New approaches to antiarrhythmic therapy
Emerging therapeutic applications of the cell biology of cardiac arrhythmias
Members of the Sicilian Gambit

Cardiac arrhythmias complicate many diseases affecting the heart and the circulation, and 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- and disease-specific fashion, facilitates targeting of therapy via molecular, structural and translational biology. Additional approaches, employing 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.

Introduction
Cardiac arrhythmias are a major public health problem for which traditional pharmacological therapies have yielded disappointing results[1–5], and for which new approaches to drug development would be highly desirable. Fortunately, science and technology have evolved sufficiently to facilitate identification of new targets for drug action and tailoring of molecules to fit these targets. We approach this subject matter in three steps: first, we describe the arrhythmogenic myocardial substrate via a modelling approach that synthesizes information from molecular through organ levels. We do this because arrhythmology has become so complex that traditional linear thinking no longer integrates data at channel, cell, whole tissue or whole organ levels in ways that readily predict or describe function. We then focus on the genetic factors and environmental stresses that determine and influence normal and abnormal electrical activity and/or act as long-term modulators to remodel cardiac structural and electrical substrates. Finally, we use the principles of rational drug design to identify and discuss potential targets of new antiarrhythmic therapy.

Computational modelling to incorporate physiologic and genetic data into a framework for arrhythmogenic mechanisms (see Fig. 1)

From ion channel to single cell
The pioneering work of Hodgkin and Huxley[6] in squid axon fostered the evolution of several computational models of cardiac cellular electrical activity, including ventricular myocytes[7–11], atrial myocytes[12–14], sinus node[15,16], and Purkinje fibre[17,18]. The more recent of these models include transmembrane currents operating through ion channels and ion transfer through transporters and exchangers, and compute the dynamic changes of ion concentrations (Na⁺, Ca²⁺, K⁺) during the action potential. These models are formulated in the classical Hodgkin-Huxley scheme.

Recent developments in molecular biology and the genetics of ion channels have increased knowledge about the relationships between protein structure and electrophysiological function[19]. It is possible to simulate whole cell action potentials starting from single channel models based on discrete kinetic states of the channel protein (e.g. closed, open, inactivated states) and with this formalism to simulate genetic mutations that affect
single channel function and their arrhythmogenic consequences at the whole cell level\cite{20}. Interactions of drugs with specific channel states also can be simulated (e.g. a drug binding to a channel only in its open state). This general scheme has been used in the context of the LQT3 variant of the congenital long QT syndrome that involves the SCN5A gene and the Na$^+$ channel\cite{20}, and the LQT2 variant that involves the HERG gene and the rapid delayed rectifier K$^+$ channel\cite{21}.

**Figure 1** Factors studied at subcellular levels through the intact heart that impact on the expression of arrhythmias. See text for discussion.
overwhelming majority of clinical arrhythmias appear to be reentrant (whether initiated by automatic, triggered or reentrant impulses) [36,37]. Hence, a great deal of emphasis is currently focused on reentrant mechanisms.

Impulse propagation and arrhythmias have been studied in a variety of models ranging from the tissue as a continuous, uniform syncytium to a highly discontinuous structure that represents the discretization of the tissue into cells and its macroscopic organization [38,39]. Continuous and discontinuous models constitute two extremes incorporating distinctly different properties that are relevant to arrhythmogenesis and antiarrhythmic drug effects. Continuous properties determine arrhythmias in the absence of structural heart disease (e.g. long QT syndromes and development of torsades de pointes) [30]. Discontinuous properties determine arrhythmias in structurally altered myocardium such as remodelled tissue following myocardial infarction [31] or in chronic atrial fibrillation [32].

Another determinant of propagation is wavefront curvature [33] which can exist in both continuous and discontinuous media. The wavefront curvature affects propagation velocity [34] and ion channel involvement, particularly in the core of a spiral wave [35]. The curvature can also interact with tissue discontinuities; hence, a spiral wave can either be initiated or anchored at a site of inhomogeneity in tissue structure [36-37].

In tissue with continuous properties, functional reentry can be initiated and modified by heterogeneities that may arise functionally from passage of a premature impulse. Hence, the maintenance, perpetuation and movement of functional reentrant waves are influenced by the degree of interaction between the head of the wavefront and the repolarizing tail of the preceding wavefront (‘head–tail’ interaction) [38,39]. This type of reentry should be responsive both to drugs that modify repolarization and action potential duration (‘tail’) and to drugs that modify the action potential upstroke (‘head’). Intrinsic heterogeneities of cellular electrophysiological properties also contribute to functional reentry, with transmural inhomogeneity being introduced by heterogeneous expression of ion channels controlling repolarization (I_K, I_Na, late I_Na, I_Na,C) [45,46].

In tissues with marked structural discontinuities (e.g. advanced cell-to-cell uncoupling, marked fibrosis) conduction becomes discontinuous. Here, conduction velocity can decrease to very low values [24,47], permitting reentrant excitation to occur in very small regions (micro-reentry). Two important characteristics of discontinuous conduction determine the behaviour of reentrant waves, and most likely, their response to antiarrhythmic drugs. First, their pathways are largely determined by anatomical structure, thereby introducing excitable gaps into the circuits [39]. As a consequence, head–tail interactions are diminished and circuits may be less responsive to drugs that prolong action potential duration. A further modifier is the extent to which the ion channel remodelling that accompanies many disease processes alters drug binding and unbinding. Second, discontinuous conduction is associated with a seemingly paradoxical increase in the safety factor for propagation [24,47,49] due to decreased electrical loading of depolarizing cells. This implies a more robust depolarizing phase of the action potential (‘head’) with an increased resistance to drugs that affect depolarization [50]. Clinically, transitions can occur between continuous and discontinuous conduction during the process of tissue remodelling (e.g. evolution of atrial fibrillation from the paroxysmal to the persistent and permanent forms, scar formation in healed myocardial infarction). Hence, drugs may lose efficacy in chronic atrial fibrillation due to such remodelling-related changes in structure and function.

From tissue to the whole heart

Whole heart models are needed to study arrhythmias that critically depend on the heart’s spatial organization. Congenital or acquired disease superimposed on these spatial physiological features may also be location-dependent, e.g. localized infarct scars, regional ischaemia, apparent dominant right ventricular involvement in Brugada syndrome and arrhythmogenic right ventricular dysplasia. From the electrophysiological perspective, certain arrhythmias are uniquely expressed in the whole heart (large reentrant circuits in acute ischaemia [23], initiation of atrial fibrillation by pulmonary venous foci [51]). Whole heart models also facilitate investigation of autonomic effects which are location-dependent as a result of non-uniform innervation [52-55] and interactions of autonomic input with infarct scars and antiarrhythmic drugs.

With whole heart models arrhythmogenic behaviour can be related to its manifestations in electrograms on the cardiac surface and to the body surface ECG [56,57]. Whole heart models differ from cell and tissue models by accounting for the spatial organization of the cardiac chambers and the separation of electrically distinct and topologically defined units, such as sinus node, atrial tissue, atrioventricular node, specialized conduction systems, and ventricular myocardium. Within each of these anatomical features, which are relevant to electrical function, specific properties can be simulated in computer models, e.g. organization of pacemaker cell models into the sinus node complex [58], specific atrial structures (trabeculation, anisotropic structures such as the crista terminalis [59]), AV-nodal pathways, Purkinje network organization [60], Purkinje-muscle junctions [61], and rotational organization of ventricular muscle layers [62].

From computation to biology

A key source for the application of modelling derives from the use of ‘model organisms’. Hence, modelling is not limited to the computational approaches described above, but extends to a variety of levels of biology that impact on our understanding of arrhythmias. For example, with the advent of high throughput DNA sequencing, the genome of a number of ‘model organisms’ (e.g. yeast, fly, round worm, and human) has been sequenced. Such model organisms may provide critical
insights into the pathogenesis of arrhythmias and related basic biology. For example, studies in humans may identify a number of potential candidate modifier genes for LQTS, but it may not be possible to pinpoint which gene is the most important modifier by studying human populations alone. However, one can test genetic interactions of candidate genes by genetic complementation, suppressor assays, genetic backcross and other methods readily available in model organism systems.

Despite differences in heart size and physiology, the mouse model recapitulates at least some features of human disease. For example, first- and second-degree AV block occur in homozygous mutant connexin40 mice\(^{63}\) while mice heterozygous for connexin43 deletion exhibit reduced ventricular conduction velocity\(^{64}\). Those homozygous for minK deletion exhibit a tachyarrhythmia with characteristics of atrial fibrillation\(^{65}\), and ventricular arrhythmia susceptibility has been seen in a number of mice in which major repolarizing currents have been disrupted\(^{66–68}\).

The main advantage of mouse models is that the consequences of altering a single gene can be studied. A very important caveat is that disruption of a single gene may lead to altered expression or function of other genes. Nevertheless, these experiments demonstrate the utility of genetically-modified animals to provide new arrhythmia models and identify the altered genes as candidate contributors to the arrhythmias.

**Genetic modifiers of cardiac arrhythmias**

The multiple genetic factors that contribute to arrhythmia susceptibility in patients with inherited and acquired disorders have been reviewed in detail elsewhere\(^{69–72}\). Importantly, even related individuals who carry the same disease-associated mutation(s), can manifest substantial differences in phenotypic expression ranging from life-threatening to asymptomatic. This phenomenon, which may be consequent to incomplete penetrance (lack of disease manifestations in mutation carriers in families with an inherited disease)\(^{73}\) or variable expressivity (varying severity or spectrum of disease manifestations)\(^{74}\) has seen a variety of explanations, including the existence of ‘modifier genes’. These are genetic factors separate from a primary mutation that protect from or aggravate an underlying condition. Although inherited arrhythmias are rare, genetic modifiers of congenital arrhythmias may be relevant to more common acquired disorders of cardiac rhythm.

The human genome includes a vast array of DNA polymorphisms, most often single nucleotide polymorphisms (SNPs, or ‘snips’), in which a single nucleotide varies in a population. SNPs may be non-functional, or may occur in regulatory regions that alter gene expression, may result in changes in the sequence of the encoded proteins, may alter phenotype expression only under pathological conditions (e.g. ischaemia) and/or may directly alter a protein’s function. The human genome probably includes >3 000 000 SNPs.

**Figure 2** shows a general approach to identifying DNA variants that modulate physiology, pathophysiology, and responses to stresses such as sympathetic activation, ischaemia, or drugs. The first step in this process (Fig. 2–1) is to define a phenotype. This involves assigning each individual in a kindred as affected, unaffected, or uncertain and describing the anomaly. In studies to identify modifier genes, a phenotype might be defined as the presence or absence of symptoms such as syncope or signs such as QT prolongation in known mutation carriers. In studies to define genes that modify response to drugs (‘pharmacogenomics’), the phenotype might be the presence or absence of a beneficial drug response, or an adverse effect such as torsades de pointes or hepatotoxicity. In each example, it is important to establish, to whatever extent possible, that the phenotype includes a genetic component before attempting to identify that component.

The next step (Fig. 2–2) is to identify candidate genes, i.e. those in which DNA variants ‘might’ account for variability in the pre-defined phenotype (e.g. in genes affecting sympathetic responsiveness in patients with the long QT syndromes). Polymorphisms in these genes might reasonably be expected to modulate the phenotype. Further, we might expect different sets of polymorphisms to modulate different subtypes of LQTS, based on their variable relationships to sympathetic activation. Identifying candidate genes therefore requires an understanding of the molecular pathophysiology of the phenotype being studied. Identification of polymorphisms in candidate genes (Fig. 2–3) may then be followed by studies designed to establish whether such polymorphisms, alone or in combination, can be implicated as modulating the phenotype (Fig. 2–4). This may require characterization of function of variant proteins in vitro, in genetically modified animals, or computer simulations, as well as further genetic epidemiological and statistical analyses. If a relationship between a DNA variant, or a set of DNA variants, and the pre-specified phenotype is defined, further studies (Fig. 2–5) should then (a) validate such relationships prospectively, and (b) use this information to refine the phenotype.

An alternative approach is to conduct a genome-wide search to identify loci linking (in the formal genetic sense) to the phenotype. Once such loci are identified, each gene within the locus becomes a ‘candidate’. This positional candidate approach has been used successfully in many diseases (e.g. LQT\(^{75}\), cystic fibrosis\(^{76}\), Alzheimer’s\(^{77}\), and has been suggested as an alternative means to identify genes that modify drug responses\(^{78}\).

Such approaches may help us detect mutations in the various molecules considered potential candidate modifier genes for inherited arrhythmias. These include genes determining action potential duration (e.g. voltage-gated ion channels, ion transporters and pumps), molecules involved with intracellular signalling (e.g. kinases, phosphatases and proteins involved in intracellular calcium homeostasis), and those involved in cell responses to extracellular factors (e.g. adrenergic and other hormone receptors, gap junctions, components of
the cytoskeleton, anchoring proteins on the extracellular matrix). In addition to selecting candidates based upon known physiological and biochemical associations with cardiac conduction and excitability, gene scans using newer expression profiling techniques (e.g. microarray technology) may provide information implicating many more unanticipated factors.

One example of genetic variability is seen in the autonomic nervous system, where a polymorphism (Ile164) of the β2-adrenergic receptor is associated with increased mortality among patients with heart failure[79]. This polymorphism produces a substantial decrease in basal and epinephrine-stimulated adenylyl cyclase activities[80]. These studies have highlighted the issue of combinations of polymorphisms (haplotype) in a given gene. In the case of the β2-adrenergic receptor, 13 single nucleotide polymorphisms have been identified in the coding and regulatory regions, leading to 8192 possible haplotypes, and over 33 million possible genotypes. The vast majority of these combinations do not occur (i.e. polymorphisms are linked to each other), although preliminary data suggest that response to inhaled β2 agonists in asthma varies as a function of which of the 12 identified haplotypes is present[81]. It seems reasonable to surmise that relatively common genetically controlled alterations in autonomic function — by increasing or decreasing either the amount of norepinephrine released during sympathetic activation or its effects — may ‘modify’ the propensity toward life-threatening arrhythmias due to mutations in genes encoding cardiac ion channels[82].

An important mandate for confirming the relevance of genetic polymorphisms to the physiological disturbances underlying arrhythmia susceptibility is the demonstration of functional consequences of an identified gene sequence variant. Association studies provide the first level of evidence linking a genetic variation with a phenotype, but extensive characterization of the variant sequence (protein or regulatory element) is critical to validate its culpability and to understand its mechanistic implications. Demonstrating the functional significance of polymorphisms linked to arrhythmia susceptibility will require increasingly powerful and new approaches to develop appropriate phenotyping skills as well as molecular, physiological, cell biological, and computational tools.

*Figure 2 A strategy for discovery of DNA variants modulating physiology, pathophysiology and response to drugs and other ‘stressors’. See text for discussion.*

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**Refine the phenotype**
- Prospective study

**Define a phenotype**
- adverse drug reaction
- beneficial drug response
- symptoms in a genetic syndrome

**Is there evidence that the phenotype is genetically determined?**

**Identify candidate genes**

**Identify polymorphisms in candidate genes**

**Relate the identified polymorphisms to the phenotype**
- functional studies
  - *in vitro*
  - model systems
  - modeling
- clinical; genetic epidemiology

**Is there evidence that the phenotype is genetically determined?**

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Environmental stresses leading to remodelling of the cardiac phenotype and to arrhythmogenic substrates

A variety of stresses impact on the heart, whose adaptation to these has been described as ‘remodelling’ (Fig. 3). The stresses can be physiological — as in the left ventricular hypertrophy that attends closure of the ductus arteriosus in the newborn, or it can be pathological, as in the hypertrophy that accompanies hypertension. Although the latter form of remodelling may actually provide short-term benefit to cardiac function, in the long run it is deleterious and arrhythmogenic. Remodelling can be expressed in altered structure/function of ion channels, signalling molecules, structural proteins, etc., of his heart and nervous system. Environmental stresses incorporate, among others, neural stimuli and factors such as smoking and stress. A variety of hormonal systems can induce structural and electrical remodelling of the myocardium and/or acutely trigger arrhythmias. Remodelling is believed to trigger arrhythmias and/or facilitate their occurrence via a number of steps, of which a central one is the enhancement of heterogeneity of repolarization. Moreover, once arrhythmias occur they can induce further remodelling by altering heart rate and/or activation sequence.

Elements in remodelling
Remodelling may be usefully considered in terms of effects on ‘upstream’ and ‘downstream’ elements. By upstream, we refer to those long-term modulators of structure/function in Fig. 3 that change expression of the molecules that contribute to the arrhythmic substrate. Reducing or limiting the progression of heart disease would be expected to prevent remodelling and hence, arrhythmias. Although upstream therapy would seem to lessen the arrhythmia burden, the manifold mechanisms by which this can occur vary with the underlying disease and its specific treatment. For example, treating heart failure could reduce arrhythmias induced by activation of mechanical stretch receptors, excess catecholamine release and other.
factors that influence the arrhythmic substrate acutely or chronically.

Those modulators likely to be involved in arrhythmic remodelling include but are not limited to catecholamines, free radicals, angiotensin converting enzyme, angiotensin II, cytokines, and nitric oxide, each operating via specific signalling cascades to alter the cardiac phenotype. Evidence that upstream approaches to therapy should be rewarding is already evident from clinical trials of statins[89], spironolactone[91] and ACE inhibitors[87,92,93]. Regrettably, there are relatively few studies of these non-traditional ‘antiarrhythmic’ agents on numerous aspects of electrogensis (e.g. ion channels, receptors, gap junctions, or membrane physiology). Yet for angiotensin II evidence does exist of short- and long-term modulation of ion channel and gap junctional structure and function[94–97]. Hence, the upstream focus suggested here seems to take advantage of antiarrhythmic effects of agents not routinely considered as part of the arrhythmic armamentarium. The following examples highlight important new leads in this approach:

Fibrosis and extracellular matrix Hearts with extensive fibrosis exhibit very slow conduction[31]. The low macroscopic conduction velocities have been explained by microscopically zig-zagging circuits[113] or the special conduction characteristics of tissue with a discontinuous, branching architecture[91]. Tissue with very slow and discontinuous conduction can explain reentrant excitation in paths of small dimensions (a few millimetres in diameter)[24,98,99].

A crucial consideration in the evolution of fibrosis is the non-uniform mechano-electric coupling that occurs in a dilated or scarred chamber. Several considerations may shed light on this. First, fibroblasts manifest mechano-electric coupling[100], and fibrotic tissue containing fibroblasts could actively contribute to an arrhythmogenic substrate. Second, much depends on how fibrosis restructures a cardiac chamber wall[87]. It is not difficult to visualize a geometry in which contractile dispersion (one segment stretching another) in a scarred matrix influences electrophysiological dispersion to initiate arrhythmias. Alternatively, fibrotic tissue could shield myocytes from abnormal stress and strain, depending on the geometrical arrangement.

Mechanical stress and angiotensin II The myocardium contains connective tissue elements that link not only to the myocardial wall but to the cytoskeleton, effectively tethering the myocytes within a matrix[87]. Therefore, cardiac activation that is directionally altered not only alters contractile motion of regions of myocardium with respect to one another, but of cells as well, subjecting them to altered stress-strain relationships[87,101,102]. The result is activation of mechanotransduction mechanisms that can have profound electrophysiological effects. For example, altered stretch of cardiac cells in culture induces synthesis of angiotensin II, the availability of which alters cellular structure and electrophysiology[96,103–106]. Angiotensin II can initiate the electrical remodelling that modifies repolarization[96,107] and the structural remodelling that influences impulse conduction[108]. Actions to promote fibrosis and to enhance norepinephrine release from intracardiac nerves have also been documented[109]. Examples of clinical applicability of modulating angiotensin II include the effects of ACE inhibitors to reduce the occurrence of atrial fibrillation in post-myocardial infarction patients with left ventricular dysfunction[109,110] and some secondary prevention ACE inhibitor trials which have shown a reduction in sudden cardiac death[92,93].

Aldosterone synthesis, stimulated by angiotensin II, can result in prolonged, progressive changes in myocyte and fibroblast proliferation, hypertrophy and collagen deposition, independent of its effects on salt and water homeostasis. These changes are transduced via classical, nuclear-mediated mineralocorticoid receptor pathways[111]. The potential importance to arrhythmia prevention of reversing the genetic reprogramming induced by aldosterone is seen in the RALES trial in which spironolactone reduced sudden arrhythmic deaths by 20–30%[91]. Moreover, stretch can directly activate the sensory endings of sympathetic nerve fibres leading to excitatory sympathetic reflexes and local catecholamine release. Hence, mineralocorticoid receptor antagonists alone or in combination with ACE inhibitors or β-adrenergic antagonists seem likely to lead to amelioration of arrhythmias.

Additional factors Inflammation and acute and chronic ischaemia cause rapid release of cytokines, whose gene expression has been characterized in the post-ischaemic ventricle[112]. Within 15 min of experimental coronary occlusion, cardiac mRNAs for interleukin 1β, TNFα and TNFβ are measurable and sustained for several hours. TNFα is an autocrine contributor to myocardial dysfunction and cell death in the ventricle[112]. Little is known about the acute effects of these inflammatory agents on the function of ion channels, but there are reports of reduced L-type Ca2+ current amplitude[113]. Reactive oxygen species also have direct effects on cellular structure and function, and may be critical signalling transducers in the setting of myocardial infarction and the ensuing remodelling[114]. However, the molecular mechanisms by which OH and other O radicals alter the function of ion channel proteins are not fully known. Other studies have suggested energy balance may be an important consideration, as noted especially in the atrial[115,116].

Downstream elements ‘Downstream’ elements are those considered to impact most directly on initiation and perpetuation of an arrhythmia.

Ion channel remodelling A vast literature considers the ion channel changes that accompany myocardial infarction, cardiac failure, myocardial hypertrophy, atrial fibrillation, and other cardiac pathologies[117–119]. Among the ion channel abnormalities characterizing the failing ventricular myocardium are reduction in density of the transient outward current, $I_{to}$ and the inward rectifier, $I_{K1}$. Interestingly, $I_{to}$ density is also reduced...
in peri-infarct fibres of ventricular myocardium\textsuperscript{[118]}. This suggests that different pathological settings may share a common denominator in a particular ion channel’s response to stress, although non-pathological interventions such as cardiac pacing and resultant altered activation also can reduce $I_{\alpha}$ density\textsuperscript{[120]}. In all these settings, $I_{\alpha}$ reverts to a property characteristic of the neonatal heart, which has no $I_{\alpha}$ \textsuperscript{[121]}. Given this commonality of change regardless of the inciting intervention, we should probably focus more attention on the dedifferentiation of cell properties that accompany a variety of stresses, and the types of interventions that might be most appropriate for restoring them to normal or to yet another state that supports normal function.

Remodelling of intracellular Ca\textsuperscript{2+} homeostasis Ca homeostasis is sustained via maintenance of [Ca\textsuperscript{2+}]\textsubscript{i} in a range from ~100 nM during diastole to a peak of ~1000 nM in systole\textsuperscript{[122]}. Aberrant Ca fluxes may occur as a result of inappropriate sarcomplasmic reticular Ca release during diastole (when the R\textsubscript{S}R\textsubscript{2} channel should be closed), and of prolonged, excessive Ca influx via $I_{\text{Ca,L}}$ during a long action potential. Moreover, reverse-mode operation of the Na/Ca exchanger — which occurs especially when intracellular Na\textsuperscript{+} concentrations are high as in tachycardias — will bring still more Ca into the cell. Both the aberrant release of sarcoplasmic reticular Ca during diastole and excessive Ca influx via $I_{\text{Ca,L}}$ channel during late systole (when the action potential is repolarizing) can initiate afterdepolarizations\textsuperscript{[123]} which can in turn generate extrasystoles\textsuperscript{[124]}. The association of mutations in the R\textsubscript{S}R\textsubscript{2} receptor with clinical cardiac arrhythmias attributed to triggered activity (as in catecholamine-sensitive ventricular tachycardias\textsuperscript{[125]}\textsuperscript{[126]}) stresses the importance of sarcomplasmic reticulum-based mechanisms in modulating arrhythmias.

Remodelling of cell-to-cell coupling Remodelling in cardiac gap junctions can result from alterations in connexin transcription, and/or connexin protein synthesis or degradation. The turnover of at least two cardiac gap junctional proteins, Cx43 and Cx45, is rapid; in adult rat heart Cx43 turnover has a half-life of less than 90 min\textsuperscript{[125]} which could facilitate remodelling in cardiac disease states.

Although a number of agents influence cardiac connexin expression, relatively few mechanistic pathways have been established. The effects of several hypertrrophic stimuli on Cx43 expression in cultured neonatal myocytes have been investigated, including dibutyryl cyclic AMP\textsuperscript{[126]}; angiotensin II\textsuperscript{[127]} and myotrophin\textsuperscript{[127]}. In each case, Cx43 mRNA and/or protein levels increased several fold. Also, connexin43 is upregulated after short periods of mechanical stress. Conversely, several factors repressing Cx43 expression have been described, including the tumour necrosis factor, TNF\textsubscript{α}, suggesting that Cx43 expression may be regulated by circulating cytokines\textsuperscript{[128]}. How these factors play into the physiological and pathological modulation of gap junctional function and the extent to which this can be manipulated to predictably modify cardiac rhythm remains to be seen.

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, currently estimated at 100 000–350 000 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, grouped according to their level in the integrated electrical behaviour of the heart:

(A) Ion channels as direct mediators of cardiac electrogensis,

(B) Other molecules that maintain ion homeostasis and cell–cell coupling; e.g. ion-motive ATPases, electroneutral exchangers, Ca-release channels, and connexins,

(C) Modulators of the proteins in sets A and B; e.g. G-proteins, calmodulin, kinases, phosphatases, cytoskeletal elements, etc.,

(D) Upstream regulators and mediators of remodelling; e.g. ACE inhibitors, etc.

(A) Direct mediators of cardiac electrogensis. Classical antiarrhythmic drugs that block those ion channels that are essential for normal electrical function are of limited clinical value\textsuperscript{[129–131]}. Although many of these drugs have fallen into disfavour since the CAST\textsuperscript{[1]} and SWORD\textsuperscript{[2]} 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 sulphonamides. A special problem with the classical 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 level 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\textsuperscript{[132–136]} 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 are active in pathological processes, and are potential therapeutic targets. For example, block of T-type Ca channels may be useful in modifying cardiac hypertrrophy or its
Current views on antiarrhythmic drug action and channel targeting

Electrical consequences. Stretch-activated channels and surface membrane or mitochondrial KATP channels may open only during pathological states. Complete block of these channels may leave normal electrogenesis unaltered, but suppress arrhythmogenesis under pathological conditions. Similarly, specific block of late I_{Na} and/or cardioselective block of I_{Ks} may provide targets that minimize electrical heterogeneity and prevent arrhythmogenesis under a variety of pathophysiological conditions[157,158].

Currently used antiarrhythmic drugs exhibit complex interactions with channel pores and gating states[139]. Such interactions are epitomized by lidocaine’s well-known preference for the inactivated states of the Na channel, producing its characteristic and clinically useful use-dependence[140]. 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[141]. However, drugs that modify channel gating instead of blocking the pore might be fruitfully exploited utilizing rational drug design.

An alternative to classical pore-blocking behaviour 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[142], Na channels[143], and KATP channels[144]. Some arrhythmias seen in cardiomyopathies and long QT syndrome may respond to K channel activators, rather than blockers. Shift of the voltage range of rectification of I_{K1} or the activation range of the pacemaker I_{K1} channel could subtly alter excitability. Most channels essential for electrogenesis have multiple subunits[139], 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.

(B) 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 (SR) Ca-release channel, Ca ATPase and phospholamban, Na–H exchange transporters, connexins, Ca-activated Cl channels, non-selective cation channels, and mitochondrial KATP channels. Because intracellular Ca is a key factor in both 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, SR Ca uptake and release, and possibly mitochondrial Ca uptake, all appropriate targets. Stretch can activate non-selective cation channels, influencing membrane potential levels and allowing Ca influx[145], providing yet another target.

(C) Modulators of channels and transporters.

A different approach to change of electrical behaviour is modulation rather than block of the proteins involved in electogenesis. For example, β-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 favourable 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[146–148], and modulation of these channels may be accomplished by targeting their calmodulin response.

A new area of interest that impacts on several ion channels, including stretch-activated, voltage-gated, and KATP channels. For example, actin polymerization regulates Na channel function by altering its kinetics to resemble long QT syndrome[149]. The opening of the KATP channel and its sensitivity to ATP-induced inhibition are both affected by mechanical distortion of the membrane[150,151]. Thus, the cytoskeletal system may become a target for antiarrhythmic drug development, particularly in ischemia.

(D) Upstream regulators and mediators of remodelling.

Interventions that target a variety of G-protein coupled receptors, notably β-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 signalling pathways involved in hypertrophy and the substrate for arrhythmias (see remodelling, above). K channel expression is altered by angiotensin II, possibly at the transcriptional level[146]. This action may have important implications for antiarrhythmic treatment in hypertrophy and constrictive heart failure, where expression of K channels is altered. The challenge at this time is to identify molecules involved only in disease-initiated cascades, in order to limit drug action to the diseased or damaged region.

Rational design of antiarrhythmic drugs

The first step in rational drug design[152] is to select a molecular target relevant to the disease, presenting a therapeutic opportunity, and 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 the 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 a full effect. In many ways targeting regulatory sites may provide effective modulation of channel function without risk of excessive channel inhibition or 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 functional activity of the target molecule are a secondary essential component of rational drug design. Assays must be implemented in at least semi-automated form, so that 100,000 or more compounds can be screened to identify a selection of positive leads for subsequent determination of their binding constants. Classical 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 of 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 read-out, by yeast two-hybrid methods, and by NMR. 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 principal in rational drug design. Combinatorial chemistry has increased the chemical diversity of compound libraries substantially, so that 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 three-dimensional 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 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 three-dimensional 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 use of structure-based drug-design methods to yield a new generation of channel modulating drugs.

Utilizing 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 addition of 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 three-dimensional arrangement in the target molecule are crucially important steps toward 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, NMR and modelling have greatly improved. However, identification of the three-dimensional structure of membrane proteins still faces formidable obstacles, because high level expression is difficult, large molecular size prevents NMR 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

(A) 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 folding of the protein and co-assembly of multiple pore-forming subunits (e.g., K channels) and/or accessory subunit proteins that 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 three-dimensional 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’ probably of between a few hours and perhaps 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 LQT2, where 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[161,162]. 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 to human subjects has not yet been tested. Its possible applicability now seems likeliest in genetic ion channel diseases where 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’.

(B) 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[163]. 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[164], and at least some ion channels 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[165].

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; e.g. thyroid hormone and steroid hormones bind to nuclear receptors and affect transcription of channel[166], 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.

(C) Gene therapy for arrhythmias.

Gene therapy is defined here as the transfer of nucleic acids to somatic cells with therapeutic intent. In contrast to 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)[167,168], and overexpression of inhibitory G proteins to modify AV nodal conduction as a means of slowing heart rate in atrial fibrillation[169]. 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 as well as to efficacy.

Concluding remarks

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 man. Complicating the picture is that making a solitary change in a non-linear system will probably restore normal function only if the defect is truly isolated and is the direct cause of the phenotypic response, and 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.
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References


Appendix

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