New Approaches to Antiarrhythmic Therapy, Part II
Emerging Therapeutic Applications of the Cell Biology of Cardiac Arrhythmias

Members of the Sicilian Gambit

Abstract—Cardiac arrhythmias complicate many diseases affecting the heart and circulation, and they incorporate a multiplicity of underlying mechanisms. The evolution of scientific knowledge has made the complex changes produced by cardiovascular disease sufficiently understood at the organ, cellular, and molecular levels such that there is a diversity of therapeutic targets for pharmacological therapy and/or prevention. Moreover, the approach of rational drug design in mechanism-specific and disease-specific fashions facilitates the targeting of therapy using the methods of molecular, structural, and translational biology. Additional approaches, using similar drug design strategies but based on gene therapy and transcriptional and translational modification, are on the horizon. Hence, there is reason to be optimistic regarding the design, testing, and clinical availability of novel antiarrhythmic therapies. (Circulation. 2001;104:2990-2994.)

Key Words: molecular biology ▪ gene therapy ▪ genes ▪ electrophysiology ▪ pharmacology

Identification of Targets and Drug-Target Interactions

Therapeutic Targets
The human genome contains ~30,000 distinct genes, and that diversity is further amplified by alternative splicing of mRNA and post-translational processing modifications, which are currently estimated at 100,000 to 350,000 proteins. The subset of proteins that are important in the pathogenesis of arrhythmias is smaller, but there are potentially a very large number of possible drug targets. Unfortunately, our present insight into upstream pathogenetic mechanisms is limited, so we must focus on a restricted number of molecular therapeutic targets, which are grouped according to their level in the integrated electrical behavior of the heart: (1) ion channels as direct mediators of cardiac electrogensis, (2) other molecules that maintain ion homeostasis and cell-cell coupling (eg, ion-motive ATPases, electrogenic exchangers, Ca-release channels, and connexins), (3) modulators of the proteins of ion channels and other molecules (eg, G-proteins, calmodulin, kinases, phosphatases, cytoskeletal elements, etc), and (4) upstream regulators and mediators of remodeling (eg, ACE inhibitors).

Direct Mediators of Cardiac Electrogenesis
Classic antiarrhythmic drugs that block those ion channels that are essential for normal electrical function are of limited clinical value.1-4 Although many of these drugs have fallen into disfavor since the Cardiac Arrhythmia Suppression Trial (CAST)5 and Survival With Oral D-sotalol (SWORD)6 trials, to conclude that such drugs can never be useful antiarrhythmic agents would be similar to deciding in the 1930s that antibiotics are not generally useful in antimicrobial therapy on the basis of our experience with arsenicals and sulfonamides. A special problem with the classic antiarrhythmic drugs is that they have a relatively low affinity and their therapeutic range is on the lower end of the dose-response curve for channel block. Hence, small changes in tissue levels result in either inadequate or excessive block. Moreover, recent mapping of receptor sites on the voltage-dependent channels reveals a common character and shared location in the pore beneath the selectivity filter.6-10 This similarity of binding site may allow considerable cross-reactivity for various channels and consequent unwanted side effects.

In addition, there are several channels that are not essential contributors to normal electrogensis but that are active in pathological processes, and they are potential therapeutic targets. For example, blocking T-type Ca channels may be useful in modifying cardiac hypertrophy or its electrical consequences. Stretch-activated channels and surface membrane or mitochondrial K_ATP channels may open only during pathological states. Complete block of these channels may leave normal electrogensis unaltered, but suppress arrhythmogenesis under pathological conditions. Similarly, specific block of late I_{Na} and/or cardioselective block of I_{K} may...
provide targets that minimize electrical heterogeneity and prevent arrhythmogenesis under a variety of pathophysiological conditions.\textsuperscript{11,12}

Currently used antiarrhythmic drugs exhibit complex interactions with channel pores and gating states.\textsuperscript{13} Such interactions are epitomized by lidocaine’s well-known preference for the inactivated states of the Na channel, which produces its characteristic and clinically useful use-dependence.\textsuperscript{14} Other interactions are not always beneficial; for example, reverse use-dependent effects on action potential duration markedly restrict the use of K-channel blocking drugs in tachyarrhythmias.\textsuperscript{15} However, drugs that modify channel gating instead of blocking the pore might be fruitfully exploited using rational drug design.

An alternative to classic pore-blocking behavior is to alter channel kinetics and/or voltage dependence or to alter ion gradients involved in electrogenesis. “Agonist” drugs enhance channel opening, as has been demonstrated for L-type Ca channels,\textsuperscript{16} Na channels,\textsuperscript{17} and K\textsubscript{ATP} channels.\textsuperscript{18} Some arrhythmias seen in cardiomyopathies and long-QT syndrome may respond to K channel activators, rather than blockers. Shifting the voltage range of rectification of I\textsubscript{K1} or the activation range of the pacemaker I\textsubscript{K} channel could subtly alter excitability. Most channels essential for electrogenesis have multiple subunits,\textsuperscript{19} and some of these auxiliary subunits are appropriate targets for channel modification. Hence, we believe it unwise to discard ion channels completely as therapeutic targets, because a rich variety of modifications is available that are relatively unexplored.

Other Molecules Involved in Cell Homeostasis
The search for new approaches can logically be extended to include ion transport molecules such as the sarcoplasmic reticulum Ca-release channel (Ca ATPase) and phospholamban, Na-H exchange transporters, connexins, Ca-activated Cl channels, nonselective cation channels, and mitochondrial K\textsubscript{ATP} channels. Because intracellular Ca is a key factor in both the modulation of ion channel function and in cell adaptation to disease, proteins involved in Ca homeostasis are important potential targets. Ca overload results from complex interactions between Ca channels, Na/Ca exchange, sarcoplasmic reticulum Ca uptake and release, and possibly mitochondrial Ca uptake, which are all appropriate targets. Stretch can activate nonselective cation channels, influencing membrane potential levels and allowing Ca influx,\textsuperscript{19} thus providing yet another target.

Modulators of Channels and Transporters
A different approach to changing electrical behavior is modulation rather than block of the proteins involved in electrogenesis. For example, \textbeta-adrenergic receptor blockers can modulate ion channel or transporter function by changing the level of channel phosphorylation. Because modulation can be complete without impairing normal electrogenesis, these agents offer a favorable dose-response relation. Calmodulin is thought to be a major Ca sensor for L-type Ca channels, the slowly rectifying K channel, the pacemaker channel, and probably other proteins,\textsuperscript{20–22} and modulation of these channels may be accomplished by targeting their calmodulin response.

Cytoskeletal biology is another expanding area of interest that impacts several ion channels, including stretch-activated, voltage-gated, and K\textsubscript{ATP} channels. For example, actin polymerization regulates Na channel function by altering its kinetics to resemble long-QT syndrome.\textsuperscript{23} The opening of the K\textsubscript{ATP} channel and its sensitivity to ATP-induced inhibition are both affected by mechanical distortion of the membrane.\textsuperscript{24,25} Thus, the cytoskeletal system may become a target for antiarrhythmic drug development, particularly in ischemia.

Upstream Regulators and Mediators of Remodeling
Interventions that target a variety of G-protein-coupled receptors, notably \beta-adrenergic blockers, have led to unexpected benefits as antiarrhythmic agents. There are many such receptors, and a large number of lead compounds are available. As noted, cytokines are additional potential targets. However, as an example of upstream regulation, the renin-angiotensin-aldosterone system is perhaps the most dramatic. This system plays a pivotal role not only in blood pressure regulation and ion homeostasis, but also in hypertrophy of myocardial cells. Angiotensin II induces various signaling pathways involved in hypertrophy and the substrate for arrhythmias (see information on remodeling in Part II of this article). K channel expression is altered by angiotensin II, possibly at the transcriptional level.\textsuperscript{26} This action may have important implications for antiarrhythmic treatment in hypertrophy and congestive heart failure, in which the expression of K channels is altered. The challenge at this time is to identify molecules involved only in disease-initiated cascades to limit drug action to the diseased or damaged region.

Rational Design of Antiarrhythmic Drugs
The first step in rational drug design\textsuperscript{27} is to select a molecular target that is (1) relevant to the disease, (2) presents a therapeutic opportunity, and (3) is sufficiently well-defined molecularly to allow specific drug screening. It is ideal if the molecular target is specifically expressed in the target tissue and cell type and is specifically involved in the pathway to be modulated. Inhibition or stimulation of target molecule activity should be expected to have the therapeutic effect without unacceptable mechanism-based side effects. A specific gene product and alternative splice isoform should be identified as a screening target.

The ability to apply this approach for ion channels has greatly increased as the traditional ion channel targets have been defined in the past decade and new ones have been characterized at the molecular level. These include many new channel isoforms and associated auxiliary subunits and alternative splice forms expressed in a tissue-specific and cell-specific manner. Moreover, regulatory proteins with specific anchoring sites have been described. Each cardiac pore-forming unit is a potential target for modulation. This includes the Na, Ca, and K channels, as well as novel targets such as cyclic-nucleotide–gated and mechanosensitive ion channels. The interaction sites between the principal subunit and each auxiliary subunit is also a potential target site. In the
same way, sites of interaction of regulatory proteins with the ion channels are novel targets, including those for kinase and phosphatase anchoring, for G-protein subunit interaction, and for Ca-calmodulin interaction, either blocking an undesired interaction or creating full effect. In many ways targeting regulatory sites may provide effective modulation of channel function without the risk of excessive channel inhibition or the production of undesirable effects. This approach allows specific intervention in cellular transduction pathways by ubiquitous messengers like Ca and cAMP, which have other and essential modulatory functions.

Rapid and accurate assays for the functional activity of the target molecule are a second essential component of rational drug design. Assays must be implemented in at least semi-automated form, so \( \geq 100,000 \) compounds can be screened to identify a selection of positive leads for subsequent determination of their binding constants. Classic electrophysiological techniques are insufficient, and new methods are required. However, considerable progress has been made that may help solve this problem. Cell lines exist that express unique cardiac channel subunits. New fluorescent methods for monitoring membrane potential and other cellular functions have been developed and are easily adapted to mass screening. Protein-protein interactions are amenable to drug discovery by screening with ELISA assays with optical readout, by yeast 2-hybrid methods, and by nuclear magnetic resonance. Combinations of these methods with the rapidly developing definition of second messenger interaction sites on ion channels and other proteins involved in excitatory phenomena will provide screens broad and rapid enough for drug development in the arrhythmia field.

Examining a wide range of compounds covering broad molecular and conformational space is another principle in rational drug design. Combinatorial chemistry has increased the chemical diversity of compound libraries substantially, so the diversity of drug structures that can be synthesized is rarely a limiting factor for drug discovery, at least by large pharmaceutical firms. Access to such libraries by smaller companies or academic laboratories is, however, more problematic.

Structural information on ion channels is also beginning to appear. The 3D structure of the pore-forming region of a bacterial potassium channel has been determined at near-atomic resolution, providing a general guide for analysis of structure-function relationships of pore-blocking drugs for all related ion channels. The binding sites for the pore-blocking drugs of sodium, calcium, and potassium channels have been mapped, providing a template for understanding drug-receptor interactions. Cytoplasmic domains of ion channels have been determined, including the sodium channel inactivation gate and the potassium channel oligomerization domain. Much more information is needed, and it is likely that detailed 3D structures of the transmembrane domains of ion channels will be slow to emerge, especially for the large sodium and calcium channels and intracellular channels like the ryanodine-sensitive calcium release channel of the sarcoplasmic reticulum. However, the intracellular domains of these channels are involved in subunit interactions and regulation by second messenger processes, which may be more amenable to structural analysis. Three-dimensional structures of subunit interaction sites and regulatory sites may allow for the use of structure-based drug design methods to yield a new generation of channel modulating drugs.

Using structure-based design, optimization of the affinity and specificity of a drug candidate for its molecular target is best achieved by comparing high-resolution structural information on the target in the free and drug-bound forms with structure-function studies of drug effects. Drug structure is then tailored by adding appropriate functional groups to provide new points of molecular contact with the target site to increase the affinity and specificity of interaction. Determination of the amino acid residues that are involved in drug binding and analysis of their 3D arrangement in the target molecule are crucially important steps toward the rational design of more potent and specific drugs. When lead compounds have been identified by screening, their site of action and the critical amino acid residues within that locus can be mapped by site-directed mutagenesis and functional analysis of the resulting mutants. As for structural determinations, x-ray crystallography, nuclear magnetic resonance, and modeling have greatly improved. However, identification of the 3D structure of membrane proteins still faces formidable obstacles, because high level expression is difficult, large molecular size prevents nuclear magnetic resonance analysis, crystallization is unpredictable, and analysis of the resulting small, poorly ordered crystals by x-ray diffraction is uncertain.

**Emerging Leads for New Drug Development**

**Post-Translational Modification of Ion Channel Trafficking**

Conventional antiarrhythmic drugs generally target the end product of ion channel synthesis, the mature channel protein. An alternative approach would be to target steps in protein synthesis and in translational and post-translational processing of these proteins. Immature proteins undergo a series of complex biochemical steps, including the folding of the protein and coassembly of multiple pore-forming subunits (e.g., K channels), and accessory subunit proteins are usually required to confer normal function. Nascent proteins are thought to come into contact with a variety of chaperone molecules, enzymes that, for example, progressively add and/or modify sugar moieties, and small molecules that participate in protein folding and stabilize 3D structure. Such drug chaperones might ultimately be developed to preferentially modify protein processing to increase or decrease the number of mature channels in the cell membrane.

Mature ion channel proteins undergo degradation by different pathways, and the mature functional proteins have “life spans” of probably between a few hours and a few days. The concept of targeting processing steps involved in an ion channel’s protein synthesis comes, in part, from increased understanding of diseases such as cystic fibrosis and LQTS, in which gene mutations frequently produce mutant ion channel proteins that are retained in the endoplasmic reticulum for degradation. Yet functional channels can be formed if the mutant channel proteins can reach the surface membrane.
In LQT2 studied in HEK cells, it was recently shown that the trafficking of some HERG mutations can be corrected (“rescued”) by drugs that bind with high affinity to the HERG molecule, presumably by stabilizing the protein configuration that can traffic normally to the surface membrane.36,37 Although much remains to be learned about specific targets within cells, these experiments validate the concept that functional ion channel density can potentially be modified pharmacologically by manipulating post-translational protein processing. The potential for this approach in human subjects has not yet been tested. Its possible applicability now seems most likely in genetic ion channel diseases in which a specific defect causing a functional protein to be misprocessed is to be corrected. It also may be possible, using this approach, to manipulate the subunit composition of an ion channel protein, thereby altering regulatory steps, functional expression levels, and “phenotype.”

Targeting Gene Regulation as an Antiarrhythmic Strategy

Another means to target arrhythmias would be to alter the myocardial substrate by controlling gene expression at the transcriptional level. The concept is attractive for a number of reasons. (1) Gene expression is finely controlled in nature; for example, the sinus node expresses different genes, and the same genes at different levels, than the surrounding atrium.38 This observation demonstrates that fine discrimination among adjacent tissues is biologically tenable. (2) Protein turnover for relevant gene products is rapid. Connexins, for example, typically turn over within an hour or less,39 and at least some ion channels turn over within a few days. Because proteins do not linger long, cardiac excitability could theoretically be reprogrammed within a matter of hours to days. (3) Many transcription factors have been cloned, sequenced, and crystallized, and canonical regulatory DNA sequences are well-recognized. (4) Ion channel gene promoters contain numerous regulatory elements, as do the genes for other potential targets.40

Despite these reasons for optimism, enthusiasm for targeting gene regulation is tempered by a number of practical limitations. The control of gene expression in nature is complex and poorly understood. Many transcription factors are ubiquitous, necessitating localized “therapy.” Existing paradigms portend generalized effects, eg, thyroid hormone and steroid hormones bind to nuclear receptors and affect channel transcription,41 but do so in a complex multisystem manner. Such considerations confer a significant risk of unintended consequences if gene regulation were attempted with existing technology. In any case, much more fundamental insight is required to advance this promising antiarrhythmic strategy.

Gene Therapy for Arrhythmias

Gene therapy is defined here as the transfer of nucleic acids to somatic cells with therapeutic intent. In contradistinction with the immediately preceding discussion of transcriptional regulation, gene therapy is quite general: transcription may be targeted, but much more commonly the gene of interest would not be directly involved in transcriptional control.

Instances of gene therapy for arrhythmias in which there are plausible precedents include potassium channel expression in the ventricle to offset long-QT syndromes (either inborn or acquired)42,43 and overexpression of inhibitory G proteins to modify atrioventricular nodal conduction as a means of slowing heart rate in atrial fibrillation.44 Given the plethora of potential targets, possible applications are limited only by the imagination. Practical implementation for clinical use must, however, await refinements in gene delivery methods and vector design. In addition, extensive attention must be given to safety and to efficacy.

Conclusions

There is a great deal of promise and excitement in the possibilities for new antiarrhythmic therapies now afforded us. Yet, however logical and feasible, the therapies that can be created will remain speculative until tested first in biological and computer models and ultimately in humans. Complicating the picture is that making a solitary change in a nonlinear system will likely restore normal function only if the defect is truly isolated and is the direct cause of the phenotypic response and if the repair is complete. The presence of minor associated abnormalities or an incomplete restoration might constitute an important residual arrhythmic substrate such that proarrhythmic effects might not necessarily be eliminated.

Appendix

Meeting Organizers

The meeting was organized by Edward Carmeliet, MD, PhD, University of Leuven, Belgium; Harry A. Fozzard, MD, University of Chicago, Ill; Masayasu Hiraoaka, MD, Tokyo Medical and Dental University, Tokyo, Japan; Michiel J. Janse, MD, Cardiovascular Research, Amsterdam, the Netherlands; Satoshi Ogawa, MD, Keio University, Tokyo, Japan; Dan M. Roden, MD, Vanderbilt University School of Medicine, Nashville, Tenn; Michael R. Rosen, MD, Columbia University, New York, NY; Yoram Rudy, PhD, Case Western Reserve University, Cleveland, Ohio; and Peter J. Schwartz, MD, Policlinico S. Matteo IRCCS, Pavia, Italy. It was chaired by Dr Rosen and cochaired by A. John Camm, MD, St George’s Hospital Medical School, London, UK, and Drs Fozzard, Janse, Roden, and Rudy.

Participating in the meeting and sharing in authorship of the article are the above individuals as well as Charles Antzelevitch, PhD, Masonic Medical Research Laboratory, Uitca, NY; Penelope A. Boyd, PhD, Columbia University, New York, NY; William A. Catterall, PhD, University of Washington, Seattle; Glenn I. Fishman, Mts Sinai School of Medicine, New York, NY; Alfred L. George, MD, Vanderbilt University Medical Center, Nashville, Tenn; Seigo Izumo, MD, Beth Israel Deaconess Medical Center, Boston, Mass; José Jalife, MD, SUNY Syracuse, Syracuse, NY; Craig T. January, MD, PhD, University of Wisconsin, Madison; André G. Kléber, MD, Universitaet Bern, Bern, Switzerland; Eduardo Marban, MD, PhD, the Johns Hopkins University, Baltimore, Md; Andrew R. Marks, MD, Columbia University, New York, NY; Peter M. Spooner, PhD, NIH/NHLBI, Bethesda, Md; Albert L. Waiido, MD, Case Western Reserve University, Cleveland, Ohio; James M. Weiss, MD, UCLA Cardiovascular Research Laboratory, Los Angeles, Calif; and Douglas P. Zipes, MD, Krannert Institute of Cardiology, Indianapolis, Ind.

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References


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