Despite a remarkable decline in cardiovascular mortality over the past 40 years and in spite of the effectiveness of implantable cardioverter defibrillators (ICDs) in aborting ventricular arrhythmias, sudden cardiac death (SCD) has remained a major medical challenge. Every year more than 350,000 individuals succumb to SCD in the USA alone. The underlying cardiac rhythm abnormality is commonly polymorphic ventricular tachycardia degenerating into ventricular fibrillation and less commonly, bradycardia and asystole. Despite the terminal event being an arrhythmic episode, the majority of such patients have an underlying structural heart disease, most commonly coronary artery disease and less commonly primary cardiomyopathies. SCD also occurs in the absence of discernible structural heart disease and is due to primary abnormalities in ion channels. The latter group comprises single gene disorders affecting cardiac ion channels, such as the long QT syndrome, short QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT) among the others. The apparent distinction between the two underlying substrates, however, is rather phenomenological, as myocardial structural abnormalities might affect ion channel biophysical and physiological properties and conversely, ion channel abnormalities could influence myocardial function. It is these complex interactions between the substrate and the ion channels that have been at the core of clinical management of patients at the risk of SCD.

There has been considerable progress in the management of patients with supraventricular tachycardias, such as catheter-based ablation of certain supraventricular arrhythmias. Atrial fibrillation has remained the most common significant cardiac arrhythmia, affecting approximately 1 to 2% of the general population in the US. Atrial fibrillation, which typically occurs in the presence of an underlying structural heart disease, imparts considerable impacts on the clinical outcomes of the affected individuals including stroke and other thromboembolic events.

Elucidation of the molecular genetic basis of monogenic cardiac rhythm disorders in conjunction with characterization of ion channels biophysical properties in patients with structural heart disease and model organisms have provided considerable insights into the molecular basis of cardiac arrhythmias. Yet, development of effective anti-arrhythmic pharmacological agents that have proven clinical success and are effective without pro-arrhythmic and off-target effects, has been extremely difficult, even though ion channels are among the most common targets in the current pharmacopeia. Indeed, not a single effective new pharmacological agent for the treatment of ventricular arrhythmias has been developed during the last two decades. The challenge of developing a successful pharmacological therapy for cardiac arrhythmias and prevention of SCD partly reflects the diversity and abundance of ion channels in each cell and organ as well as the complex transcriptional and post-translational regulation of the channels.
The diversity is best reflected by the fact that human genome contains about 3,000 genes that code for ion channels, each transcribed into multiple alternatively spliced variants and some shared among multiple organs including the heart and the brain. The complexity poses considerable risk for off-target effects of any candidate pharmacological agent and hence, renders the challenge of developing effective new drugs that target a specific ion channel even more daunting. Thus, gargantuan efforts are needed to better delineate the structural and molecular changes that are responsible for cardiac arrhythmias and SCD, whether they are involved in ion channels directly or indirectly through accessory proteins or post-translational regulation of the expressed proteins. The editors of Circulation Research are enthusiastic about facilitating and disseminating scientific advances regarding the discovery of the molecular and cellular basis of cardiac arrhythmias, the identification of novel pharmacological targets, and the development of novel agents to prevent and treat cardiac arrhythmias and abrogate SCD. Indeed, Circulation Research has been a leading forum for the dissemination of clinical, translational and molecular mechanistic studies in cardiac arrhythmias and SCD.

The following represent a selection of recently published Circulation Research articles on cardiac arrhythmias and electrophysiology, presented in their reverse order of publication. Articles highlighted in yellow represent the top 10 most read original research articles selected based on the number of Full Text/PDF downloads, adjusted to compensate for differences in the length of time since online publication.

Connexin45 Provides Optimal Atrioventricular Nodal Conduction in the Adult Mouse Heart; Frank et al

What Is Known?

- Gap junction channels in the mammalian heart are formed by connexin (Cx) proteins and contribute to electrical impulse propagation and coordinated contraction of cardiomyocytes.
- Cx45 is strongly expressed in the conduction system of the mouse and human heart.
- The lack of Cx45 in mouse embryos leads to early embryonic death attributable to cardiovascular defects.

What New Information Does This Article Contribute?

- For the first time, the functional contribution of Cx45 to impulse propagation in the conduction system of the adult mouse heart has been clarified and its interaction with Cx30.2 has been highlighted.
- Cx45 and Cx30.2 provide optimal impulse propagation through the atrioventricular (AV) node.

Conclusions

Cx45 is required for optimal impulse propagation in the atrioventricular node and stabilizes the level of the coexpressed Cx30.2 protein in the adult mouse heart. In contrast to the embryo, Cx45 is not essential for the viability of adult mice.

TMEM16A/ANO1 Channels Contribute to the Myogenic Response in Cerebral Arteries; Bully et al

What Is Known?

- Intravascular pressure stimulates arterial smooth muscle cell membrane depolarization, leading to voltage-dependent calcium (Ca2+) channel activation, an elevation in intracellular Ca2+ concentration, and vasoconstriction (the myogenic response).
- Smooth muscle cell nonselective cation channels, including transient receptor potential (TRP) channels, contribute to pressure-induced membrane depolarization.
- Arterial smooth muscle cell chloride (Cl−) channels may also regulate vascular contractility and contribute to the myogenic response, but defining physiological functions of these channels in the vasculature has been hindered by uncertain identity of protein(s) involved and a lack of selective modulators.

What New Information Does This Article Contribute?

- Cell swelling and pressure-induced membrane stretch activate TMEM16A, a Ca2+-activated Cl− channel, in cerebral artery smooth muscle cells.
- Membrane stretch activates TMEM16A currents through a mechanism that involves upstream induction of nonselective cation channels that generate a local intracellular Ca2+ signal to stimulate TMEM16A.
- Intravascular pressure stimulates smooth muscle cell TMEM16A channels, leading to membrane depolarization and vasoconstriction.

Conclusions

Membrane stretch activates arterial myocyte TMEM16A channels, leading to membrane depolarization and vasoconstriction. Data also provide a mechanism by which a local Ca2+ signal generated by nonselective cation channels stimulates TMEM16A channels to induce myogenic constriction.

Redox-Sensitive Sulfenic Acid Modification Regulates Surface Expression of the Cardiovascular Voltage-Gated Potassium Channel Kv1.5; Svoboda et al

What Is Known?

- Multiple potassium channels, which play an essential role in maintaining normal cardiac rhythm and blood vessel contractility, are regulated by oxidative stress.
- Modification of cysteine residues of proteins to sulfenic acids alters the expression and function of many proteins under oxidative stress.
- Diseases such as chronic atrial fibrillation and hypoxic pulmonary hypertension (HPH) are characterized by both oxidative stress and reduced expression of the oxygen-sensitive, voltage-gated potassium (Kv) channel Kv1.5.
What New Information Does This Article Contribute?

• Chronic atrial fibrillation in human patients is accompanied by a global increase in sulfenic acid–modified proteins in the heart.
• Sulfenic acid containing Kv1.5 protein is present in normal heart, and the level of this modification increases under oxidative stress.
• Sulfenic acid formation in Kv1.5 reduces the level of the channel protein on the cell surface, disrupts its normal trafficking, and promotes its degradation in cardiac myocytes.

Conclusions
Sulfenic acid modification to proteins, which is elevated in diseased human heart, regulates Kv1.5 channel surface expression and stability under oxidative stress and diverts channel from a recycling pathway to degradation. This provides a molecular mechanism linking oxidative stress and downregulation of channel expression observed in cardiovascular diseases.

Calcium Leak Through Ryanodine Receptors Leads to Atrial Fibrillation in 3 Mouse Models of Catecholaminergic Polymorphic Ventricular Tachycardia; Shan et al13

What Is Known?
• Chronic atrial fibrillation (AF) is associated with increased diastolic sarcoplasmic reticulum (SR) Ca2+ leak in atrial cardiac myocytes.
• Catecholaminergic polymorphic ventricular tachycardia (CPVT)-linked ryanodine receptor 2 (RyR2) mutations cause diastolic SR Ca2+ leak in ventricular cardiac myocytes.
• Recent reports show that CPVT patients have increased prevalence of AF.

What New Information Does This Article Contribute?
• Diastolic SR Ca2+ leak probably plays a critical role in initiating AF in murine models of human CPVT-linked RyR2 mutations.
• S107 (Rycal) significantly inhibits diastolic SR Ca2+ leak in atrial myocytes and prevents pacing-induced AF in models of human CPVT-linked RyR2 mutations.

Conclusions
The present study demonstrates that RyR2-mediated diastolic SR Ca2+ leak in atrial myocytes is associated with AF in murine mice. Moreover, the Rycal S107 inhibited diastolic SR Ca2+ leak through RyR2 and pacing-induced AF associated with CPVT mutations.

Could Plugging the Oxidation-Mediated Ca2+ Leak Stem the Tide of the Atrial Fibrillation Epidemic? [Editorial]; Backx14

Abstract
As the most common sustained arrhythmia, atrial fibrillation (AF) afflicts >2 million patients in the United States alone, and this number is predicted to double by the year 2050.1 Although AF is not life-threatening, it is linked to a 2-fold increase in mortality when present as a comorbidity in heart disease patients.2,3 AF can have devastating consequences, such as stroke, impaired cardiac performance, and promotion of cardiomyopathy. The treatment of AF can also produce life-threatening side effects, such as ventricular arrhythmias and hemorrhage. AF patients are generally classified as paroxysmal (episodes lasting <7 days), persistent (lasting >7 days but treated to restore sinus rhythm), or permanent (no attempt to restore sinus rhythm).2,3 AF is usually secondary to other conditions such as hypertension, heart failure, valvular disease, sleep apnea, hyperthyroidism, and diabetes. Most of these AF-inducing conditions are associated with elevated atrial pressure and atrial stretch, as well as increased oxidative stress and inflammation, which lead to structural and electric changes (remodeling) that create a substrate for supporting AF induction and maintenance.15

Functional NaV1.8 Channels in Intracardiac Neurons: The Link Between SCN10A and Cardiac Electrophysiology; Verkerk et al15

What Is Known?
• The sodium channel isoform NaV1.8 (encoded by the SCN10A gene) is highly expressed in neurons of the dorsal root ganglia and cranial sensory ganglia, where it is involved in generating and maintaining action potentials and controlling neuronal firing patterns.
• Several recent genome-wide association studies have linked SCN10A to PR interval and QRS duration on the ECG, but the precise localization and role of NaV1.8/SCN10A in the heart remains unknown.

What New Information Does This Article Contribute?
• NaV1.8-based sodium channels are absent in cardiomyocytes but are present in the intracardiac neurons of the murine heart.
• NaV1.8 blockade markedly reduces action potential firing frequency in intracardiac neurons.
• NaV1.8/SCN10A might affect myocardial electrophysiological properties through regulation of cardiac neural activity.

Conclusions
Our findings demonstrate the functional presence of SCN10A/NaV1.8 in intracardiac neurons, indicating a novel role for this neuronal sodium channel in regulation of cardiac electric activity.

Blocking Scn10a Channels in Heart Reduces Late Sodium Current and Is Antiarrhythmic; Yang et al16

What Is Known?
• Genome-wide association studies have implicated variation in the sodium channel gene SCN10A as modulators of cardiac conduction (PR and QRS durations).
SCN10A was originally cloned from dorsal root ganglion, and the encoded channel (termed Nav1.8) is thought to play a role in pain perception.

What New Information Does This Article Contribute?
- A specific Nav1.8 blocker eliminates the persistent sodium current recorded after long depolarizing pulses in mouse and rabbit cardiomyocytes and shortens the action potential duration.
- These effects are absent in myocytes from Scn10a knockout mice.
- The Nav1.8 blocker reverses arrhythmogenic early afterdepolarizations elicited by experimental conditions that increase persistent sodium current.

Conclusions
Scn10a expression contributes to late sodium current in heart and represents a new target for antiarrhythmic intervention.

High-Resolution 3-Dimensional Reconstruction of the Infarct Border Zone: Impact of Structural Remodeling on Electrical Activation; Rutherford et al.17

What Is Known?
- The border zone (BZ) surrounding a healed myocardial infarction (MI) is a region in which the normal organization of cardiac cells is disrupted.
- The infarct BZ can provide a substrate for reentrant electric activity, giving rise to propagation delays and unidirectional conduction block.

What New Information Does This Article Contribute?
- We have imaged the 3-dimensional (3D) arrangement of myocytes and collagen in the BZ and normal myocardium adjacent to healed rat infarcts at higher spatial resolution and across larger tissue volumes than previously reported.
- We have quantitatively determined the topology and physical coupling of myocytes from these images and interrogated the functional impact of BZ structure on electric activation using computer models.
- We have demonstrated that in the BZ following MI, the 3D cellular organization alone is sufficient to provide the necessary conditions for sustained reentrant electric activity.

Conclusions
We have used a detailed image-based model of the infarct BZ to demonstrate that structural heterogeneity provides a dynamic substrate for electric reentry.

Whither Art Thou, SCN10A, and What Art Thou Doing? [Editorial]; London18

Abstract
Since its initial cloning in 1992, SCN5A (Nav1.5) has become known as “the” cardiac sodium channel.1 SCN5A encodes a tetrodotoxin (TTX)-resistant channel that is responsible for the inward sodium current (INa), which initiates the cardiac action potential. Its RNA and protein expression dominate the sodium channel landscape in the ventricle, atrium, and specialized conduction system. Mice heterozygous for a targeted Scn5a deletion (Scn5a+/− mice) have slowed conduction along with atrial and ventricular arrhythmias, whereas homozygous targeted deletion in mice is embryonic lethal.2 In humans, pharmacological agents that block SCN5A such as flecainide, developed as antiarrhythmics, are proarrhythmic and increase mortality in patients with structural heart disease.3 In addition, SCN5A mutations cause a potpourri of diseases that lead to sudden cardiac death including long-QT syndrome type 3 (LQT3, gain-of-function mutations that slow inactivation and increase late sodium current, INa,L), Brugada syndrome (BRS1, loss of function mutations that decrease peak INa), conduction disease, atrial fibrillation, and dilated cardiomyopathy.4 More recently, changes in SCN5A (expression, splicing, posttranslational modifications including CaMKII phosphorylation) and/or its β-subunits that decrease peak INa or increase INa,L have been implicated as potential contributing factors for more common types of sudden death from acquired heart diseases such as heart failure.5–8

Epistatic Rescue of Nkx2.5 Adult Cardiac Conduction Disease Phenotypes by Prospero-Related Homeobox Protein 1 and HDAC3; Risebro et al.19

What Is Known?
- Nkx2.5 is an essential cardiac transcription factor with conserved roles in the developing and adult cardiac conduction system.
- Dominant mutations in NKX2.5 in humans result in cardiac defects which include delayed or blocked atrioventricular conduction.

What New Information Does This Article Contribute?
- Nkx2.5 plays a critical role in maintaining the adult conduction system.
- The homeobox factor Prox1, acting with HDAC3, acts as a direct negative regulator of Nkx2.5 and haploinsufficiency for Prox1 rescues anatomical, molecular, and functional conduction defects in Nkx2.5 heterozygous mutant mice.
- Prox1 is a direct upstream regulator of Nkx2.5 and the Prox1-HADC3=>NKX2.5 signaling pathway may be a novel therapeutic target in human conduction disease.

Conclusions
Here we identify Prox1 as a direct upstream modifier of Nkx2.5 in the maintenance of the adult conduction system and rescue of Nkx2.5 conduction disease phenotypes. This study is the first example of rescue of Nkx2.5 function and establishes a model for ensuring electrophysiological function within the adult heart alongside insight into a novel Prox1-HDAC3-Nkx2.5 signaling pathway for therapeutic targeting in conduction disease.
Myofilament Ca Sensitization Increases Cytosolic Ca Homeostasis, and Causes Pause-Dependent Ca-Triggered Arrhythmia; Schober et al20

What Is Known?

- Familial hypertrophic cardiomyopathy (FHC) is an inherited disease caused by mutations in sarcomeric proteins that is associated with a high risk for ventricular arrhythmia and sudden death.
- FHC-linked mutations often increase myofilament calcium sensitivity, which has been linked to increased arrhythmia susceptibility.
- Myofilaments are the dominant cytosolic calcium buffer, binding about 50% of calcium entering the cytosol during a normal heart beat.

What New Information Does This Article Contribute?

- Calcium-sensitizing FHC mutants increase the cytosolic calcium binding affinity (Kd) and cause excess cytosolic calcium accumulation during physiological heart rates, which shifts into the sarcoplasmic reticulum during longer diastolic intervals.
- The pause-dependent excessive sarcoplasmic reticulum calcium uptake and subsequent release causes action potential prolongation, early afterdepolarizations and triggered beats that can be prevented by myofilament calcium desensitization.
- Acute calcium sensitization with EMD 57033 mimics the effects of calcium-sensitizing FHC mutants and produces pause-dependent ventricular arrhythmia after acute myocardial infarction.

Conclusions

Myofilament Ca sensitization increases cytosolic Ca binding affinity. A major proarrhythmic consequence is a pause-dependent potentiation of Ca release, action potential prolongation, and triggered activity. Increased cytosolic Ca binding represents a novel mechanism of pause-dependent arrhythmia that may be relevant for inherited and acquired cardiomyopathies.

Noise-Free Visualization of Microscopic Calcium Signaling by Pixel-Wise Fitting; Tian et al21

What Is Known?

- Fast confocal imaging of beating cardiac myocytes is traditionally performed only in one spatial dimension thereby restricting representativeness and spatial information.
- Ultrafast 2-dimensional confocal data are noisy, and noise-removing procedures inevitably alter the data and its information content.
- Calcium alternans in single cardiac myocytes correlate of T-wave alternans in the ECG.

What New Information Does This Article Contribute?

- This new method for image processing allows noise removal from microscopic imaging of cardiac myocytes producing noise-free “movies” of beating cells.
- Very locally occurring alternans (“microscopic alternans”) are precursors of previously known alternans (“macroscopic alternans”).

Conclusions

Pixel-wise fitting provides novel insights into cardiac excitation-contraction coupling. Specifically, it revealed microscopic calcium alternans on the level of individual coupling sites. Microscopic calcium alternans is an early precursor of cellular alternans and as such will shed more light onto this mechanism leading to cardiac arrhythmia.

Ruled by the Clock [Commentary]; Tomaselli22

Abstract

The ability of organisms to respond to time allows for anticipation of cyclic changes in the environment that provide a survival advantage. The time variance of environmental influences on an organism may occur over years, months, or days. Biological clocks are ubiquitous and hierarchical in mammals and provide cell autonomous, transcriptionally mediated mechanism(s) to regulate function over several time scales.1 The master clock in mammals is in the suprachiasmatic nucleus (SCN) of the hypothalamus, which is regulated by external cues (zeitgebers), prominently light. Circadian rhythmicity is maintained by endogenous 24-hour cycle clocks that can be regulated by external signals to produce diurnal behaviors. Circadian clocks are characteristic of most mammalian tissues and are coupled to produce complex diurnal behaviors.2

Calmodulin-Dependent Protein Kinase II: Linking Heart Failure and Arrhythmias [Review]; Swaminathan et al23

Abstract

Understanding relationships between heart failure and arrhythmias, important causes of suffering and sudden death, remains an unmet goal for biomedical researchers and physicians. Evidence assembled over the past decade supports a view that activation of the multifunctional Ca2+ and calmodulin-dependent protein kinase II (CaMKII) favors myocardial dysfunction and cell membrane electrical instability. CaMKII activation follows increases in intracellular Ca2+ or oxidation, upstream signals with the capacity to transition CaMKII into a Ca2+ and calmodulin-independent constitutively active enzyme. Constitutively active CaMKII appears poised to participate in disease pathways by catalyzing the phosphorylation of classes of protein targets important for excitation-contraction coupling and cell survival, including ion channels and Ca2+ homeostatic proteins, and transcription factors that drive hypertrophic and inflammatory gene expression. This
rich diversity of downstream targets helps to explain the potential for CaMKII to simultaneously affect mechanical and electrical properties of heart muscle cells. Proof-of-concept studies from a growing number of investigators show that CaMKII inhibition is beneficial for improving myocardial performance and for reducing arrhythmias. We review the molecular physiology of CaMKII and discuss CaMKII actions at key cellular targets and results of animal models of myocardial hypertrophy, dysfunction, and arrhythmias that suggest CaMKII inhibition may benefit myocardial function while reducing arrhythmias.

Local β-Adrenergic Stimulation Overcomes Source-Sink Mismatch to Generate Focal Arrhythmia; Myles et al24

What Is Known?

- In isolated cardiac myocytes, β-adrenergic stimulation can cause spontaneous Ca2+ release from the sarcoplasmic reticulum, which may depolarize the membrane and lead to triggered action potentials.
- In the intact heart, strong electrotonic coupling exists between cells, which means that spontaneous Ca2+ release in a single cell cannot produce sufficient change in membrane potential to trigger propagating action potentials and arrhythmia (‘source-sink mismatch’).
- For spontaneous Ca2+ release to trigger arrhythmias in the intact heart, it must be synchronized across many cells, but little is known about how many are required, or the mechanism of synchrony.

What New Information Does This Article Contribute?

- Localized β-adrenergic stimulation by norepinephrine injection leads to Ca2+-mediated focal arrhythmia in the intact rabbit heart.
- Local β-adrenergic stimulation synchronizes spontaneous Ca2+ release from the sarcoplasmic reticulum across thousands of cells, overcoming the source-sink mismatch.
- Source-sink interactions are critically important in the generation of Ca2+-mediated focal arrhythmia.

Conclusions

These data provide the first experimental demonstration that localized β-adrenergic receptor stimulation produces spatiotemporal synchronization of sarcoplasmic reticulum Ca2+ overload and release in the intact heart and highlight the critical nature of source-sink balance in initiating focal arrhythmias.

Electrical Coupling and Propagation in Engineered Ventricular Myocardium With Heterogeneous Expression of Connexin43; Beauchamp et al25

What Is Known?

- Connexin (Cx) proteins Cx43 and Cx45 in the ventricular myocardium are responsible for intercellular electric low-resistance pathways and assure normal electric propagation.
- In human cardiac failure, ventricular remodeling of Cx43 leading to heterogeneous Cx43 expression has been associated with arrhythmogenesis.
- Mouse models of cardiac-restricted and heterogeneous Cx43 ablation have a high incidence of ventricular arrhythmias if electric propagation velocity decreases <50% of normal, with average Cx43 immunofluorescence signals as low as 18%.

What New Information Does This Article Contribute?

- Cardiac strands engineered with mixtures of Cx43 wild-type (WT) and Cx43-null cells show a marked decrease of electric velocity to <50% of normal velocity if the proportion of Cx43-null cells is increased >50%. The average Cx43 immunosignal underestimates the proportion of Cx43 WT cells.
- At the cellular level, microscopic propagation is characterized by a combination of fast propagation meandering across Cx43 WT cell clusters and discontinuous delayed propagation within areas of Cx43-null cells.
- The electric conductance at the interface between cell pairs engineered from Cx43 WT and Cx43-null ventricular myocytes is very low (<10% of normal) and determined by the presence of mixed gap junction channels formed from heteromeric Cx43/Cx45 and homomeric Cx45 connexons.

Conclusions

Heterogeneous ablation of Cx43 leads to a marked decrease in propagation velocity in tissue strands composed of <50% cells with WT Cx43 expression and marked dissociation of excitation at the cellular level. However, the small residual electric conductance between Cx43 and WTGFP myocytes assures excitation of Cx43−/− cells. This explains the previously reported undisturbed contractility in tissues with spatially heterogeneous downregulation of Cx43 expression.

Orai1 Determines Calcium Selectivity of an Endogenous TRPC Heterotetramer Channel; Cioffi et al26

What Is Known?

- Transient receptor potential channel (TRPC) proteins TRPC1 and TRPC4 mediate endothelial cell store-operated calcium entry.
- Orai1 is also a component of endothelial cell store-operated calcium channels.
- Calcium entry through TRPC1- and TRPC4-containing channels disrupts the endothelial cell barrier, but it is not clear whether Orai1 interacts with TRPC protein to alter ion channel properties and endothelial permeability.
What New Information Does This Article Contribute?

- Orai1 interacts with TRPC4 and is a component of the TRPC1- and TRPC4-containing channel in endothelium, both in vitro and in vivo.
- Orai1 increases calcium selectivity of, and calcium permeation through, the TRPC channel.
- Orai1 is required for calcium influx through store-operated calcium channels to induce interendothelial cell gaps.

Conclusions

Orai1 interacts with TRPC4 in the endogenous channel complex, where it controls TRPC1/4 activation and channel permeation characteristics, including calcium selectivity, important for control of endothelial cell barrier function.

Tuning Electrical Conduction Along Endothelial Tubes of Resistance Arteries Through Ca2+-Activated K+ Channels; Behringer & Segal²⁷

What Is Known?

- The endothelium conducts electrical signals along resistance vessels to coordinate relaxation of smooth muscle cells.
- Gap junctions provide a low-resistance pathway for current to flow between endothelial cells.
- Small and intermediate Ca2+-activated K+ channels (SKCa/IKCa) are highly expressed in endothelial cells and initiate hyperpolarization.

What New Information Does This Article Contribute?

- Electrical conduction of hyperpolarization and depolarization along the endothelium produce equivalent (but opposite) changes in membrane potential.
- Irrespective of gap junctions or charge polarity, opening voltage-insensitive ion channels dissipates electrical signals along the endothelium.
- Activation of SKCa/IKCa channels effectively “tunes” the ability of the endothelium to conduct electrical signals.

Conclusions

These findings illustrate a novel role for SKCa/IKCa activity in tuning electrical conduction along the endothelium of resistance vessels by governing signal dissipation through changes in membrane resistance. Voltage-insensitive ion channels can thereby tune intercellular electrical signaling independent from gap junction channels.

Explaining Calcium-Dependent Gating of Anoctamin-1 Chloride Channels Requires a Revised Topology; Yu et al²⁸

What Is Known?

- The ion channel anoctamin-1 (Ano1) regulates vascular smooth muscle tone and participates in cardiac action potential repolarization in some species (comprising the current known as Ito2).

What New Information Does This Article Contribute?

- Ano1 conducts chloride ions and is activated by increases in intracellular calcium.

What New Information Does This Article Contribute?

- The study describes the structural determinants of calcium activation of Ano1.
- The protein domain involved in Ano1 calcium regulation was formerly thought to be extracellular; hence, a new model for the orientation of the channel in the membrane is proposed.
- The study provides insights that may be helpful in designing and evaluating new drugs that target Ano1.

Conclusions

We propose an alternative model of Ano1 topology based on mutagenesis, epitope accessibility, and cysteine-scanning accessibility. These data contradict the popular re-entrant loop model by showing that the putative fourth extracellular loop (ECL 4) is intracellular and may contain a Ca2+ binding site. These studies provide new perspectives on regulation of Ano1 by Ca2+.

Viral Gene Transfer Rescues Arrhythmogenic Phenotype and Ultrastructural Abnormalities in Adult Calsequestrin-Null Mice With Inherited Arrhythmias; Denegri et al²⁹

What Is Known?

- Recessive catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited disease that predisposes to cardiac arrest and sudden death, and it is caused by mutations in the calsequestrin 2 (CASQ2) gene, leading to CASQ2 reduction or disappearance.
- CASQ2 reduction decreases levels of triadin (TrD) and junctin (JnC), which are important components of the sarcoplasmic reticulum (SR) calcium-release channel complex leading to impaired SR calcium release, junctional sarcoplasmic reticulum (jSR) structural abnormalities, and life-threatening arrhythmias.

What New Information Does This Article Contribute?

- Administration of the CASQ2 gene by in vivo viral transfer infects 50% of myocytes and increases the tissue levels of CASQ2, TrD, and JnC to 80% to 90% of control.
- Exogenous CASQ2 rescues ultrastructural abnormalities, reverts electrophysiological instability of myocytes, and abolishes ventricular tachycardia.

Conclusions

We have proven the concept that induction of CASQ2 expression in knockout mice reverts the molecular, structural, and electric abnormalities and prevents life-threatening arrhythmias in CASQ2-defective catecholaminergic polymorphic ventricular tachycardia mice. These data support the view that development of CASQ2 viral gene transfer could have clinical application.
Optical Imaging of Voltage and Calcium in Cardiac Cells & Tissues [Review]; Herron et al

Abstract
Cardiac optical mapping has proven to be a powerful technology for studying cardiovascular function and disease. The development and scientific impact of this methodology are well-documented. Because of its relevance in cardiac research, this imaging technology advances at a rapid pace. Here, we review technological and scientific developments during the past several years and look toward the future. First, we explore key components of a modern optical mapping setup, focusing on: (1) new camera technologies; (2) powerful light-emitting-diodes (from ultraviolet to red) for illumination; (3) improved optical filter technology; (4) new synthetic and optogenetic fluorescent probes; (5) optical mapping with motion and contraction; (6) new multiparametric optical mapping techniques; and (7) photon scattering effects in thick tissue preparations. We then look at recent optical mapping studies in single cells, cardiomyocyte monolayers, atria, and whole hearts. Finally, we briefly look into the possible future roles of optical mapping in the development of regenerative cardiac research, cardiac cell therapies, and molecular genetic advances.

Shortened Ca2+ Signaling Refractoriness Underlies Cellular Arrhythmogenesis in a Postinfarction Model of Sudden Cardiac Death; Belevych et al

What Is Known?

- In multiple pathologies associated with both genetic and acquired defects in the cardiac ryanodine receptor (RyR2) channel, arrhythmias result from aberrant Ca2+ release from the sarcoplasmic reticulum (SR) in the form of spontaneous diastolic Ca2+ waves.
- Normal control of SR Ca2+ release involves Ca2+-dependent activation of RyR2s followed by their store-dependent deactivation rendering the RyR2s refractory during diastole.
- Spontaneous diastolic Ca2+ waves are thought to arise when [Ca2+] in the SR ([Ca2+]SR) exceeds a critical threshold level thereby directly activating RyR2s; however, direct experimental conformation of this proposed mechanism is lacking.

What New Information Does This Article Contribute?

- Intra-SR [Ca2+] during arrhythmogenic Ca2+ waves are recorded in cardiac myocytes isolated from post–myocardial infarction (MI) canine hearts prone to ventricular fibrillation (VF).
- Ca2+ waves do not arise immediately on [Ca2+]SR reaching its final Ca2+ level, but rather occur with a distinct time delay that is markedly shorter in myocytes from post-MI hearts.
- Increased predisposition toward Ca2+ waves in myocytes from post-MI hearts is due to diminished/shortened refractoriness of RyR2, caused by reduced ability of RyR2s to become deactivated by a decline in luminal Ca2+ after systolic SR Ca2+ release.
- Impaired refractory behavior of RyR2s is attributable to posttranslational modification of the RyR2 protein by both Ca2+/calmodulin-dependent protein kinase (CaMKII)-dependent phosphorylation as well as oxidation.

Conclusions
The attainment of a certain threshold [Ca2+]SR is not sufficient for the generation of DCWs. Postrelease Ca2+ signaling refractoriness critically influences the occurrence of DCWs. Shortened Ca2+ signaling refractoriness due to RyR2 phosphorylation and oxidation is responsible for the increased rate of DCWs observed in VF myocytes and could provide a substrate for synchronization of arrhythmogenic events at the tissue level in hearts prone to VF.

Inhibition of CaMKII Phosphorylation of RyR2 Prevents Induction of Atrial Fibrillation in FKBP12.6 Knockout Mice; Li et al

What Is Known?

- Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia.
- Increased open probability of ryanodine receptors (RyR2) contributes to defective intracellular Ca2+ handling in AF.
- Ca2+/calmodulin-dependent kinase II (CaMKII) is upregulated in patients with chronic AF.

What New Information Does This Article Contribute?

- Genetic inhibition of CaMKII phosphorylation of RyR2 prevents induction of AF in FKBP12.6-deficient mice.
- CaMKII phosphorylation of RyR2 promotes spontaneous Ca2+ waves, activation of inward Na+/Ca2+ exchange current, and delayed afterdepolarizations in atrial myocytes from FKBP12.6-deficient mice.

Conclusions
FKBP12.6 mice exhibit AF caused by SR Ca2+ leak, Na+/Ca2+-exchanger activation, and DADs, which promote triggered activity. Genetic inhibition of RyR2-S2814 phosphorylation prevents AF induction in FKBP12.6/− mice by suppressing SR Ca2+ leak and DADs. These results suggest suppression of RyR2-S2814 phosphorylation as a potential anti-AF therapeutic target.

Development, Maturation, and Transdifferentiation of Cardiac Sympathetic Nerves [Review]; Kimura et al

Abstract
The heart is electrically and mechanically controlled as a syncytium by the autonomic nervous system. The cardiac nervous system comprises the sympathetic, parasympathetic, and sensory nervous systems that together regulate heart function on demand. Sympathetic electric activation was initially considered the main regulator of cardiac function; however,
modern molecular biotechnological approaches have provided a new dimension to our understanding of the mechanisms controlling the cardiac nervous system. The heart is extensively innervated, although the innervation density is not uniform within the heart, being high in the subepicardium and the special conduction system. We and others showed previously that the balance between neural chemoattractants and chemorepellents determine cardiac nervous development, with both factors expressed in heart. Nerve growth factor is a potent chemoattractant synthesized by cardiomyocytes, whereas Sema3a is a neural chemorepellent expressed specifically in the subendocardium. Disruption of this well-organized molecular balance and innervation density can induce sudden cardiac death due to lethal arrhythmias. In diseased hearts, various causes and mechanisms underlie cardiac sympathetic abnormalities, although their detailed pathology and significance remain contentious. We reported that cardiac sympathetic rejuvenation occurs in cardiac hypertrophy and, moreover, interleukin-6 cytokines secreted from the failing myocardium induce cholinergic transdifferentiation of the cardiac sympathetic system via a gp130 signaling pathway, affecting cardiac performance and prognosis. In this review, we summarize the molecular mechanisms involved in sympathetic development, maturation, and transdifferentiation, and propose their investigation as new therapeutic targets for heart disease.

Acidosis Dilates Brain Parenchymal Arterioles by Conversion of Calcium Waves to Sparks to Activate BK Channels; Dabertrand et al34

What Is Known?

- Cerebral blood flow is highly regulated by pH, with acidification causing profound vasodilation.
- In arteries on the surface of the brain (pial arteries), activation of large conductance potassium calcium-activated channels (BKCa) channels by local Ca2+ signals (Ca2+ sparks) through ryanodine receptors opposes vasoconstriction.
- Unlike pial arteries, inhibition of BKCa channels has little effect on the diameter of (parenchymal) arterioles within the brain, even though their smooth muscle cells have a significant BKCa channel density.

What New Information Does This Article Contribute?

- Ca2+ waves are the predominant spontaneous Ca2+ signals in parenchymal arteriolar smooth muscles cells.
- Acidosis reshapes spontaneous Ca2+ waves into Ca2+ sparks that activate BKCa channels.
- This mechanism accounts for 60% of acidosis-induced dilation of parenchymal arterioles.

Conclusions

These results support the novel concept that acidification, by converting Ca2+ waves to sparks, leads to the activation of BKCa channels to induce dilation of cerebral PAs.

The Role of Fibroblasts in Complex Fractionated Electrograms During Persistent/Permanent Atrial Fibrillation: Implications for Electrogram-Based Catheter Ablation; Ashihara et al35

What Is Known?

- Electrogram-based catheter ablation, targeting complex fractionated atrial electrograms (CFAEs), is empirically known to be effective in halting persistent/permanent atrial fibrillation (AF).
- Myocardial tissue is mainly composed of myocytes and collagen-producing fibroblasts. Fibroblasts proliferate as part of the atrial structural remodeling associated with persistent/permanent AF.

What New Information Does This Article Contribute?

- Fibroblast proliferation and electrotonic interactions between atrial myocytes and fibroblasts in fibrotic regions lead to the formation of CFAEs during persistent/permanent AF.
- Catheter ablation targeting fibroblast-derived CFAEs can terminate AF by suppressing spiral wave breakup and pushing spiral waves out of the fibroblast proliferation areas.

Conclusions

Fibroblast proliferation in atria might be responsible for the genesis of CFAEs during persistent/permanent AF. Our findings could contribute to better understanding of the mechanisms underlying CFAE-targeted AF ablation.

Dominant-Negative Control of cAMP-Dependent IKs Upregulation in Human Long-QT Syndrome Type 1; Heijman et al36

What Is Known?

- The slowly activating delayed-rectifier K+ current (IKs) plays a major role in ventricular repolarization, particularly during conditions of increased sympathetic tone when IKs is upregulated through cAMP-dependent signaling.
- Loss-of-function mutations in the pore-forming KCNQ1 subunit cause long-QT syndrome type 1 (LQT1), predisposing mutation carriers to ventricular tachyarrhythmias, particularly during exercise or arousal.
- The hot-spot mutation A341V in the S6 transmembrane segment of KCNQ1 (KCNQ1-A341V) results in an unusually severe clinical phenotype with adrenergic receptor polymorphisms as independent arrhythmia risk modifiers.

What New Information Does This Article Contribute?

- KCNQ1-A341V causes a dominant-negative suppression of cAMP-dependent IKs upregulation on top of a dominant-negative reduction in baseline current when the IKs macro-molecular complex is expressed in Chinese hamster ovary cells.
- Loss of cAMP-dependent IKs upregulation is due to loss of phosphorylation at Serine 27 (S27) but not due to reduced
KCNQ1/Yotiao interaction or hindrance of current upregulation after alterations at S27.

- Heterozygous expression of the C-terminal mutation KCNQ1-G589D (“Fin mutation”), as well as heterozygous expression of the phospho-inhibitory substitution KCNQ1-S27A (in the absence of other pathogenic mutations), also results in loss of cAMP-dependent IKs upregulation, indicating that cAMP/PKA-dependent IKs upregulation is under strong dominant-negative control of KCNQ1 phosphorylation at S27.

**Conclusions**

Our results indicate the involvement of the KCNQ1-S6 region at/or around A341 in cAMP-dependent stimulation of IKs, a process that is under strong dominant-negative control, suggesting that tetrameric KCNQ1 phosphorylation is required. Specific long-QT1 mutations, including heterozygous A341V, disable this regulation.

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**Adrenergic Signaling Controls RGK-Dependent Trafficking of Cardiac Voltage-Gated L-Type Ca2+ Channels Through PKD1; Jhun et al**

**What Is Known?**

- RGK proteins are strong inhibitors of voltage-gated calcium channels.
- One of the RGKs, Rem1, is highly expressed in heart.
- The physiological role of Rem1 and its upstream regulation is unknown.

**What New Information Does This Article Contribute?**

- The α-adrenergic receptor stimulation dramatically attenuates Rem1-mediated inhibition of VLCC function and promotes T-tubular localization in cardiomyocytes.
- PKD1-dependent Rem1 phosphorylation of Rem1 (S18) mediates the α-adrenergic regulation of VLCC.
- Stimulation of α-adrenergic-PKD1 and endogenous Rem1 signaling regulates cardiac VLCC channels, demonstrating for the first time a physiological role of Rem1 in the heart.

**Conclusions**

The α1-adrenergic stimulation releases Rem1 inhibition of VLCCs through direct phosphorylation of Rem1 at Ser18 by protein kinase D1, resulting in an increase of the channel activity and transverse-tubule expression. Our results uncover a novel molecular regulatory mechanism of VLCC trafficking and function in the heart and provide the first demonstration of physiological regulation of RGK function.

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**Role of K\textsubscript{ATP} Channels in the Maintenance of Ventricular Fibrillation in Cardiomyopathic Human Hearts; Farid et al**

**What Is Known?**

- Ventricular fibrillation (VF) is the most common cause of sudden cardiac death.
- High-voltage electric shock is the most common procedure to terminate VF.

**What New Information Does This Article Contribute?**

- During VF in myopathic human hearts, there is spatio-temporal heterogeneity in refactoriness across the left ventricular myocardium.
- Blockade of K\textsubscript{ATP} channels by glibenclamide attenuates spatio-temporal heterogeneity in refactoriness, causing spontaneous termination of VF.

**Conclusions**

K\textsubscript{ATP} channel subunit gene expression is heterogeneously altered in the cardiomyopathic human heart. Blockade of KATP channels promotes spontaneous defibrillation in cardiomyopathic human hearts by attenuating the ischemia-dependent spatiotemporal heterogeneity of refactoriness during early VF.

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**Hydrogen Sulfide as Endothelium-Derived Hyperpolarizing Factor Sulfhydrates Potassium Channels; Mustafa et al**

**What Is Known?**

- Hydrogen sulfide (H2S) is a gaseous signaling molecule. It is synthesized by cystathionine γ-lyase (CSE), which is confined predominantly to the vascular endothelium.
- Mice lacking H2S are hypertensive and demonstrate impaired endothelium-dependent vasorelaxation. Thus, H2S acts as an endothelium-derived relaxing factor that mediates vascular relaxation and lowers blood pressure.
- The effects of H2S, unlike those of nitric oxide, are mediated, in part, by the activation of the ATP-sensitive potassium channels (KATP) but are independent of cyclic GMP.

**What New Information Does This Article Contribute?**

- H2S causes a redox-sensitive posttranslational modification, sulfhydration, of a single cysteine, C43, in the Kir 6.1 subunit of the KATP channel.
- Hence, cholinergic, endothelium-dependent vasorelaxation and hyperpolarization are significantly reduced in vessels in which CSE is inhibited, in vessels from CSE/- mice, or in which the KATP channel has been inhibited.
• Sulfhydration of the calcium-dependent intermediate conductance potassium channel (IKca) contributes to H2S-dependent hyperpolarization of endothelial cells.

Conclusions
H2S is a major EDHF that causes vascular endothelial and smooth muscle cell hyperpolarization and vasorelaxation by activating the ATP-sensitive, intermediate conductance and small conductance potassium channels through cysteine S-sulfhydration. Because EDHF activity is a principal determinant of vasorelaxation in numerous vascular beds, drugs influencing H2S biosynthesis offer therapeutic potential.

Splice Variant Specific Modulation of CaV1.2 Calcium Channel by Galectin-1 Regulates Arterial Constriction; Wang et al

What Is Known?
• The CaV1.2 channels play critical roles in vascular smooth muscle contraction and arterial constriction. Alternative splicing provides a posttranscriptional mechanism for exquisite diversity of CaV1.2 function in muscle physiology.
• Galectin-1 (Gal-1) is reported to be involved in the regulation of vascular smooth muscle cells (VSMCs), and play a role in the pathogenesis of pulmonary hypertension.

What New Information Does This Article Contribute?
• Gal-1 binds to the I-II loop of CaV1.2 channels to regulate its expression. Gal-1 modulation of CaV1.2 channel function is influenced by alternative exon 9*. Splice-variant specific inhibition of CaV1.2 by Gal-1 provides tissue-selective regulation.
• Gal-1 binds to the endoplasmic reticulum (ER) export signal on the C-terminus of exon 9 to prevent surface expression. However, the presence of exon 9 may increase the likelihood of this region to form an α-helical structure to prevent binding by Gal-1, thus suggesting a plausible explanation for splice-variant specific regulation by Gal-1.
• Overexpression of Gal-1 inhibits, while knock-down of Gal-1 increases, CaV1.2 currents, indicating that Gal-1 regulates the function of VSMCs via CaV1.2 channels. Down-regulation of Gal-1 increases arterial constriction, suggesting that Gal-1’s regulation in blood vessels may play a role in hypertension.

Conclusions
The above data indicated that Gal-1 regulates ICa,L via decreasing the functional surface expression of CaV1.2 channels in a splice variant selective manner and such a mechanism may play a role in modulating vascular constriction.

Abolishing Myofibroblast Arrhythmogeneity by Pharmacological Ablation of α-Smooth Muscle Actin Containing Stress Fibers; Rosker et al

What Is Known?
• Following insults to the heart like mechanical overload and infarction, normal resident fibroblasts can undergo a phenotype switch to myofibroblasts (“activated fibroblasts”), which are characterized by de novo expression of α-smooth muscle actin containing stress fibers (α-SMA-SFs).
• In addition to their role in cardiac fibrotic remodeling, in vitro data show that myofibroblasts induce arrhythmogenic slow conduction and ectopic activity in cardiac tissue.
• Myofibroblast arrhythmogeneity is based on depolarizing current flow (“injury current flow”) from moderately polarized myofibroblasts to well-polarized cardiac myocytes following establishment of heterocellular gap junctional coupling.

What New Information Does This Article Contribute?
• Pharmacological ablation of α-SMA-SFs in myofibroblasts with actin-targeting drugs (ATDs: Cytochalasin D, Latrunculin B, Jasplakinolide) abolishes their arrhythmogenic interactions with cardiomyocytes in vitro.
• Suppression of myofibroblast arrhythmogeneity is likely due to a hyperpolarization of cells undergoing disruption of α-SMA-SFs, which, in turn, causes a reduction in arrhythmogenic “injury current flow.”
• α-SMA-SFs, the structural hallmark of myofibroblasts, are instrumental for this cell type to exert adverse arrhythmogenic effects on cardiac tissue.

Conclusions
The results suggest that α-SMA containing stress fibers importantly contribute to myofibroblast arrhythmogeneity. After ablation of this cytoskeletal component, cells lose their arrhythmic effects on cardiomyocytes, even if heterocellular electrotonic coupling is sustained. The findings identify α-SMA containing stress fibers as a potential future target of antiarrhythmic therapy in hearts undergoing structural remodeling.

p63RhoGEF Couples Gaq/11-Mediated Signaling to Ca2+ Sensitization of Vascular Smooth Muscle Contractility; Momotani et al

What Is Known?
• The small GTPase RhoA is activated by multiple agonists and significantly contributes to vascular contractility under physiological as well as pathophysiological conditions such as hypertension.
• Multiple GTP exchange factors (GEFs) are expressed in smooth muscle raising the possibility that specific agonists of specific G-protein coupled receptors (GPCRs) may be associated with distinct RhoGEFs.
• Angiotensin II has been shown to signal through Gq/11 in cultured smooth muscle cells but the role of other agonists in intact blood vessels is unknown, particularly agonists that regulate basal vascular tone.

What New Information Does This Article Contribute?

• p63RhoGEF is selectively activated by agonists such as α-adrenergic and endothelin-1 that signal through Gq/11 in blood vessels and maintain normotensive blood pressure.
• Knock down of p63RhoGEF decreases RhoA activity leading to increased myosin phosphatase activity, decreased myosin phosphorylation and decreased force development in blood vessels.
• We demonstrate in vivo that the molecular mechanism of action of p63RhoGEF is consistent with a model derived from crystallographic studies.
• p63RhoGEF is a potential selective therapeutic target for decreasing peripheral resistance and blood pressure.

Conclusions
We demonstrate that p63RhoGEF selectively couples Gq/11 but not G12/13, to RhoA activation in blood vessels and cultured cells and thus mediates the physiologically important Ca2+ sensitization of force induced with Gq/11-coupled agonists. Our results suggest that signaling through p63RhoGEF provides a novel mechanism for selective regulation of blood pressure.

IrX3: A Conductor of Conduction [Commentary]; Kelly

Extract:
Despite major clinical importance as a cause of arrhythmias, how the transcriptional program of the ventricular conduction system is controlled remains obscure. In a recent article published in PNAS, Zhang et al identify an evolutionarily conserved role for the transcription factor Irx3 in regulating gap junction gene expression and fast propagation of electrical signal in the vertebrate ventricular conduction system.

Human Atrial Action Potential and Ca2+ Model: Sinus Rhythm and Chronic Atrial Fibrillation; Grandi et al

What is Known?
• Atrial cells exhibit electrophysiological characteristics that differ from those of ventricular cells due to structural differences and specific combinations of ion channel/transporter expression and function.
• During chronic atrial fibrillation (AF), electrical and structural remodeling contributes to the development of the AF substrate, and abnormalities in intracellular Ca2+ cycling have emerged as key mediators in AF pathophysiology.
• Detailed models of myocyte Ca2+ cycling have typically focused on ventricular rather than atrial myocytes, in part because of limited appropriate experimental data (especially from human atrial myocytes).

What New Information Does This Article Contribute?

• Based on recent data from human atrial cells, we have developed a new mathematical model of the human atrial myocyte that accounts for the electrophysiological and Ca2+ handling properties of atrial cells in both normal and chronic AF conditions.
• Simulations indicate that heart rate-dependent action potential duration (APD) shortening in healthy atrial cells involves the accumulation of intracellular [Na+] at high frequencies that causes outward shifts in Na+/Ca2+ exchange and Na+/K+ pump currents, whereas ionic and Ca2+ handling remodeling lead to reduced Na+ accumulation in chronic AF, which causes a blunted APD rate-dependent response.
• Our modeling suggests that IKur is a key component of the adrenergic response of human atrial cells, as its loss (such as in Kv1.5 channelopathy) results in predisposition to early afterdepolarizations in the presence of isoproterenol and may help explain the bouts of stress mediated AF observed in these patients.

Conclusions
Our study provides a novel tool and insights into ionic bases of atrioventricular AP differences, and shows how Na+ and Ca2+ homeostases critically mediate abnormal repolarization in AF.

Differential Protein Kinase C Isoform Regulation and Increased Constitutive Activity of Acetylcholine-Regulated Potassium Channels in Atrial Remodeling; Makary et al

What is Known?
• Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder. It remodels atrial electric function in a way that promotes its own maintenance and causes increasing resistance to therapy.
• An important component of AF-related remodeling is increased ligand-independent (constitutive) activity of a ligand-gated potassium channel that is normally operated by acetylcholine. Protein kinase C (PKC) modulates the activity of several ion channels.

What New Information Does This Article Contribute?

• Conventional (Ca2+-dependent) PKC isoforms inhibit, whereas the novel (Ca2+-independent) PKC isoforms enhance the activity of atrial acetylcholine-regulated potassium channels.
• Rapid activation of atrial cardiomyocytes to mimic AF reduces the expression of the conventional PKCα isoform and enhances membrane localization of the novel PKCe isoform, shifting the balance of PKC action toward channel activation.
• Tachycardia-induced changes in PKCα isoform expression require cell Ca2+ loading, likely via the Ca2+-dependent proteolytic enzyme calpain.

Conclusions
PKC isoforms differentially modulate IKACH, with conventional Ca2+-dependent isoforms inhibiting and novel isoforms enhancing activity. ATR causes a rate-dependent PKC isoform switch, with Ca2+/calpain-dependent downregulation of inhibitory PKCα and membrane translocation of stimulatory PKCε, enhancing IKACHC. These findings provide novel insights into mechanisms underlying IKACHC dysregulation in AF.

Phosphodiesterase 4D Regulates Baseline Sarcoplasmic Reticulum Ca2+ Release and Cardiac Contractility, Independently of L-Type Ca2+ Current; Beca et al46

What Is Known?
• Cyclic nucleotide phosphodiesterases (PDEs) are a complex family of enzymes encoded by 23 distinct genes that degrade cAMP/cGMP.
• PDEs are typically found in macromolecular complexes allowing tight spatial and temporal control of cAMP-dependent signaling in cellular microdomains.
• Family-specific PDE inhibitors are used clinically for inotropic support in heart failure patients; however, their prolonged use increases mortality.

What New Information Does This Article Contribute?
• PDE4D is tethered to the sarcoplasmic reticular (SR) Ca2+ ATPase type 2a (SERCA2a), thereby suppressing baseline cAMP/protein kinase A–dependent Ca2+ cycling.

Conclusions
PDE4D regulates basal cAMP levels in SR microdomains containing SERCA2a-PLN, but not L-type Ca2+ channels or ryanodine receptor. Because whole-cell Ca2+ transient amplitudes are reduced in failing human myocardium, these observations may have therapeutic implications for patients with heart failure.

HCN3 Contributes to the Ventricular Action Potential Waveform in the Murine Heart; Fenske et al47

What Is Known?
• Hyperpolarization-activated cyclic nucleotide-gated channels (HCN channels) are transmembrane proteins that conduct sodium and potassium ions. The current passed by these channels is called Ih or If.
• In spontaneously beating cells of the sinoatrial node (SAN), Ih plays a key role in the generation of the pacemaker potentials. Consequently, Ih is a major determinant of cardiac automaticity.
• The HCN channel family comprises four members (HCN1–HCN4) that assemble to homotetrameric and heterotetrameric complexes.

What New Information Does This Article Contribute?
• HCN3 is a component of ventricular Ih.
• In ventricular myocytes, HCN3 is constitutively open at resting membrane potential. During the time course of the ventricular action potential (AP), HCN3 channels do not close (deactivate) because these channels display ultraslow deactivation kinetics. HCN3 channels generate a depolarizing current during late repolarization that prolongs the action potential.

Conclusions
We propose that HCN3 together with other members of the HCN channel family confer a depolarizing background current that regulates ventricular resting potential and counteracts the action of hyperpolarizing potassium currents in late repolarization. In conclusion, our data indicate that HCN3 plays an important role in shaping the cardiac action potential waveform.

Distribution and Functional Role of Inositol 1,4,5-trisphosphate Receptors in Mouse Sinoatrial Node; Ju et al48

What Is Known?
• Cardiac pacemaking in the sinoatrial node relies not only on voltage-dependent currents in the membrane but also on the intracellular activity of Ca2+.
• Heart rate slows when Ca2+ release is inhibited from the major Ca2+ release channels (ryanodine receptors) in cardiac tissue.
• Release of Ca2+ from another minor group of Ca2+ release channels—inositol 1,4,5 trisphosphate receptors (IP3Rs)—has been implicated in arrhythmia and cardiac hypertrophy.

What New Information Does This Article Contribute?
• In the mouse sinoatrial node, the type II IP3Rs (IP3R2) is the predominant IP3R isoform.
• Increasing the release of Ca2+ from IP3R2s increases the heart rate, whereas inhibiting it slows the heart rate.

Conclusions
This study provides new evidence that functional IP3R2s are expressed in the mouse SAN and could serve as an additional Ca2+-dependent mechanism in modulating cardiac pacemaker activity as well as other Ca2+-dependent processes.

Cardiomyocytes Obtained From Induced Pluripotent Stem Cells With Long-QT Syndrome 3 Recapitulate Typical Disease-Specific Features In Vitro; Malan et al49

What Is Known?
• The pathophysiological consequences of ion channel mutations that cause long-QT syndrome (LQTS) cannot be analyzed directly in cardiomyocytes from patients.
Induced pluripotent stem (iPS) cells can be generated from skin biopsy samples of patients and differentiated into cardiomyocytes.

**What New Information Does This Article Contribute?**

- Disease-specific iPS cells can be generated from murine fibroblasts that carry a human mutation of the Na+ channel that causes LQTS 3.
- Cardiomyocytes can be differentiated in the culture dish from LQTS 3–specific iPS cells and show the known biophysical features of the cardiac Na+ channel mutation.
- Action potential durations of LQTS 3 cardiomyocytes were found to be prolonged at slow heart rates, which is the pathognomonic feature of LQTS 3.

**Conclusions**

We demonstrate that disease-specific iPS cell–derived cardiomyocytes from an LQTS 3 mouse model with a human mutation recapitulate the typical pathophysiological phenotype in vitro. Thus, this method is a powerful tool to investigate disease mechanisms in vitro and to perform patient-specific drug screening.

**Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Long QT Syndrome: Is Personalized Medicine Ready for Prime Time? [Editorial]; Priori et al.**

**Extract:**

The study by Malan et al in this issue of the *Circulation Research* presents elegant data about induced pluripotent stem cell (iPSC)-derived myocytes from a mouse model of long QT syndrome (LQTS) type 3. The study is an important contribution that adds to a highly innovative field that is trying to define the role of iPSC technology in the understanding of inherited arrhythmias. In this accompanying editorial, I provide an overview of the previous studies in the field, comment on the contribution of Malan et al, and conclude by discussing some of the challenges in the field.

**Fibroblast Growth Factor Homologous Factor 13 Regulates Na+ Channels and Conduction Velocity in Murine Hearts; Wang et al.**

**What Is Known?**

- Fibroblast growth factor homologous factors (FHFs), a subfamily of fibroblast growth factors (FGFs), do not function as traditional FGFs.
- FHFs are intracellular modulators of voltage-gated Na+ channels and have been linked to neurodegenerative diseases.
- Certain FHFs have been found in embryonic heart.

**What New Information Does This Article Contribute?**

- FGF13 binds directly to, and colocalizes with, the major cardiac Na+ channel, NaV1.5, in the sarcolemma of adult mouse ventricular myocytes.
- Knockdown of FGF13 in adult mouse ventricular myocytes results in a loss-of-function of NaV1.5 characterized by reduced Na+ current (INa) density, decreased Na+ channel availability, and slowed INa recovery from inactivation.
- Knockdown of FGF13 decreases NaV1.5 at the sarcolemma but does not reduce whole-cell NaV1.5 protein or NaV1.5 mRNA levels.
- Knockdown of FGF13 slowed conduction velocity and reduced maximum capture rate in neonatal rat ventricular myocyte monolayers.

**Conclusions**

These findings show that FHFs are potent regulators of Na+ channels in adult ventricular myocytes and suggest that loss-of-function mutations in FHFs may underlie a similar set of cardiac arrhythmias and cardiomyopathies that result from NaV1.5 loss-of-function mutations.

**Fluorescence Resonance Energy Transfer–Based Sensor Camui Provides New Insight Into Mechanisms of Calcium/Calmodulin-Dependent Protein Kinase II Activation in Intact Cardiomyocytes; Erickson et al.**

**What Is Known?**

- Calcium/calmodulin-dependent kinase II (CaMKII) translates a broad range of upstream signaling mechanisms to downstream physiological effects in the heart.
- Activation of CaMKII is a critical step in the transition to arrhythmia and heart failure.
- CaMKII activity is regulated by several mechanisms, including calcium transient frequency and redox potential.

**What New Information Does This Article Contribute?**

- We present a novel method for dynamic real-time monitoring of CaMKII activity in intact cardiac myocytes using the fluorescent biosensor Camui.
- Camui allows spatial and temporal resolution of CaMKII activation state in living cells.
- Signaling mechanisms known to enhance CaMKII activity do so through distinct molecular mechanisms.
- Camui represents a critical tool in the translation of CaMKII research into clinical applications.

**Conclusions**

Camui is a novel, nondestructive tool that allows spatiotemporally resolved measurement of CaMKII activation state in physiologically functioning myocytes. This represents a first step in using Camui to elucidate key mechanistic details of CaMKII signaling in live hearts and myocytes.
Flecainide Exerts an Antiarrhythmic Effect in a Mouse Model of Catecholaminergic Polymorphic Ventricular Tachycardia by Increasing the Threshold for Triggered Activity; Liu et al

What Is Known?

- Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal inherited arrhythmogenic disease. Present therapy is inadequate.
- Abnormal diastolic calcium leak from the mutant cardiac ryanodine receptor (RyR2) is responsible for the induction of triggered activity, which is the pivotal arrhythmogenic mechanism in CPVT.
- Flecainide, a sodium channel blocker, prevents ventricular arrhythmias in CPVT patients and in a CPVT transgenic mouse model. The leading hypothesis is that flecainide blocks RyR2 and therefore abolishes the abnormal diastolic calcium leak that generates cardiac arrhythmias.

What New Information Does This Article Contribute?

- Flecainide prevents triggered activity by reducing Na+ channel availability and increasing the threshold for triggered activity.
- Flecainide does not prevent abnormal diastolic calcium leak in RyR2R4496C+/− myocytes.

Conclusions

Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in RyR2R4496C+/− mice; however, at variance with previous reports, we observed minimal effects on intracellular Ca2+ homeostasis. Our data suggest that the antiarrhythmic activity of the drug is caused by reduction of Na+ channel availability and by an increase in the threshold for triggered activity.

Restoration of Normal L-Type Ca2+ Channel Function During Timothy Syndrome by Ablation of an Anchoring Protein; Cheng et al

What Is Known?

- A single amino acid substitution in CaV1.2 L-type Ca2+ channels causes long QT syndrome 8 (LQT8).
- CaV1.2-LQT8 channels are characterized by an abnormally slow rate of inactivation and by exhibiting a high frequency of coordinated openings between nearby channels.
- The A-kinase anchoring protein 150 (AKAP150) is a CaV1.2 channel–associated scaffolding protein that regulates CaV1.2 channel function and excitation–contraction (EC) coupling by targeting adenyl cyclase 5, protein kinase A, and calcineurin near these channels.

What New Information Does This Article Contribute?

- AKAP150 is required for the expression of the LQT8 phenotype in a mouse model of this disease.
- AKAP150 functions like an allosteric modulator of CaV1.2-LQT8 channels that increases the opening time and also facilitates coupled gating between these channels in LQT8 cardiac myocytes.
- AKAP150 directly modulates the gating of CaV1.2-LQT8 without the aid of kinases.

Conclusions

We propose that AKAP150-dependent changes in CaV1.2-LQT8 channel gating may constitute a novel general mechanism for CaV1.2-driven arrhythmias.

Phenotypic Manifestations of Mutations in Genes Encoding Subunits of Cardiac Potassium Channels [Review]; Shimizu & Horie

Abstract

Since 1995, when a potassium channel gene, hERG (human ether-a-go-go-related gene), now referred to as KCNH2, encoding the rapid component of cardiac delayed rectifier potassium channels was identified as being responsible for type 2 congenital long-QT syndrome, a number of potassium channel genes have been shown to cause different types of inherited cardiac arrhythmia syndromes. These include congenital long-QT syndrome, short-QT syndrome, Brugada syndrome, early repolarization syndrome, and familial atrial fibrillation. Genotype-phenotype correlations have been investigated in some inherited arrhythmia syndromes, and as a result, gene-specific risk stratification and gene-specific therapy and management have become available, particularly for patients with congenital long-QT syndrome. In this review article, the molecular structure and function of potassium channels, the clinical phenotype due to potassium channel gene mutations, including genotype-phenotype correlations, and the diverse mechanisms underlying the potassium channel gene–related diseases will be discussed.

A Model of Canine Purkinje Cell Electrophysiology and Ca2+ Cycling: Rate Dependence, Triggered Activity, and Comparison to Ventricular Myocytes; Li & Rudy

What Is Known?

- Cardiac Purkinje cells (Pcell) are thought to be more prone to arrhythmic activity than ventricular myocytes (Vcell).
- Documented Pcell participation in arrhythmia includes catecholaminergic polymorphic ventricular tachycardia and ventricular fibrillation (VF).
- The electrophysiological profile and calcium (Ca) cycling properties of Pcell are considerably different from Vcell.

What New Information Does This Article Contribute?

- We developed a mathematical model of Pcell that represents its unique electrophysiological and Ca handling properties, and we conducted a Pcell–Vcell comparison.
• Rate dependence of action potential (AP) and Ca transient (CaT) is steeper in Pcell, consistent with greater vulnerability to arrhythmia.
• Pcell is more prone to the development of arrhythmogenic delayed afterdepolarizations (DAD).
• Pcell mechanisms and properties of AP and CaT alternans and of early afterdepolarizations (EAD) differ from those of Vcell.

Conclusions
Steeper rate dependence of action potential and Ca2+ transients, central peripheral heterogeneity of Ca2+ cycling, and distinct ion channel profile underlie greater arrhythmic vulnerability of Pcell compared to Vcell.

At the Source: Treating Heart Failure by Altering Muscle Motor Function [Commentary]: James & Robbins87
Extract:
A new study in Science provides proof of the principle for directly modulating the cardiac muscle’s motor, myosin, as a therapeutic target in heart failure.

Phosphatase-Resistant Gap Junctions Inhibit Pathological Remodeling and Prevent Arrhythmias; Remo et al88
What Is Known?
• Gap junctions comprise intercellular channels that electrotonically couple cardiomyocytes with one another and facilitate impulse propagation and normal rhythmicity in the heart.
• Diverse disease-causing stimuli promote abnormal expression of gap junctions, ie, pathological gap junction remodeling (GJR), and various lines of experimental evidence indicate that GJR contributes to increased arrhythmic propensity.
• Connexin43 (Cx43), the major cardiac gap junction protein, is posttranslationally phosphorylated by numerous kinases, and these posttranslational events are thought to regulate channel assembly, membrane trafficking, gating, and turnover.
• In vitro studies indicate that phosphorylation of Cx43 by casein kinase 1δ (CK1δ) at serines 325, 328, and 330 may promote gap junction assembly, which suggests this event may be an important regulator of intercellular coupling and cardiac rhythmicity.

What New Information Does This Article Contribute?
• Using genetically engineered knock-in mice with site-specific mutations introduced into the Cx43 gene, we demonstrate that phosphomimetic mutants of Cx43 at CK1δ-dependent target sites enhance gap junction formation and confer resistance both to pathological GJR and to the induction of ventricular arrhythmias.

Conversely, inhibition of phosphorylation at these same target sites diminishes gap junction formation and confers enhanced arrhythmic susceptibility.

Conclusions
These data demonstrate a mechanistic link between posttranslational phosphorylation of Cx43 and gap junction formation, remodeling, and arrhythmic susceptibility.

Small Heat Shock Protein 20 Interacts With Protein Phosphatase-1 and Enhances Sarcoplasmic Reticulum Calcium Cycling; Qian et al89
What Is Known?
• Heat shock proteins (Hsp) are important mediators of cell survival under stress conditions.
• Hsp20 is a small Hsp that protects the heart against ischemic injury, β-agonist remodeling, and apoptosis.
• Acute expression of Hsp20 in cardiomyocytes stimulates contractility, but the underlying in vivo mechanisms are not currently known.

What New Information Does This Article Contribute?
• Hsp20 enhances cardiac function accompanied by increased sarcoplasmic reticulum (SR) calcium (Ca) cycling.
• The enhanced contractility is associated with specific increase in phospholamban (PLN) phosphorylation by Hsp20.
• The stimulatory effects of Hsp20 are ascribed to inhibition of protein phosphatase 1 (PP1) activity by its direct physical interaction, indicating that Hsp20 represents a novel regulator of PP1.

Conclusions
Hsp20 is a novel regulator of sarcoplasmic reticulum Ca cycling by targeting the PP1–PLN axis. These findings, coupled with the well-recognized cardioprotective role of Hsp20, suggest a dual benefit of targeting Hsp20 in heart disease.

Metabotropic Regulation of RhoA/Rho-Associated Kinase by L-type Ca2+ Channels: New Mechanism for Depolarization-Evoked Mammalian Arterial Contraction; Fernandez-Tenorio et al60
What Is Known?
• L-type Ca2+ channels constitute an important pathway for extracellular Ca2+ influx and vascular smooth muscle contraction.
• In the absence of extracellular Ca2+, Ca2+ channels in vascular myocytes act as voltage sensors that couple membrane depolarization to G-protein/PLC/InsP3 synthesis and Ca2+ release from the sarcoplasmic reticulum (SR) (calcium channel-induced calcium release [CCICR]). Both agonist stimulation and membrane depolarization can evoke Ca2+-dependent RhoA/Rho kinase activation and arterial contraction.
What New Information Does This Article Contribute?

- Depolarization or agonist (ATP)-evoked sustained arterial contraction requires metabotropic Ca2+ release from the SR.
- L-type Ca2+ channel activation and metabotropic Ca2+ channel-induced Ca2+ release play an essential role in depolarization-evoked RhoA/Rho kinase activation and sustained myocyte contraction.
- Depolarization-evoked sustained RhoA activation does not depend on the change in membrane potential itself or the mere release of Ca2+ from the SR, but it requires the simultaneous activation of voltage-gated calcium channels (VGCC) and the downstream stimulation of a metabotropic pathway, leading to InsP3 synthesis and Ca2+ release.

Conclusions
These findings reveal that calcium channel-induced Ca2+ release has a major role in tonic vascular smooth muscle contractility because it links membrane depolarization and Ca2+ channel activation with metabotropic Ca2+ release and sensitization (RhoA/ROCK stimulation).

Chronic Electrical Neuronal Stimulation Increases Cardiac Parasympathetic Tone by Eliciting Neurotrophic Effects; Rana et al61

What Is Known?

- Chronic electric vagal stimulation improves ventricular function in heart failure and decreases the risk of ventricular arrhythmias.
- Nerve growth factor (NGF) promotes pathological sympathetic hyperinnervation which is known to accelerate the development of lethal arrhythmias after myocardial infarction and heart failure.
- Because there is evidence for an increase of baseline parasympathetic tone during chronic parasympathetic stimulation, neurotrophic effects might be operative.

What New Information Does This Article Contribute?

- In vivo, chronic electric stimulation of intracardiac parasympathetic ganglia induces neuronal growth, which is accompanied by an increase in the expression of NGF and neurotrophin (NT)-3.
- In vitro, electric stimulation of intrinsic cardiac parasympathetic neurons increases neuronal cellular growth, which is mediated by NGF.
- In vitro, electric stimulation induces NT-3–mediated but growth-independent upregulation of choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VACChT) expression, and acetylcholine (ACh) excretion.

Conclusions
FKBP12 is a critical regulator of the heart rhythm and the cardiac voltage-gated sodium current in mice; Maruyama et al62

What Is Known?

- FK506 binding protein (FKBP)12 and FKBP12.6 are cytosolic cis-trans peptidyl prolyl isomerases that bind to the immunosuppressants FK506 (tacrolimus) and rapamycin.
- Unlike FKBP12.6, which regulates the function of type 2 ryanodine receptor (RyR2) calcium release channel, the biological function of FKBP12 in cardiomyocytes is unknown.
- Treatment of transplant recipients with tacrolimus could lead to drug-induced long QT syndrome, sinus arrest, and sudden death.

What New Information Does This Article Contribute?

- FKBP12 plays an important role in regulating the electric properties of the heart, primarily via modulation of the cardiac voltage-gated sodium current, INa. FKBP12 overexpression can lead to cardiac arrhythmias.
- FKBP12-deficient cardiomyocytes exhibit increases in peak INa density and the maximal phase 0 upstroke velocity of the action potential.
- Cardiomyocytes that overexpress FKBP12 display decreased peak INa density and increased late INa density, which in turn led to a significant deceleration of the maximal phase 0 upstroke velocity and prolongation of the action potential prolongation, respectively.

Conclusions
FKBP12 is a critical regulator of INa and is important for cardiac arrhythmogenic physiology. FKBP12-mediated dysregulation of INa may underlie clinical arrhythmias associated with FK506 administration.

Small-Conductance Calcium-Activated Potassium Channel and Recurrent Ventricular Fibrillation in Failing Rabbit Ventricles; Chua et al63

What Is Known?

- Electrical storm describes a clinical condition in which the patients experience recurrent spontaneous ventricular fibrillation (SVF) requiring multiple defibrillation shocks within a short period of time. Electrical storm occurs frequently in patients with heart failure (HF).
- We developed a model of electrical storm in failing rabbit ventricles that develops acute but reversible postshock action potential duration (APD) shortening, leading to late phase 3 early afterdepolarizations (EADs), triggered activity and recurrent SVF.
• Small-conductance Ca2+-activated K+ (SK) channels are present both in the atria and in the ventricles. Although these channels are active in the normal atria, they conduct little or no current in normal ventricles.
• Apamin is a neurotoxin that selectively blocks SK channels.

What New Information Does This Article Contribute?
• We found that HF heterogeneously increases the sensitivity of the apamin-sensitive K+ current (IKAS) to intracellular Ca2+, leading to upregulation of IKAS, postshock APD shortening, late phase 3 EAD, triggered activity, and recurrent SVF.
• These new findings suggest that IKAS is a possible new target for preventive therapy in both atrial and ventricular arrhythmias.

Conclusions
Heart failure heterogeneously increases the sensitivity of IKAS to intracellular Ca2+, leading to upregulation of IKAS, postshock APD shortening, and recurrent SVF.

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel [Review]; Wilde & Brugada

Abstract
Variations in the gene encoding for the major sodium channel (Nav1.5) in the heart, SCN5A, has been shown to cause a number of arrhythmia syndromes (with or without structural changes in the myocardium), including the long-QT syndrome (type 3), Brugada syndrome, (progressive) cardiac conduction disease, sinus node dysfunction, atrial fibrillation, atrial standstill, and dilated cardiomyopathy. Of equal importance are variations in genes encoding for various subunits and regulatory proteins interacting with the α-subunit Nav1.5 and modifying its function. Based on detailed studies of genotype–phenotype relationships in these disease entities, on detailed studies of the basic electrophysiological phenotypes (heterologous expressed wild-type and mutant sodium channels and their interacting proteins), and on attempts to integrate the obtained knowledge, the past 15 years has witnessed an explosion of knowledge about these disease entities.

Inherited Dysfunction of Sarcoplasmic Reticulum Ca2+ Handling and Arrhythmogenesis [Review]; Priori & Chen

Abstract
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease occurring in patients with a structurally normal heart: the disease is characterized by life-threatening arrhythmias elicited by stress and emotion. In 2001, the ryanodine receptor was identified as the gene that is linked to CPVT; shortly thereafter, cardiac calsequestrin was implicated in the recessive form of the same disease. It became clear that abnormalities in intracellular Ca2+ regulation could profoundly disrupt the electrophysiological properties of the heart. In this article, we discuss the molecular basis of the disease and the pathophysiological mechanisms that are impacting clinical diagnosis and management of affected individuals. As of today, the interaction between basic scientists and clinicians to understand CPVT and identify new therapeutic strategies is one of the most compelling examples of the importance of translational research in cardiology.

Dynamic Calcium Movement Inside Cardiac Sarcoplasmic Reticulum During Release; Picht et al

What Is Known?
• Free intra–sarcoplasmic reticulum (SR) [Ca2+] ([Ca2+]SR) is a critical factor in regulating the strength of the heartbeat, the normal termination of SR Ca2+ release, and the initiation and propagation of arrhythmogenic Ca2+ waves; however, there are limited direct measurements of [Ca2+]SR.
• The SR network is connected throughout the myocyte, and the lumen of the SR also communicates with the nuclear envelope.

What New Information Does This Article Contribute?
• [Ca2+]SR was imaged during normal cardiac myocyte contractions (synchronous release from all sites) and during spontaneous local Ca2+ sparks or blinks when only local Ca2+ release occurs.
• Ca2+ diffuses rapidly within the SR network. During normal Ca2+ transients there are only very small [Ca2+]SR gradients between the nonjunctional and junctional SR regions.
• During spontaneous local Ca2+ release, large gradients of [Ca2+]SR develop, creating nonuniformity that could impact arrhythmogenicity.
• Observations quantitatively describe intra-SR Ca2+ regulation, permitting the development of a mathematical model of SR Ca movements.
• Compared with the cytosol, diffusion within the SR is 3 to 4 times slower and is faster in the longitudinal rather than the transverse direction.

Conclusions
Intra-SR Ca diffusion is rapid, limiting spatial [Ca]SR gradients during excitation–contraction coupling. Spatiotemporal [Ca] SR gradients are apparent during Ca sparks, and these observations constrain models of dynamic Ca movement inside the SR. This has important implications for myocyte SR Ca handling, synchrony, and potentially arrhythmogenic spontaneous contraction.
A Peptide Mimetic of the Connexin43 Carboxyl Terminus Reduces Gap Junction Remodeling and Induced Arrhythmia Following Ventricular Injury; O’Quinn et al

**What Is Known?**

- Pathological changes to gap junctions between myocytes occur in a thin layer of heart muscle next to myocardial infarcts called the infarct border zone.
- These pathological changes to gap junctions are thought to be a causal factor in fatal arrhythmias and sudden cardiac death.
- Serine 368 phosphorylation of connexin (Cx)43 has been associated with gap junctions becoming more resistant to arrhythmia-causing changes in organization.

**What New Information Does This Article Contribute?**

- A short peptide based on the Cx43 carboxyl terminus increased S368 phosphorylation in the border zone when administered to an injured heart.
- Associated with treatment with the Cx43 mimetic peptide, pathological changes to gap junction organization were inhibited.
- Treated injured hearts electrically activated more efficiently and were more resistant to developing arrhythmias.
- Evidence is provided that the Cx43 peptide works by enhancing the action of the protein kinase (PKC-ε) responsible for S368 phosphorylation.
- Technical innovations include a protocol for generating a nontransmural cryoinjury and an adherent methylcellulose membrane for peptide delivery in vivo.

**Conclusions**

cCT1 increases Cx43-pS368 in vitro in a PKC-ε-dependent manner and in the IBZ in vivo acutely following ventricular injury. cCT1-mediated increase in Cx43-pS368 phosphorylation may contribute to reductions in inducible-arrhythmia following injury.

Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Voltage–Dependent L-Type Calcium Channel [Review]; Napolitano & Antzelevitch

**Abstract**

The L-type cardiac calcium channel (LTCC) plays a prominent role in the electric and mechanical function of the heart. Mutations in the LTCC have been associated with a number of inherited cardiac arrhythmia syndromes, including Timothy, Brugada, and early repolarization syndromes. Elucidation of the genetic defects associated with these syndromes has led to a better understanding of molecular and cellular mechanisms and the development of novel therapeutic approaches to dealing with the arrhythmic manifestations. This review provides an overview of the molecular structure and function of the LTCC, the genetic defects in these channels known to contribute to inherited disorders, and the underlying molecular and cellular mechanisms contributing to the development of life-threatening arrhythmias.

Modelling Long QT Syndrome With iPSCs: Be Still, My Beating Heart … [Commentary]; Tiscornia et al

**Extract:**

Recent publications by Itzhaki et al and Moretti et al introduce the latest improvement for in vitro disease modelling of cardiac arrhythmias. iPSC lines derived from patients with LTQ1 and LTQ2 can be differentiated into cardiomyocytes, showing the disease’s characteristic electrophysiologic signature, establishing a convenient and powerful system for studying mechanisms of pathogenesis and testing therapeutic compounds.

Reactive Oxygen Species–Activated Ca/Calmodulin Kinase IIδ Is Required for Late INa Augmentation Leading to Cellular Na and Ca Overload; Wagner et al

**What Is Known?**

- Heart failure (HF) is associated with increased reactive oxygen species (ROS) and Ca2+/calmodulin-dependent protein kinase (CaMK)II expression.
- ROS can activate CaMKII by oxidation.
- ROS and CaMKII can increase late INa and intracellular Na concentration.

**What New Information Does This Article Contribute?**

- Ca2+ release from the sarcoplasmic reticulum (SR) is required for ROS-dependent CaMKII oxidation and autophosphorylation.
- ROS-activated CaMKII enhances late INa, leading to cellular Na+ and Ca2+ overload.
- ROS-activated CaMKII is arrhythmogenic.

**Conclusions**

Free [Ca2+] and a functional SR are required for ROS activation of CaMKII. ROS-activated CaMKIIδ enhances late INa, which may lead to cellular Na and Ca overload. This may be of relevance in heart failure, where enhanced ROS production meets increased CaMKII expression.

Nuclear Factor κB Downregulates the Transient Outward Potassium Current Ito,f Through Control of KChIP2 Expression; Panama et al

**What Is Known?**

- The transient outward potassium current (Ito,f) plays an important role in cardiac excitation–contraction coupling and arrhythmogenesis.
- Ito,f is consistently decreased in cardiac disease, but the underlying mechanisms are unclear.
• The transcription factor nuclear factor (NF)-κB is activated in cardiac hypertrophy and disease, and many of the same stimuli that reduce Ito,f also activate NF-κB.

What New Information Does This Article Contribute
• NF-κB strongly decreases Ito,f current by repressing the expression the potassium channel interacting protein (KChIP)2, a critical β-subunit necessary for Ito,f channel expression in the heart.
• Ito,f subunits are differentially regulated by NF-κB: the α-subunit Kv4.2 is upregulated by NF-κB, whereas the Kv4.3 is unaffected.

Conclusions
NF-κB regulates KChIP2 and Kv4.2 expression. The reductions in Ito,f observed following α-adrenergic receptor stimulation or tumor necrosis factor α application require NF-κB–dependent decreases in KChIP2 expression.

Reciprocal Control of hERG Stability by Hsp70 and Hsc70 With Implication for Restoration of LQT2 Mutant Stability; Li et al69

What Is Known?
• The human ether-a-go-go-related gene (hERG) encodes the potassium channel α-subunit, IKr, and its hereditary dysfunction causes long QT syndrome type 2 (LQT2).
• Heat shock protein (Hsp)70 stabilizes hERG protein to increase IKr.
• Heat shock cognate (Hsc)70, because of its high degree of sequence homology to Hsp70, may also influence hERG protein.

What New Information Does This Article Contribute?
• We found that Hsc70 destabilizes hERG proteins to decrease IKr, indicating that Hsc70 and Hsp70 reciprocally control the maturation of hERG proteins. Hsp70 competes with Hsc70 in the binding with hERG and facilitates its maturation.
• Heat shock–induced Hsp70 increases the level of the mature form of missense mutant hERG causing LQT2.

Conclusions
These results indicate reciprocal control of hERG stability by Hsp70 and Hsc70. Hsc70 is a potential target in the treatment of LQT2 resulting from missense HERG mutations.

Genetic Determinants of Cardiac (Electric) Conduction [Commentary]; Priori70

Extract:
Sotoodehinia et al investigated the role of common genetic variations (single nucleotide polymorphisms; SNPs) in determining QRS duration. The authors performed a meta-analysis of 14 genome-wide association studies of QRS duration in a total of 40 407 individuals (after adjustment for clinical variables) using a cut off of P=5×10−8. The discovery phase was followed by a validation study consisting of 7170 newly genotyped European individuals. The most significant association has been identified in a region of chromosome 3 corresponding to the gene SCN10A encoding for the Nav1.8 voltage gated sodium channel, thus confirming results of two previous genome-wide association studies that investigated the genetic component of heritability of QRS.

SAP97 and Dystrophin Macromolecular Complexes Determine Two Pools of Cardiac Sodium Channels Nav1.5 in Cardiomyocytes; Petitprez et al71

What Is Known?
• Cardiac sodium channel Nav1.5 plays an essential role in action potential initiation and impulse propagation.
• Hundreds of mutations in the gene encoding Nav1.5, SCN5A, have been found in patients with various cardiac disorders such as congenital long QT syndrome, Brugada syndrome, and dilated cardiomyopathy.
• Many regulatory proteins have been found to interact with Nav1.5 and form macromolecular complexes.

What New Information Does This Article Contribute?
• Cardiac sodium channels are parts of at least 2 distinct macromolecular complexes in cardiac cells: one localized at lateral membranes with the dystrophin complex and one at the intercalated discs.
• Absence of dystrophin leads to a specific downregulation of lateral Nav1.5 channels and impulse propagation slowing.
• The scaffolding protein SAP97, which is predominantly found at the intercalated discs, interacts with Nav1.5 and regulates its membrane density, hence forming another macromolecular complex.

Conclusions
These data support a model with at least 2 coexisting pools of Nav1.5 channels in cardiomyocytes: one targeted at lateral membranes by the syntrophin-dystrophin complex, and one at intercalated discs by SAP97.

Quirky Calcium Release in the Heart; Brochet et al72

What Is Known?
• Ca2+ sparks represent the elemental units of Ca2+ release from the sarcoplasmic reticulum (SR) of the cardiac myocyte.
• Ca2+ depletion from the junctional (j)SR during a Ca2+ spark can be measured and has been termed a “Ca2+ blink.”
• Clusters of type 2 ryanodine receptors (RyR2s) can be different sizes but could share the same jSR.

What New Information Does This Article Contribute?
• Imaging jSR and cytosomal Ca2+ simultaneously enables the detection of subtle Ca2+ release events that otherwise would be difficult to discriminate from noise.
• Using this method, we detected low amplitude, solitary Ca2+ release events, referred to as quarky Ca2+ release (QCR), which occurred either independently or during the declining phase of a full amplitude Ca2+ spark.
• QCR events, but not the primary spark-mediated Ca2+ release, were suppressed by the slow Ca2+ buffer EGTA, indicating that they were triggered by a Ca2+-induced Ca2+ release (CICR) mechanism. The stochastic recruitment of QCR events spilled by the Ca2+ spark plausibly explains the variability of spark duration.
• In paced myocytes, QCR events were so frequent that the SR Ca2+ leak from these events could be equal to that through Ca2+ sparks.
• QCR events not associated Ca2+ sparks could contribute to “invisible” Ca2+ leak in health and disease.

Conclusions
QCR events play an important role in shaping elemental Ca2+ release characteristics and the nonspark QCR events contribute to “invisible” Ca2+ leak in health and disease.

Blink and You’ll See It: How to Detect Ca2+ Quarks [Editorial]; Macquaide & Sipido

Extract:
Nearly 2 decades ago, local Ca2+ release events during diastole were first observed and named Ca2+ sparks. These were originally attributed to large releases from single ryanodine receptor (RyR) channels. Subsequently, Lipp and Niggli proposed that Ca2+ sparks could be the summation of smaller releases recruited from within the same cluster and coined the term calcium quarks. Arduous experiments from this group and others have indeed recorded very small release events proposed to be from smaller numbers of RyRs; however, such reports have remained limited. In this issue of Circulation Research, evidence is presented to show how small release events from within a RyR cluster are a key component of sarcoplasmic reticulum (SR) Ca2+ release.

Atrial Fibrillation Induces Myocardial Fibrosis Through Angiotensin II Type 1 Receptor–Specific Arkadia-Mediated Downregulation of Smad7; He et al

What Is Known?
• The renin–angiotensin system and, more specifically, angiotensin II (Ang II), is involved in the genesis of the atrial fibrosis induced by excessively rapid heartbeat during atrial fibrillation (AF).

What New Information Does This Article Contribute?
• Rapid atrial pacing induces atrial fibrosis in adult rabbit heart through release of Ang II.
• Rapid atrial pacing–induced stimulation of Ang II type 1 (AT1) receptor increases expression of TGF-β1, ERK, Smad2/3, Smad4, and collagen I but significantly decreases Smad7 through activation of the Arkadia-mediated protein degradation.

Conclusions
Ang II/AT1 receptor–specific activation of Arkadia-mediated poly-ubiquitination and degradation of Smad7 may decrease the inhibitory feedback regulation of TGF-β1/Smad signaling and serves as a key mechanism for AF-induced atrial fibrosis.

Alternans and Arrhythmias: From Cell to Heart [Review]; Weiss et al

Abstract
The goal of systems biology is to relate events at the molecular level to more integrated scales from organelle to cell, tissue, and living organism. Here, we review how normal and abnormal excitation–contraction coupling properties emerge from the protein scale, where behaviors are dominated by randomness, to the cell and tissue scales, where heart has to beat with reliable regularity for a lifetime. Beginning with the fundamental unit of excitation–contraction coupling, the couplon where L-type Ca channels in the sarcolemmal membrane adjoin ryanodine receptors in the sarcoplasmic reticulum membrane, we show how a network of couplons with 3 basic properties (random activation, refractoriness, and recruitment) produces the classic physiological properties of excitation–contraction coupling and, under pathophysiological conditions, leads to Ca alternans and Ca waves. Moving to the tissue scale, we discuss how cellular Ca alternans and Ca waves promote both reentrant and focal arrhythmias in the heart. Throughout, we emphasize the qualitatively novel properties that emerge at each new scale of integration.

Integrative Systems Models of Cardiac Excitation–Contraction Coupling [Review]; Greenstein & Winslow

Abstract
Excitation–contraction coupling in the cardiac myocyte is mediated by a number of highly integrated mechanisms of intracellular Ca2+ transport. The complexity and integrative nature of heart cell electrophysiology and Ca2+ cycling has led to an evolution of computational models that have played a crucial role in shaping our understanding of heart function. An important emerging theme in systems biology is that the detailed nature of local signaling events, such as those that occur in the cardiac dyad, have important consequences at higher biological scales. Multiscale modeling techniques have revealed many mechanistic links between microscale events, such as Ca2+ binding to a channel protein, and macroscale phenomena, such as excitation–contraction coupling gain. Here, we review experimentally based multiscale computational models of excitation–contraction coupling and the insights that have been gained through their application.

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