Ion Channel Trafficking: A New Therapeutic Horizon for Atrial Fibrillation

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Abstract

Atrial fibrillation (AF) is a common cardiac arrhythmia with potentially life-threatening complications. Drug therapies for treatment of AF that seek long-term maintenance of normal sinus rhythm remain elusive due in large part to proarrhythmic ventricular actions. Kv1.5, which underlies the atrial specific I_{Kur} current, is a major focus of research efforts seeking new therapeutic strategies and targets. Recent work has shown a novel effect of antiarrhythmic drugs where compounds that block Kv1.5 channel current can also alter ion channel trafficking. This work further suggests that the pleiotropic effects of antiarrhythmic drugs may be separable. Although this highlights the therapeutic potential for selective manipulation of ion channel surface density, it also reveals an uncertainty regarding specificity of modulating trafficking pathways without risk of off-target effects. Future studies may show that specific alteration of Kv1.5 trafficking can overcome the proarrhythmic limitations of current pharmacotherapy and provide an effective method for long-term cardioversion in AF.

Keywords

Cardiovascular; Atrial fibrillation; Cardioversion; Kv1.5; Trafficking; Channels

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia affecting an estimated 2.2 million adults in the in the United States.[1] AF is caused by the rapid and irregular activation of the atria by electrical sources outside the normal sinus node, and can be classified as paroxysmal, persistent, or long-standing persistent.[2] The occurrence of atrial fibrillation increases dramatically with age, affecting less than 1% of individuals under age 50 to approximately 10% of individuals over age 80.[1, 3] Importantly, over the past two decades the age standardized death rate (per 100,000 in the US) has increased from 27.6 to 69.8.[4] Therefore, AF presents a significant increasing health risk with an untold burden for healthcare costs.

The combination of inefficient atrial contraction and irregular ventricular rate can lead to serious complications. AF related deaths are due primarily to the increased risk of stroke and heart failure. AF is associated with a nearly 5-fold increase in the risk of embolic stroke with nearly one fourth of all strokes in patients over 80 attributable to AF.[5, 6] This increased rate of stroke is due to the rapid, uncoordinated atrial rhythm that leads to inefficient contraction

of the atria, resulting in the pooling of blood in the atria and promotion of thrombo-emboli formation. The presence or absence of several other risk factors significantly contributes to the risk of stroke, such as recent cardiac failure, hypertension, age, diabetes, or a history of stroke or transient ischemic attack (TIA).[7, 8] In addition to an increased risk of stroke, atrial thrombo-emboli can propagate to regions other than the brain, such as the kidneys, mesenteric circulation, or the heart itself where they may induce myocardial infarction. AF also participates reciprocally with several comorbid conditions including congestive heart failure, thyrotoxic heart disease, and hypertension.[9]

Current therapy for AF is aimed at rate control or rhythm control.[10] In rate control, the goal is to maintain the ventricular rate within a physiological range by slowing atrioventricular conduction. In rhythm control, treatment aims to restore normal sinus rhythm through pharmacological cardioversion or through electrical cardioversion or catheter ablation. Catheter ablation is considered a 2nd-line treatment for AF with published success rates of 22-85%. Higher success rates are often seen for patients with paroxysmal AF.[2, 11-13] While approximately half of these patients remain asymptomatic, nearly 30% require a second procedure and 10-25% require additional pharmacological therapy in order to maintain normal sinus rhythm post ablation therapy.[14, 15] However, the long-term effectiveness of this technique remains to be fully determined.[16, 9]

Pharmacological cardioversion makes use of antiarrhythmic drugs targeting cardiovascular ion channels to achieve normal sinus rhythm control in the treatment of AF.[17-20] Pharmacological cardioversion has an advantage over catheter ablation in that it is not invasive, however it has been reported to be less effective.[21] If class I or III antiarrhythmic agents are administered within the first 24 hours of onset of AF, the reported success rate is 47-84%; however, this drops sharply for AF that persists longer than 48 hours in that the antiarrhythmic therapy can only achieve cardioversion in 15-30% of patients.[22]

A common negative side effect for antiarrhythmic drug therapy is proarrhythmia in the ventricles due to non-selective ion channel block and/or overlapping expression of ion channels in both the atria and ventricles. More recently, there has been a shift in both academia and industry to target atrial specific currents such in order to terminate AF and maintain normal sinus rhythm while avoiding proarrhythmic risk in the ventricles. One of the main targets in this research effort is the voltage-gated potassium channel Kv1.5 that underlies the I current. Importantly, I_{Kur} is selectively reported only in the human atria [17, 20, 23, 24] where it contributes to repolarization and participates in the control of action potential duration. In the human atria, Kv1.5 is a predominant channel mediating repolarization and alterations in its expression level have been demonstrated in pathophysiological states such as persistent and paroxysmal AF and chronic pulmonary arterial hypertension.[17, 25] More specifically, there is a marked reduction in Kv1.5 channel protein expression in these pathophysiological states.[26,27, 28] perhaps as an endogenous compensatory mechanism. Given the atrial specific expression of Kv1.5 and its known alterations in cardiovascular disease, it is no surprise that the development of Kv1.5-specific blockers has been a target of both academic and industrial research efforts for the treatment of AF.[29-31] While several compounds have been developed, these antiarrhythmic drugs have been limited by a lack of channel or tissue selectivity, or by poor bioavailability. Therefore, there remains an unmet need for the development of safe new compounds with both atrial selectivity and clinical efficacy for the long-term treatment of AF.

New therapeutic strategies that focus on the regulation of ion channel surface density are emerging from basic research at the bench (Figures 1 and 2).[32, 33] Traditional antiarrhythmic drugs target the ion permeability of channels; however, as highlighted above this approach has not yet yielded a satisfactory outcome. There are two ways to decrease I_{Kur}, through a direct effect on the conduction properties (classically pore block) of channel subunits or through

alterations in surface density of the protein. The steady-state cell surface density of proteins is determined by the balance between the anterograde and retrograde trafficking pathways. Anterograde trafficking ensues only after proper synthesis and processing in the endoplasmic reticulum and golgi, including quality control mechanisms, glycosylation, and post-translation modification (Figure 1).[34] Retrograde movement initiates with endocytosis after which internalized proteins can follow multiple routes to different intracellular fates (Figure 1).[35] One well-recognized fate is the targeting of internalized proteins to lysosomes or proteasomes followed by degradation (Figure 1). Alternatively, trafficking through recycling endosomes allows proteins to return to the plasma membrane and protects them from degradation (Figure 1).[36] Sorting at early endosomes to Rab-GTPase specific compartments is now established as an important event determining the intracellular fate of internalized proteins.[37-39] Another important component of the endocytic machinery regulating protein surface levels is the coordinated movement of molecular motors. In general, protein trafficking is highly coordinated between long-range events, involving the microtubule-based kinesin and dynein motors, and short-range events using unconventional myosin motors.[40-43] There is a significant and growing body of literature about ion channel trafficking from synthesis to sorting to degradation in multiple tissues and cells systems that has been reviewed previously.[44-48] Our discussion will center mainly on recent work focused on the acute modulation of ion channel density at the plasma membrane in the heart where relatively little is known about protein trafficking.

While the precise mechanisms regulating plasma membrane localization and targeting of Kv1.5 in atrial myocytes have not been fully elucidated, several key components and steps are now known. The formation of functional Kv1.5 begins in the endoplasmic reticulum where tertiary folding is coupled to formation of the quaternary structure through tetramerization of the T1 domain in the amino terminus of this channel.[49] The first transmembrane segment (S1) of Kv1.5 has also been implicated in the co-assembly of homo- and heterotetrameric K^+ channels. [50] In addition to protein folding and assembly, subunit composition and post-translational modification can play a crucial role in determining the plasma membrane levels of functional Kv1.5.[51-60] At the plasma membrane, localization to specific membrane microdomains and association with scaffolding proteins into macromolecular signaling complexes may contribute to the stability and biological function of Kv1.5.[61-67] Despite association with scaffolding proteins, Kv1.5 has been shown to undergo dynamic trafficking at the plasma membrane through constitutive internalization and recycling.[68] Internalization of Kv1.5 occurs via a dynein-mediated, microtubule dependent pathway.[68, 69] Following internalization, sorting of Kv1.5 into specific, rab-dependent endocytic compartments determines the intracellular fate of the channel. Specifically, association of Kv1.5 with rab4- or rab11-containing endocytic vesicles is associated with recycling of the channel back to the plasma membrane, whereas association with rab7-containing vesicles denotes channel degradation.[68-70] In addition, ubiquitin modification of Kv1.5 has been described in agreement with data implicating the proteasome in channel degradation.[32, 52, 71-73] As the molecular machinery and endogenous regulatory mechanisms of Kv1.5 surface density begin to emerge, one new therapeutic horizon is the extrinsic manipulation of Kv1.5 surface levels.

The concept of drugs modulating ion conduction and/or surface density of channels it not new. For example, the antiarrhythmic agents ketoconazole and fluoxetine have been shown to reduce hERG density by at least 50% following 48 hours of treatment,[74-77] whereas pentamidine and probucol reduce cell-surface hERG without affecting ion conduction.[77-80] Research into the therapeutic potential of antiarrhythmic drugs that alter channel trafficking has primarily focused on hERG. Alterations in hERG mediated I_{Kr} current, whether drug-induced or a result of the over 200 naturally occurring mutations of this channel, may induce or contribute to the development of long QT syndrome. In particular, long QT syndrome type II which results from retention of misfolded hERG in the quality control pathways of the ER.[81, 82] Nearly 70% of

these mutant channels can be rescued to the plasma membrane by antiarrhythmic drugs such as E4031.[81, 83] These drugs likely act to stabilize misfolded protein through facilitation of quality control machinery in the ER such as Hsc70 and Hsp90 that have been shown to exist in a macromolecular complex with hERG and facilitate its maturation and export from the ER. [82, 84] Thus far research has focused primarily on hERG folding and maturation with little investigation into the molecular mechanisms regulating anterograde trafficking from the ER to the plasma membrane. None-the-less, these studies give credence to the idea that antiarrhythmic drugs may be developed to manipulate specific ion channel trafficking pathways as a novel therapeutic approach for treating cardiac arrhythmias.

Recently, we have reported a previously unrecognized mechanism of antiarrhythmic drug action in the acute modulation of Kv1.5 channel trafficking.[32] Using quinidine, an antiarrhythmic agent which has both class Ia actions[85, 86] and class III actions in mammalian atrium and ventricle, we demonstrated that channel blockers can both inhibit ion conduction and regulate the stability of the channel protein within the membrane. In this study, quinidine resulted in a dose- and time-dependent internalization of Kv1.5, concomitant with channel block. Interestingly, this quinidine-induced internalization of Kv1.5 was found to be subunit-dependent and stereospecific[32] which highlights the possibility for the development of atrial selective agents that specifically modulate surface density.

Drug-induced internalization may occur for channel subunits selectively expressed in the atria. [32] Quinidine exhibits promiscuous block of cardiovascular ion channels, including several members of the Kv channel family. This lack of specificity raised the question as to whether the drug-induced internalization was specific to Kv1.5 or a general mechanism contributing to the block of current from multiple ion channels. To address this specificity, we examined the effect of quinidine on two other prominent cardiovascular potassium channel subunits expressed endogenously in the human atrium and ventricle, Kv4.2 and Kv2.1. Although ion permeability of both channels is blocked by quinidine (IC_{50} of 10 μmol/L and 20 μmol/L quinidine, respectively),[87, 88] neither Kv4.2 nor Kv2.1 internalized in response to any drug concentration tested over the time course studied. This subunit specificity may permit the development of drugs that avoid or reduce undesired proarrhythmic ventricular side effects.

The potential to separate drug-induced channel internalization from pore block stimulates further interest in the design of drugs to modulate trafficking pathways. Within the Kv1.5 channel protein there is partial, but not complete, overlap in the binding sites required for quinidine-induced internalization and pore block (Figure 3).[32] This was reveled through alanine-scanning mutagenesis of four amino acid residues within the highly conserved drug binding site of Kv channels.[89, 90] As with pore block, quinidine-induced internalization was abolished with the T480A and I508A mutations; however, unlike block, Kv1.5-L510A and V512A underwent quinidine-induced internalization indistinguishable from wild-type.[32, 89, 90] Interestingly, Kv1.5-P532L, a naturally occurring mutation (outside of the conserved drug binding site) in atrial fibrillation patients that results in a rightward shift in the dose response curve for quinidine block of channel current[91] caused an equivalent (~10-fold shift in the EC_{50} value) decrease in the sensitivity to quinidine-induced channel internalization.[32] Together, the fact that these mutations alter the open channel block and/or internalization of Kv1.5 demonstrate that quinidine is inducing its effects through direct binding to the channel and not through off-target effects such as mediators of channel trafficking. Importantly, the subunit dependence and incomplete overlap in amino acid requirements indicate that individual components of the Kv1.5 channel structure are critical for the quinidine-induced internalization. However, additional studies of alanine-scanning mutants within the putative conserved drug-binding site are necessary to compare effects on surface density to those reported for pore block (Figure 3). Nevertheless, these data highlight the possibility for

development of new agents that specifically enhance Kv1.5 channel internalization as an alternative and potentially beneficial new therapeutic strategy.

In order to develop new compounds that selectively modulate Kv1.5 surface density, the pharmacophore(s) responsible for pore block and internalization would need to be isolated. The observation that quinine, a diastereomer of quinidine, blocks Kv1.5 current in a dose-dependent fashion without enhancing internalization, suggests that a highly stereospecific binding event is required to induce channel internalization, rather than some non-stereospecific interaction with the cell membrane. Quinidine embodies structural features that enable it to induce endocytosis of Kv1.5. It should be possible to identify individual components of the quinidine structure that are critical for this stereospecific internalization/effect. In addition, these studies should be expanded to modern, more clinically relevant antiarrhythmic drugs. These structural features can be identified through the definition of structure-activity relationships (SAR), and then exploited in the design of novel chemical compounds with greater potency and selectivity for inducing endocytosis vs. direct block of the channel (Figure 3).

Another important therapeutic aspect of quinidine-induced internalization of Kv1.5 is that it is acute and reversible, further suggesting that it may be effective for acute cardioversion. In our study we found that the quinidine-induced internalization was rapid, reaching a plateau within ten minutes. Combined with the fact that this drug-mediated trafficking effect occurred at resting membrane potential in our immunocytochemical assays, these data indicate that the rate-limiting factor for this effect is likely equilibration of drug across the membrane. Importantly, the quinidine-induced internalized Kv1.5 recycled back to the plasma membrane at a rate indistinguishable from constitutive channel recycling. These data further show that the quinidine-mediated trafficking effect results in an acute increase in channel endocytosis, specifically, without altering the endogenous recycling of Kv1.5. If a drug were designed to specifically modulate Kv1.5 channel trafficking it could, in theory, be used to cause a rapid internalization of channel with recovery after drug withdrawal. Therefore, acute modulation of ion channel surface levels may offer the selective advantage for a rapid, reversible decrease in I_{Kur} with a subsequent increase in action potential duration that may terminate acute onset of AF without altering the overall pool of Kv1.5 channel. In contrast, we found that chronic treatment with quinidine diverted Kv1.5 channel to the proteasomal degradation pathway. It is unclear if this chronic decrease in surface expression is advantageous or an unwanted side-effect of the long-term drug treatment. For instance, the time course of recovery from this repression may precipitate drug-withdrawal side effects. Furthermore, long-term suppression of channel expression may contribute to remodeling of heart tissue in which a decrease in Kv1.5 channel protein levels has already been documented. The alternative is that this chronic suppression of Kv1.5 channel protein levels may overcome the current limitations of acute cardioversion which fails to terminate breakthrough AF or adequately subdue the arrhythmogenic substrate and may result in the benefit of maintained rhythm control.

An important finding that goes beyond the therapeutic potential of this approach is that the drug-induced trafficking effect is calcium-dependent and raises significant issues for drug safety screening.[32] The calcium-dependent trafficking component was responsible for a 3-fold shift in the dose response for quinidine and is therefore critically important when considering the *in vivo* effects of this drug and likely many others. It has been well established that non-antiarrhythmic drugs can have proarrhythmic effects through off target inhibition of cardiovascular ion channel currents. This has been reported for psychiatric drugs, antihistamines, antimicrobials and other compounds, many of which can induce QT prolongation with subsequent risk of torsade de pointes.[92] As a result, pharmaceutical companies and regulatory agencies mandate that cardiac ion channel testing be part of the drug safety profiling of all new compounds. Current drug safety profiling is almost entirely focused

on a drug's capacity to block ion conduction. This is commonly tested through electrophysiological measurement of current in the presence or absence of drug, using a calcium chelating agent to isolate the drug-channel interaction. Therefore antiarrhythmic, and non-antiarrhythmic, compounds that alter ion channel trafficking may show greater efficacy and potency *in vivo* than can be predicted by current drug safety profiling. This may result in a dramatic underestimation of the desired clinical effect and/or undesired side effects of a drug.

The results discussed here highlight the potential for development of new agents that selectively modulate ion conduction and/or the stability of channel protein in the membrane. In addition, manipulation of the trafficking of functional membrane channel may provide an avenue for developing new therapies with atrial selectivity and clinical efficacy and safety for the treatment of AF and potentially other cardiovascular arrhythmias.

Acknowledgments

Abbreviations

AF	Atrial fibrillation
TIA	Transient ischemic attack
SAR	structure-activity relationships

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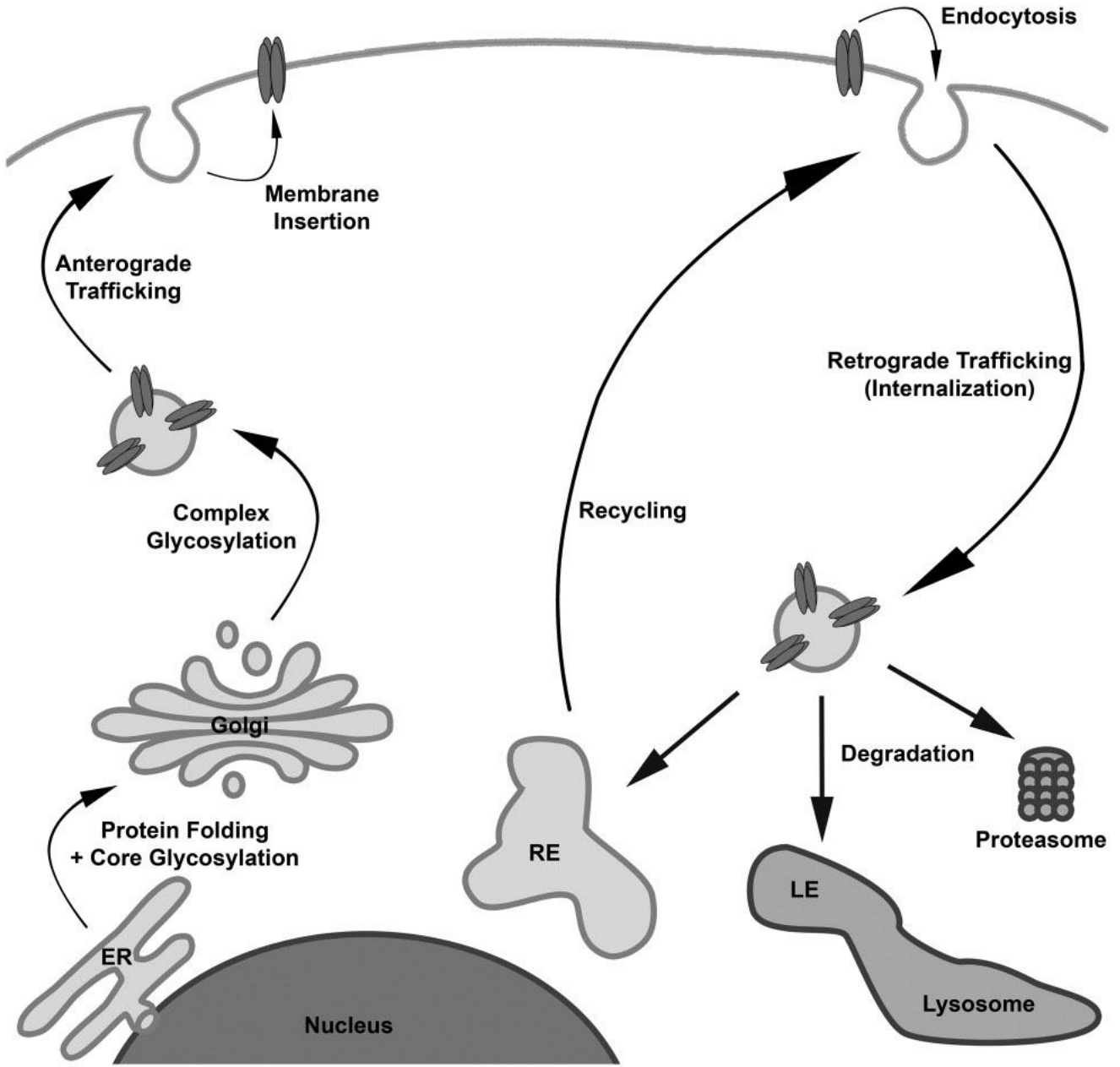
Figure 1.
Potential therapeutic intervention points in the trafficking of membrane proteins. Each arrow represents a regulatory step in the trafficking of membrane proteins that could serve as a potential therapeutic target for modulating steady-state cells surface levels of ion channels. The left half of this figure represents an area where much work has been done in the hERG field for the treatment of LQTS and other arrhythmias. The right half represents and exciting developing field for the regulation of Kv1.5 membrane levels in the treatment of atrial fibrillation. Endoplasmic Reticulum (ER); Recycling Endosome (RE); Late Endosome (LE).
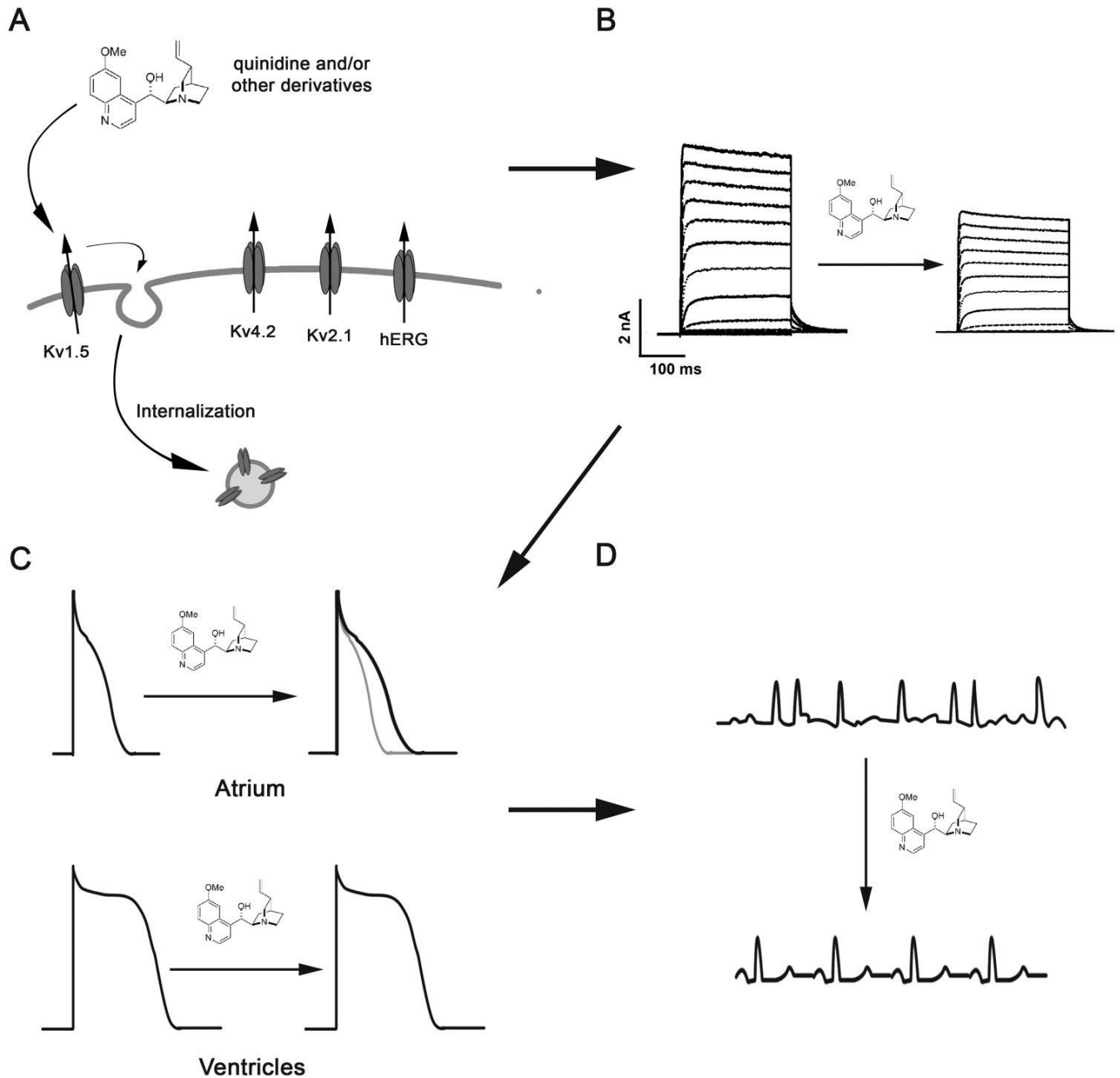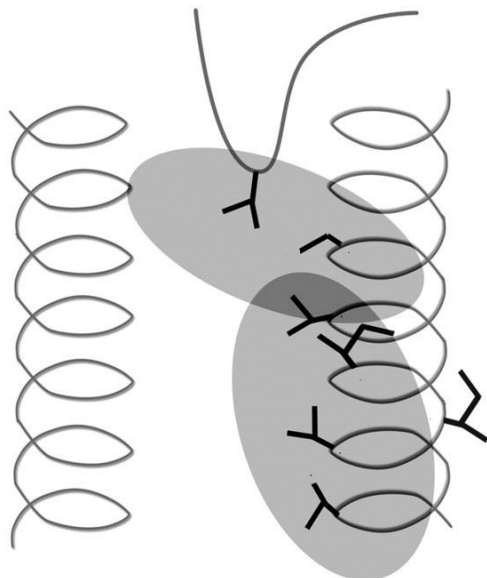
Figure 2.
Antiarrhythmic drug-induced internalization of atrial specific Kv1.5 as a novel therapeutic target for AF. **(A)** Drug-induced internalization is specific to the atrial potassium channel Kv1.5. **(B)** Kv1.5 specific internalization results in a decrease in I_{Kur} density. **(C)** Decreased I_{Kur} may result in an increase in atrial, not ventricular, action potential duration. **(D)** Increased atrial action potential duration may terminate atrial fibrillation and restore normal sinus node rhythm control.

Structure Activity Relationships for Channel Protein and Drug Molecule

1 Overlapping Sites for Pore Block and Channel Internalization within Kv1.5

2 Dissecting the Pharmacophore for Drug Block versus Channel Internalization
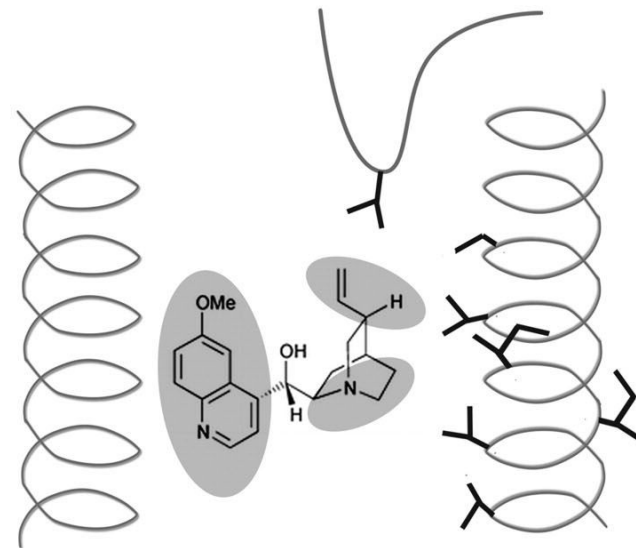
Figure 3.
Strategies to isolate drug-induced internalization from pore block. Cartoon representation of the potential drug binding site of the Kv1.5 channel pore. **(1)** Ion channel mutagenesis can be used to fully characterize the incomplete overlap in the drug binding site for pore block and channel internalization. **(2)** Multiple moieties within the quinidine molecule can be studied in structure activity analysis to identify the pharmacophore for drug-induced internalization.