± 0.04 **</td>
<td></td>
<td>7</td>
<td>4.02 ± 3.54</td>
<td>0.95</td>
<td>3.07</td>
<td>3-8</td>
</tr>
<tr>
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<td></td>
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<td>52.59 ± 7.85</td>
<td>0.88</td>
<td>3.94</td>
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<tr>
<td>T480S 0.25 ± 0.07 *</td>
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<td>52.83 ± 12.46</td>
<td>0.64</td>
<td>3.96</td>
<td>4-7</td>
</tr>
<tr>
<td>V481L 0.36 ± 0.03</td>
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<td>6</td>
<td></td>
<td></td>
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<tr>
<td>C500A 0.21 ± 0.04 **</td>
<td></td>
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<td>45.63 ± 4.06</td>
<td>0.95</td>
<td>3.42</td>
<td>3-10</td>
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<td>24.66</td>
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<tr>
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<tr>
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* p<0.05; ** p<0.01; DNE = Did not Express. Fractional Block was measured at 10 µM vernakalant.
Table II: Summary of IC\textsubscript{50} Values for Block by Flecainide for Wild-type and Mutant Kv1.5 Channels.

<table>
<thead>
<tr>
<th></th>
<th>IC\textsubscript{50} ± SEM (µM)</th>
<th>Significance</th>
<th>Fold Change</th>
<th>n</th>
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<tr>
<td>WT</td>
<td>38.14 ± 1.06</td>
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<td>6-9</td>
</tr>
<tr>
<td>T479A</td>
<td>0.19 ± 0.04</td>
<td>***</td>
<td>200</td>
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<td>I502A</td>
<td>92.04 ± 5.54</td>
<td>***</td>
<td>2.43</td>
<td>3-6</td>
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<tr>
<td>I502L</td>
<td>10.75 ± 1.09</td>
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<td>3.55</td>
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<tr>
<td>I502F</td>
<td>164.49 ± 31.36</td>
<td>***</td>
<td>4.13</td>
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<td>I508F</td>
<td>74.71 ± 5.37</td>
<td>***</td>
<td>1.96</td>
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</tr>
</tbody>
</table>

*** p<0.001
Figure 1

A) WT Kv1.5 vernakalant

B) 10 μM vernakalant

Time (s)

Current (nA)

+60 mV

-80 mV

-40 mV

0 μM

10 μM

0.4 nA

2 nA

10 ms

100 ms

0 100 200 300 400 500
Figure 2

A. Kv1.5 WT
B. Kv1.5 T479A
C. Kv1.5 T480A
D. Kv1.5 V481L
E. Kv1.5 C500A
F. Kv1.5 A501V
G. Kv1.5 I502A
H. Kv1.5 V505A
I. Kv1.5 I508A
J. Kv1.5 A509G
K. Kv1.5 V512L
L. Kv1.5 P532L

M. Fraction of block

table:

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Block Fraction</th>
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<tbody>
<tr>
<td>WT</td>
<td>0.10 ± 0.01</td>
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<tr>
<td>T479A</td>
<td>0.90 ± 0.02</td>
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<tr>
<td>T480A</td>
<td>0.80 ± 0.03</td>
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<tr>
<td>V481L</td>
<td>0.70 ± 0.04</td>
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<tr>
<td>C500A</td>
<td>0.60 ± 0.05</td>
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<tr>
<td>A501V</td>
<td>0.50 ± 0.06</td>
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<tr>
<td>I502A</td>
<td>0.40 ± 0.07</td>
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<tr>
<td>V505A</td>
<td>0.30 ± 0.08</td>
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<tr>
<td>I508A</td>
<td>0.20 ± 0.09</td>
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<tr>
<td>A509G</td>
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<tr>
<td>V512L</td>
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<tr>
<td>P532L</td>
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</tbody>
</table>

N. Fraction of block

100 ms 2 nA

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Figure 3

A

+60 mV

-80 mV

Kv1.5 T479A

1 nA

0

1

3

30

100 ms

Kv1.5 I502A

1 nA

0

30

100

300

1 mM

Kv1.5 V505A

1 nA

0

10

30

100

B

Normalized current

Concentration of vernakalant (µM)

IC50 (µM)

T479A

WT

I502A

I508A

V505A

C

IC50 (µM)

(5-23)

(5-10)

(3-10)

(3-8)

WT

T479A

I502A

V505A

I508A
Figure 4

A

Kv1.5 WT

Kv1.5 T479A

Kv1.5 I502A

Kv1.5 I508A

Kv1.5 V505A

B

Normalized current

Concentration of flecainide (µM)

IC50 (µM)

C

WT T479A I502A V505A I508A I502L

(6-9) (3-6) (4-6) (3-9) (5-10)

Normalized current

Concentration of flecainide (µM)

IC50 (µM)

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Figure 7

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Molecular Pharmacology Fast Forward. Published on September 14, 2007 as DOI: 10.1124/mol.107.039388
Figure 8

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Figure 9

The figure shows a graph with the x-axis labeled "% Change in IC50 or Block" and the y-axis labeled "% Decrease in drug potency."

- AVE0118 %Bk
- S0100176 %Bk
- Vernakalant %Bk
- Quinidine IC50
- Flecainide IC50

The graph compares the % decrease in drug potency for different compounds, with bars indicating the % change in IC50 or block for each compound.

Chemical structures of the compounds are also shown in the figure.

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Figure 10
<table>
<thead>
<tr>
<th>S5</th>
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<th>S6</th>
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<td>Shaker</td>
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