Antiarrhythmic Drug Target Choices and Screening

Michael C. Sanguinetti, Paul B. Bennett

Abstract—Most antiarrhythmic drugs are ion channel blockers, and to date, those tested in large randomized placebo-controlled clinical trials have shown no decrease in mortality outcome. This apparent lack of survival benefit may result from the significant liabilities associated with these agents that offset any long-term benefit. Despite the current success of implantable defibrillators and the future promise of gene therapy, there is still a pressing need for new antiarrhythmic drugs. An improved understanding of cardiac ion channels and novel approaches to target selection and compound screening will provide new opportunities for drug discovery in the near future. Here, we briefly review the multiple mechanisms of arrhythmia, the history of drug failures, and the possibilities that evolving technologies may provide in the search for more efficacious and safer antiarrhythmic drugs. (Circ Res. 2003;93:491-499.)

Key Words: antiarrhythmic drugs • arrhythmia • ion channels

Antiarrhythmic drugs have had limited overall success and can sometimes induce arrhythmia. Efficacy in suppressing arrhythmias is generally between 30% and 60%, and no one agent shows superiority, with the possible exception of amiodarone. Short-term antiarrhythmic benefit has been found to be offset by (depending on the specific drug) neutral or negative effects on mortality. These failures were due to multiple causes, including the facts that arrhythmias do not share a common mechanism, patient selection favored those with severe disease, and drug selection was not optimal.

Most antiarrhythmic agents are ion channel blockers and are classified on the basis of their effects on the cardiomyocyte action potential (AP). Class I agents block Na⁺ channels, decrease the rate of phase 1 depolarization of APs, and slow the rate of impulse conduction throughout the heart. Class II agents are β-adrenergic receptor antagonists that prevent the activation of adenylate cyclase and the increase in intracellular cAMP that normally occurs in response to enhanced sympathetic nervous tone. Class III agents are usually K⁺ channel blockers that prolong AP duration (APD) and increase the period of time that cardiac muscle is refractory to a premature electrical stimulus. Class IV agents slow atrioventricular nodal conduction, usually by block of L-type Ca²⁺ channels, which mediate the upstroke of nodal cell APs. The molecular identity of specific ion channels that
are affected by antiarrhythmic agents has been recently recognized (eg, human ether-a-go-go related gene [hERG] channels are blocked by dofetilide, a class III drug); however, most drugs affect numerous ion channels or have distinct kinetics of block that frustrate simple classification. Drug choice for patients is often made on an individual basis by trial and error and consideration of side effects, usually without special attention to the class of compound.

Ventricular tachycardia (VT) and ventricular fibrillation (VF) are a major cause of death in patients with myocardial infarction and a reduced left ventricular ejection fraction (LVEF). Thus, although it is not surprising that most large-scale clinical trials of ion channel–blocking drugs have focused on patients with these preexisting disorders, these cardiac abnormalities appear to accentuate the proarrhythmic tendencies of drugs previously shown to be relatively safe in preclinical animal studies and phase 1 clinical trials of normal subjects. Diseased hearts probably fibrillate more easily because of an abnormally high level of anatomic and electrophysiological heterogeneity. Therefore, it is not too surprising that clinical trials of ion channel blockers in these high-risk patients have demonstrated increased mortality (Cardiac Arrhythmia Suppression Trial [CAST] and Survival With Oral d-Sotalol [SWORD]), lack of effect (Danish Investigations of Arrhythmia and Mortality on Dofetilide [DIAMOND]), or only slight reductions in relative risk (European Myocardial Infarct Amiodarone Trial [EMIAT]).

CAST studied the suppression of ventricular ectopy with three class Ic antiarrhythmic agents: encainide, flecainide, and moricizine. Encainide and flecainide were discontinued because of excess arrhythmic mortality or acute recurrent myocardial infarction with shock. In the SWORD study of 3121 patients with LVEF ≤40%, the class III antiarrhythmic agent d-sotalol was also found to increase arrhythmic death, from 3.1% to 5.0%, compared with the placebo group. The DIAMOND trial found that dofetilide, a selective hERG K+ channel antagonist, converted and protected against the recurrence of atrial fibrillation (AF) in patients with congestive heart failure but had no effect on mortality. EMIAT assessed whether amiodarone reduced mortality and arrhythmic death in survivors of myocardial infarction with LVEF ≤40% in 1486 patients. Amiodarone is called a class III agent because it prolongs APD and the QT interval after chronic administration, but it blocks many types of ion channels. Unlike encainide, flecainide and d-sotalol, amiodarone did not alter all-cause or cardiac mortality, but it did reduce arrhythmic deaths by 35%. Thus, with the possible exception of amiodarone, large-scale efficacy studies of ion channel blockers in high-risk patients have had disappointing survival outcomes. In contrast, the β-adrenergic receptor blocker propranolol (a class II agent) reduced post–myocardial infarction mortality from 10% to 6% in a 2-year study involving almost 4000 patients, indicating that some drugs can reduce the risk of arrhythmia. Together, the results of these trials raise the issue of whether cardiac ion channels are inherently flawed drug targets or whether our understanding of ion channels and their role in arrhythmias has been inadequate. Do existing drugs have kinetic features of block that increase electrophysiological heterogeneity and are therefore proarrhythmic? Can we identify novel targets involved in disease-associated electrical remodeling or develop novel drugs that modulate the usual suspects (Na+, L-type Ca2+, and delayed rectifier K+ channels) with more favorable kinetics of interaction and thereby less proarrhythmic activity? Recent advances suggest that we can.

**New Antiarrhythmic Drugs Are Needed**

Sudden cardiac death is the most common cause of mortality in the United States, and it is usually associated with heart failure and caused by VF. Theoretically, the most effective antiarrhythmic therapy would be drugs or procedures aimed at prevention of the structural and metabolic abnormalities of the heart that predispose to life-threatening cardiac rhythms. For example, prevention of coronary heart disease with cholesterol-lowering drugs could dramatically reduce the risk of arrhythmias associated with myocardial infarction, and proper diet and regular exercise would likewise decrease the incidence of arrhythmias associated with heart failure. Unfortunately, preventative approaches are largely inconsistent with current medical practice and lifestyle choices and therefore are considered only a partial solution. Moreover, some arrhythmias are caused by inherited gene mutations and may not be amenable to such preventive measures. Implantable cardiac defibrillators are very effective at terminating fibrillation and preventing sudden death (eg, Multicenter Automatic Defibrillator Implantation Trial [MADIT] and Antiarrhythmics Versus Implantable Defibrillators [AVID] clinical trials). These devices have large psychological and lifestyle negatives and, because of their expense, are unavailable to the vast majority of the world’s population at risk.

Somatic gene transfer is another promising new alternative therapeutic approach for the treatment of arrhythmia. For example, KCNE3 is a β-subunit that can coassemble with KCNQ1 α-subunits and enhance outward current. Mazhari et al reported that 3 days after the injection of adenovirus-expressed KCNE3 into the ventricular cavity of guinea pigs, APD was shortened and the QT interval was reduced. If it is assumed that uniform and long-lasting ectopic expression can be achieved, this approach could be useful in treating long QT syndrome. Viral gene transfer has also been used to convert quiescent ventricular myocytes into pacemaker cells, a potential alternative to implantable electronic devices. Although devices are clearly effective and gene therapy has great promise, there is an obvious and continuing need for effective drugs that can be used to prevent and/or treat arrhythmias.

The multiple mechanisms of rhythm disturbances (discussed below) indicate that discovery of a “silver bullet” therapy is an unrealistic goal and that multiple types of drugs with different modes of action are needed. The discovery and development of drugs that can effectively treat arrhythmias with distinct etiologies presents a daunting challenge. Achieving this lofty goal will require a more sophisticated understanding of the mechanisms of arrhythmia and technologies that will enable detailed clinical diagnosis at the patient level. However, despite recent advances in molecular and cellular cardiology and refined electrical mapping techniques, there is still no effective method to accurately diagnose the underlying mechanism of most arrhythmias. Thus, although individualized medicine would be ideal, practical consider-
ations demand more modest goals, such as the development of drugs that affect novel targets or a combination of drugs that affect multiple targets and therefore have a broader spectrum of action.

Cardiac Arrhythmia: A Complex and Heterogeneous Disorder

Atrial and ventricular arrhythmias are commonly believed to arise primarily from inappropriate automaticity or reentrant excitation. Electrical excitation of the heart usually proceeds as a planar wave. Breakup of the planar wave of excitation (Figure 1) can lead to a spiral (2D) or scroll (3D) wave that initiates VT. Once initiated, an arrhythmia can be sustained by continuous conduction of an electrical wavefront around a nonexcitable obstacle if the path length is sufficiently long so that the excitation wavefront does not encounter refractory tissue. This can be anatomic (eg, scar tissue) or functional (eg, tissue refractory to excitation).15 In a reentrant circuit, the activating wavefront “chases” its relative refractory tail, which is separated by tissue that is excitable, the so-called excitable gap. The excitable gap can vary in length; this variation has consequences for the type of antiarrhythmic agents that will terminate the tachycardia. VT is a rapid, organized process in which the excitation travels a well-defined circuit. VF is seemingly a chaotic electrical activity resulting from the random and aperiodic propagation of multiple independent wavelets through the muscle. On the basis of these distinguishing characteristics, VF and VT have traditionally been viewed as two widely distinct processes. New data with high-resolution mapping techniques have provided insights into the mechanisms of VT and VF. In one approach, the application of nonlinear dynamic theory to the problem of wave propagation in the heart has led to the view that VT and VF are different expressions of a single mechanism. In this theory, self-organized rotating electrical spiral waves (rotors) result in either VT or VF, depending on the frequency of rotation and the resulting interaction of wave fronts with the cardiac muscle. In this model, monomorphic VT results from a stationary rotor with a rotation frequency that allows continuous (1-to-1) excitation of both ventricles.

VF results from either a single rapidly drifting rotor or a high-frequency stationary rotor, giving rise to numerous areas of intermittent conduction blockade.16 Davidenko et al17 used a potentiometric dye in combination with imaging to demonstrate, in real time, waves rotating around small arteries or bands of connective tissue in the ventricle. It was subsequently proposed that multiple wave breaks lead to multiple spiral waves or rotors, the most likely mechanism of VF initiation.18 Maintenance of fibrillation is mediated by multiple wavelets that require continual wave breaks or a single stable and rapid rotor. The breakup of spiral waves can occur in the absence of any cardiac disease–related anatomic and electrophysiological heterogeneity and is related to electrical restitution, the relationship between APD and diastolic interval (DI). The time between the end of one AP and the beginning of the next defines the DI. When cardiac tissue is electrically paced at higher rates, DI is reduced, and the AP alternates between short and long durations. APD alternans occurs only when the slope of the APD restitution relation is \( \frac{dAPD}{di} \). As discussed elsewhere,\%19 reducing the slope of the APD restitution relation to a value \(<1\) will stabilize spiral waves (allow VT but not additional wave breakup) and prevent the initiation of VF. Weiss et al\% have proposed a hypothetical relationship between dynamic instability (eg, alternans) and preexisting heterogeneity (eg, VT associated with an infarct or cardiomyopathy) that is a useful way to visualize how multiple factors interact to determine whether VT will degenerate into VF (Figure 2).
Normal hearts have a low level of preexisting heterogeneity and can tolerate a relatively high level of dynamic instability without VT converting to VF. In contrast, the diseased heart has abnormal electrical and anatomic heterogeneity that more readily allows conversion of VT into VF when APD restitution is increased. Experimental evidence indicates that reducing the slope of the APD restitution relationship to a value < 1 by drugs such as verapamil and bretylium can prevent VF\textsuperscript{20,21}; however, these drugs have serious side effects, especially decreased cardiac contractility. Recently, other pharmacological approaches to suppress APD alternans have been proposed. Gilmour\textsuperscript{19} has suggested the activation of rapid delayed rectifier K\textsuperscript{+} current (I_{Ks}) as a mechanism to modestly reduce APD and increase DI without affecting the height of the plateau phase. Maintaining a relatively normal plateau phase is important because it could avoid the decrease in contractility associated with using Ca\textsuperscript{2+} channel blockers as a means to shorten APD and lengthen DI. As a cautionary note, it should be remembered that 10 to 15 years ago, canine models indicated that I_{Ks} blockers were exceptionally effective at suppressing the induction of acute ischemia-induced VF in the setting of prior myocardial infarction.\textsuperscript{22,23} A finding that was later trumped by the rare but significant proarrhythmic activity of I_{Ks} blockers. Thus, although I_{Ks} agonists have theoretical advantages over existing drugs for the prevention or suppression of VF, proof-of-concept experiments are needed before pharmaceutical companies are likely to expend significant resources in the discovery and development of such drugs.

Arrhythmias can also be induced as an unintentional side effect of drug therapy. For example, AP prolongation by class III drugs can suppress cardiac arrhythmias by the lengthening of the tissue refractory period and the elimination of an insufficient experience to conclude that all Na\textsuperscript{+}, Ca\textsuperscript{2+}, or hERG channel block might also be less proarrhythmic in the absence of isoproterenol.\textsuperscript{33} Thus, a combination of \beta-adrenergic receptor blockade plus I_{Ks}/I_{Ko} blocking activity, either in a single molecule or achieved by combination drug therapy, might be an effective antiarrhythmic therapy.

Recent studies by Hondeghem and colleagues\textsuperscript{34,35} have also suggested that prolongation of APD is not inherently proarrhythmic. The cardiac electrophysiological effects of 702 chemicals known to block hERG channels were studied in rabbit Langendorff-perfused hearts. Beat-to-beat variability of APD, reverse frequency dependence of AP prolongation, and triangulation of AP repolarization were found to be causally related to the induction of polymorphic VT. In contrast, agents that prolonged APD without instability (ie, APD alternans) were antiarrhythmic. A drug that combines L-type Ca\textsuperscript{2+} and hERG channel block might also be less proarrhythmic.\textsuperscript{36} Together, these recent findings suggest that block of I_{Ko} is not proarrhythmic, per se, but that the specific mechanism of ion channel modulation and effects on other channels are critical.
Voltage-gated channels evolved from common ancestors and share homology in some regions. Among voltage-gated potassium (Kv) channels, the S5-P-S6 (pore) domain is relatively well conserved. Pore blockers usually bind to specific residues that line the central cavity of Kv channels. Although a single amino acid difference is sufficient to confer a unique pharmacological identity, it may be more probable to identify unique channel-selective agents that affect less conserved regions involved with gating. For example, an agent that delays or alters channel opening in this manner could produce the desired change in tissue refractoriness but with greater selectivity. Another example, R-L3, is a benzo-diazepine that enhances the KCNQ1 current37 and affects gating by binding to a site outside the central cavity. Recent studies have identified regions of Kv and other channels that show significant gating changes by interaction at regions away from the ion conduction pore.58–40 This approach has not yet been widely exploited. Other possibilities are to target specific interacting proteins (eg, β-subunits) or signaling molecules.

In principle, an agent that acted only in affected tissue (depolarized or rapidly firing) or on demand during a tachyarrhythmia would have an improved margin of safety. This is the rationale behind the development of voltage-, use-, or rate-dependent channel antagonists. In its most complete form, this would require agents that allosterically modulate ion currents, such as depicted in a modulated receptor hypothesis model.41 Pore blockers that become trapped inside the central cavity when the channel closes may show use dependence but may not dissociate rapidly enough at diastolic membrane potentials. An allosteric modifier could have high affinity for the channel under some conditions but low affinity (and, hence, rapidly dissociate) at diastolic membrane potentials. The kinetics of dissociation under physiological or pathophysiological conditions would have to be such that little inhibition occurred at normal heart rates but would accumulate during tachyarrhythmias. This mechanism may afford some functional selectivity for the channels in question and may avoid untoward actions in tissues that do not cycle electrically. It has been impossible to rationally develop channel state–dependent drugs with any certainty because of the lack of appropriate assays (ie, voltage clamp) that are of high enough capacity to measure drug-channel interactions with the volume of compounds needed for a drug development campaign. New high-throughput electrophysiological methods42,43 will greatly aid these efforts.

It is commonly believed that more selective agents will translate into greater efficacy and less side effects. However, this is not necessarily true. Amiodarone is argued to be the most effective antiarrhythmic agent in use, yet it is one of the least selective. It prolongs APD and the QT interval, but TdP is rare, emphasizing that not all drugs that prolong QT cause arrhythmias. Perhaps the efficacy of amiodarone derives from multiple molecular targets. If so, it will be exceedingly difficult to rationally discover newer agents with combined mechanisms because we do not know how to optimize chemical structures for the correct molecular targets. Drug development on a single target (eg, the L-type Ca\(^{2+}\) channel) is feasible, but it is complicated by the need to optimize for off-target safety, pharmacokinetics, and metabolism. Creating novel agents with all of these attributes and the appropriate affinity and kinetics for several ion channels will be quite challenging. One approach being used is to combine agents with desirable properties (eg, a Ca\(^{2+}\) channel antagonist and an AP-prolonging agent). The logistics of this approach are daunting as well. The pharmacokinetics, distribution, and metabolism of each agent will likely be different, making it difficult to achieve the appropriate exposure for each drug. More sophisticated molecular modeling and detailed protein structure of the target channels would facilitate compound discovery and optimization.

In summary, drugs modulating channel function rather than simply blocking the pore or drugs targeting multiple ion channels (eg, amiodarone) might be superior therapeutic agents if their safety margins can be improved. These approaches are worthy of further consideration.

New Molecular Targets to Treat Arrhythmia

Alteration of Electrical Remodeling

Tachycardia-induced electrical remodeling plays an important role in the maintenance and recurrence of AF, and Ca\(^{2+}\) overload may be an important mediator of these changes. Nattel,44–46 Morillo et al,47 and Allessie’s group (Wijffels et al48 and Tieleman et al49) have investigated the natural history of AF and put forward the concept that AF begets AF, meaning that the likelihood of remaining in AF increases with the time spent fibrillating. This observation suggests that the molecular and cellular substrates for AF become established over time. Furthermore, it seems likely that early intervention to terminate AF is important. Electrical remodeling involves changes in ion channel expression, but the cause of these changes is unknown, and several factors may be involved. Ca\(^{2+}\) overload may be an important mediator of these changes. Fareh et al50 investigated the partially selective T-type (mibebradil) and L-type (diltiazem) Ca\(^{2+}\) channel antagonists for their potential to prevent tachycardia-induced atrial remodeling in canine hearts. They found that mibebradil protected against the atrial remodeling caused by 7-day atrial tachycardia; however, diltiazem had no effect. These findings are important for understanding the role of Ca\(^{2+}\) channels and intracellular Ca\(^{2+}\) as potential mechanisms of clinically relevant atrial tachycardia–induced electrical remodeling. The observations with diltiazem suggest that L-type Ca\(^{2+}\) current inhibition alone is not sufficient to prevent heart rate–dependent electrical remodeling. Because mibebradil affects both L-type and T-type Ca\(^{2+}\) currents as well as other targets, the results do not exclude the possibility that inhibition of specific Ca\(^{2+}\) channel types is required to prevent remodeling. It may be that the prevention of Ca\(^{2+}\) overload during prolonged tachycardia (>24 hours) requires the inhibition of both Ca\(^{2+}\) entry pathways. However, complete inhibition of Ca\(^{2+}\) channels would be lethal, so it is difficult to reconcile these findings. It does not seem likely that T-type channels play a predominant role in Ca\(^{2+}\) entry in atrial tissue, although little is known about their specific roles or presence in various tissues or species. It is well appreciated that each L-type Ca\(^{2+}\) channel antagonist (eg, verapamil, diltiazem, amlodipine, and
nifedipine) has a unique pharmacodynamic profile. Indeed, even within a chemical class (eg, dihydropyridines), agents can have very different profiles against the Ca\(^{2+}\) channel, not to mention any off-target activities that are often ignored. For example, amlodipine, felodipine, and nifedipine have very different kinetic and selectivity profiles. Furthermore, it has been reported that amlodipine affects NO systems\(^{51}\) and that nifedipine can increase neurotransmitter release independent of Ca\(^{2+}\).\(^{52}\) Thus, ancillary activities of probe drugs can cloud data interpretation.

An intriguing possibility is that Ca\(^{2+}\) conducted through T-type channels may have privileged access to proteins regulating DNA transcription, as occurs with Ca\(^{2+}\) signaling pathways in neurons. Ca\(^{2+}\) entry through P/Q-, N-, and L-type Ca\(^{2+}\) channels or glutamate receptor channels affects different compartments and systems within the same neuron.\(^{53–57}\) Ca\(^{2+}\) entry through N-type channels promotes transmitter release, whereas L-type channels in the same cells are implicated in transcriptional changes. These hypotheses warrant further testing with more selective T-type antagonists and with other L-type channel antagonists.

**T-Type Ca\(^{2+}\) Channels**

T-type Ca\(^{2+}\) channels represent a novel therapeutic target for treatment of hypertension, chronic stable angina, or ischemic heart disease. As discussed above, they may play a role in atrial arrhythmias as well,\(^{50}\) but this remains to be established. T-type channels are expressed in blood vessels, sinoatrial nodes, kidneys, and adrenal glands, although the role of the channel in some of these tissues is not fully known. They are claimed to be involved in cardiac pacing (sinoatrial node),\(^{58,59}\) but they play no role in the working myocardium; they are localized in some vascular smooth muscle by electrophysiology and RNA distribution (eg, renal), but the full localization is yet unknown.\(^{50}\) They are also known to be the molecular/cellular mechanism for aldosterone synthesis/secretion coupling in adrenal glomerulosa.\(^{61}\) Target validation derives to a large degree from widespread clinical experience with mibebradil (Posicor, Hoffmann-La Roche) a partially T-type–selective Ca\(^{2+}\) channel antagonist that initially proved remarkably safe and effective. Mibebradil inhibits both T-type (CaV3) and L-type (CaV1.2, \(\alpha_{1c}\)) Ca\(^{2+}\) channels, with some selectivity for T-type over L-type channels.\(^{62–64}\) Mibebradil was first approved in 1997 for hypertension, angina pectoris, and congestive heart failure. It was withdrawn from the market a year later because it inhibited cytochrome P-450s, which caused negative drug-drug interactions, especially with statins. The reasons for withdrawal were unrelated to T-type antagonism. Further elucidation of a role for the T-type Ca\(^{2+}\) channel awaits a better characterization of its role in various tissues and more selective drugs.

**Modulation of Intracellular Ca\(^{2+}\)**

Protein kinases regulate a diverse array of target proteins in the cardiovascular system. For example, activation of cAMP-dependent protein kinase A (PKA) or CaM kinase II affects L-type Ca\(^{2+}\) channels and internal Ca\(^{2+}\) stores, thus increasing [Ca\(^{2+}\)].\(^{55–68}\) Increases in [Ca\(^{2+}\)], are implicated as a cause of afterdepolarizations, which are due in part to enhanced L-type Ca\(^{2+}\) current and [Ca\(^{2+}\)], overload.\(^{27,69}\) Ryanodine and flunarizine, both inhibitors of SR Ca\(^{2+}\) handling, prevent some arrhythmias.\(^{70,71}\)

Afterdepolarizations are associated with sympathetic nervous system activation through PKA activity.\(^{72–74}\) \(\beta\)-Adrenergic receptor antagonists prevent agonist-mediated increases in PKA activity, they are clinically useful, and they reduce sudden cardiac death.\(^{75}\) An association between triggered arrhythmias and protein kinase activation (eg, PKA or CaM kinase II) suggests that kinase inhibition may be antiarrhythmic. CaM kinase activity increases during AP prolongation, and CaM kinase inhibition prevents afterdepolarizations without shortening APD in isolated hearts.\(^{77}\) These findings suggest that if appropriate subtype targets or limited exposure could be achieved, CaM kinase inhibition may be a reasonable and novel approach for antiarrhythmic therapy. The ubiquitous nature of these kinases and the lack of tissue-specific targets raise serious questions about the feasibility and practicality of treating arrhythmias through the administration of inhibitors. Nevertheless, a drug that modestly prolonged APD without inducing afterdepolarizations and TdP would be a major advance.

PKA also phosphorylates the Ca\(^{2+}\)-release channel located on the SR. This channel (the cardiac ryanodine receptor [RyR2]) is required for cardiac excitation-contraction coupling, but it can also contribute to arrhythmogenesis in heart failure. In the diseased heart, RyR2 channels may fail to close normally during diastole, leading to an aberrant rise in [Ca\(^{2+}\)], and the initiation of DADs. The FK506 binding protein (FKBP12.6) is normally complexed with RyR2 and stabilizes the closed state of the channel. Phosphorylation of RyR2 causes dissociation of FKBP12.6 and allows channel-mediated Ca\(^{2+}\) release. Mutations in RyR2 cause a rare arrhythmia called catecholaminergic polymorphic VT.\(^{76}\) These mutations reduce the affinity of FKBP12.6 for RyR2,\(^{77}\) leading to abnormal leakage of Ca\(^{2+}\). FKBP12.6–/– mice are susceptible to fatal exercise-induced ventricular arrhythmias, and cardiac myocytes isolated from the mice exhibit isoproterenol-induced DADs.\(^{78}\) Dysfunction of the RyR2 channels in heart failure may contribute to arrhythmias in other ways. Inhibition of RyR2 causes variations in [Ca\(^{2+}\)], and leads to Ca\(^{2+}\) transient alternans,\(^{79}\) suggesting a mechanistic link between electromechanical alternans and VF.\(^{80}\) Thus, increasing FKBP12.6 binding affinity to phosphorylated RyR2 to decrease Ca\(^{2+}\) leak from the SR into the cytoplasm or direct modulation of RyR2 gating by drugs without disruption of normal function may provide new therapeutic approaches for the prevention of VT/VF in heart failure.

**Tissue-Specific Channel Blockers**

Arguably, treatment of atrial arrhythmias may benefit from the development of atrium-selective agents. This would avoid potential life-threatening drug-induced ventricular proarrhythmia. This strategy depends on the identification of atrium-specific ion channels. It is possible that tissue-specific modifier proteins (\(\beta\)-subunits) or splice variants of channels will be discovered in the atria, but whether they can be distinguished pharmacologically is an open question. Alternatively, Kv1.5 K\(^{+}\) channels,
which are expressed in atria but not ventricles, may be viable targets.\textsuperscript{8,9} Inhibition of Kv1.5 prolongs atrial refractoriness but has no effect on the ventricle. The functional roles of Kv1.5 in other tissues in which it is highly expressed (eg, smooth muscle\textsuperscript{90}) are not well understood, and whether appropriate efficacy and safety might be achieved with blockers of this channel in humans is unknown.

**Traditional and High-Throughput Methods of Antiarrhythmic Drug Screening**

In the past 20 years, antiarrhythmic drugs were typically discovered using in vitro screens that monitored changes in the maximum upstroke velocity or duration of APs or the contractility of isolated cardiac tissue. Compounds that had the desired effect of slowing maximum upstroke velocity (class I activity), lengthening APD (class III activity), or shortening APD and decreasing contractility (class IV activity) were subsequently evaluated for efficacy in isolated heart or intact animal models of arrhythmia. These approaches were gradually replaced or supplemented by measurement of ionic currents in single myocytes using whole-cell voltage-clamp techniques. However, all of these assays were tedious, had low capacity, and tended to identify “me-too” drugs that had all the same problems as previous drugs from the same class. Little attention was paid to the kinetics of block or whether the drugs altered the properties of channel gating. In the past decade, most of the traditionally targeted ion channels were cloned, enabling more refined tests of specificity and better structure-activity relationships. The trend was to discover compounds with a single predominant mechanism of action, culminating in the development of relatively specific blockers of T-type Ca\textsuperscript{2+} channels and the ultra-rapid (I \textsubscript{Kur}, Kv1.5), rapid (I \textsubscript{Kir}, hERG), and slow (I \textsubscript{Ks}, KvLQT1+minK) delayed rectifier K\textsuperscript{+} channels.

Emerging technologies for ion channel studies are based on the fact that the best way to measure channel behavior is voltage clamp. These approaches aim to automate and industrialize the patch voltage-clamp method. Some companies, such as CeNeS (now Xention), Sophion (Apatchi-1), and Flyion, have used variants of the classic electrode-based methods. Other companies (eg, Axon Instruments, Molecular Devices, Nanion, Sophion, and Cytocentrics) have adopted planar array chip–based approaches. For example, Axon Instruments, recently presented PatchXpress 7000A, an automated parallel patch-clamp system for use in ion channel drug discovery. The PatchXpress records ion channel function by patch-clamping up to 16 cells at a time on a planar electrode array. These arrays of planar electrodes provide numerous advantages relative to conventional patch-clamp electrodes, making them amenable to a stable multwell format for testing multiple cells, and include configurable electronics and fluidic systems.

The objective of microchip-based patch-clamping is to replace traditional patch electrodes with a planar array of miniaturized recording interfaces. Two chambers are separated by a substrate (silicon, polymer plastic, or glass) with a micrometer-sized hole. Cells are pulled into the holes to form high-resistance seals. This design eliminates the need for an electrode micromanipulation system, improves stability, and, therefore, has the potential to achieve high throughput. Kiss et al\textsuperscript{101} and Schroeder et al\textsuperscript{102} have described and used a plate-based electrophysiological measurement platform. The instrument is an integrated platform that consists of computer-controlled fluid handling, recording electronics, and processing tools capable of whole-cell voltage-clamp recordings from thousands of individual cells per day (Figure 3).

Advances in the techniques discussed here have been fueled by the drug discovery industry, in which increased sensitivity of ion channel assays is required to study these complex voltage- and state-dependent proteins. These demands have pushed the development of automated electrophysiology forward to allow greater numbers of compounds screened and to provide higher information content.

**Conclusions**

The last decade has provided an unprecedented wealth of information on the “parts list” of the human organism. New ion channels have been discovered, and their roles in cardiac function have been elucidated, yet the full functional wiring diagram for physiological and pathophysiological states is in a rudimentary form. It is with this information that we attempt to have an impact on existing diseases and syndromes. Electrical excitability and its disorders are particularly challenging because of circuit complexity, feedback loops, evolving disease states, and multiple risk factors. Practical considerations include identification of an antiarrrhythmic target molecule (receptor, enzyme, channel, or vulnerable parameter) and the development of assays to drive quantitative chemical structure-activity relationships. Because of the complex dynamics of arrhythmias, a completely reductionist approach does not fully address the problem. Nevertheless, a molecular approach is one that we must pursue for ultimate success. As a practical matter, thousands of chemical entities...
must be screened in a search through chemical space to identify a few candidate drugs. Massive screening of ion channels is challenging because of their complexity, but evolving technologies will aid in the discovery of new ion channel–directed drugs. The molecular, functional, and technical advances made recently have led to significant insights, and proteins that modulate ion channel function (eg, FKBP12.6 and CaM kinase II) represent novel targets. Nonetheless, ion channels remain important drug targets for arrhythmias. Understanding their complexity and diversity will lead to novel drugs for the prevention and treatment of arrhythmias.

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References

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