Thematic Synopses

The goal of Thematic Synopses is to provide our readers with a concise but comprehensive overview of the work published in Circulation Research, which we hope will keep our readers abreast of recent scientific discoveries and facilitate discussion, interpretation, and integration of the findings. These collections of articles are organized thematically and the papers listed in chronological order, beginning with the most recent ones. In each synopsis, the top ten downloaded original research articles (normalized to time since publication) are highlighted in yellow. Review articles are also included with titles highlighted in blue and the summary of each is provided. Instead of using abstracts, we have elected to publish the Novelty and Significance section of each article, which we believe provides a clear précis of the salient findings and their implications in a language that is easily understandable by the non-initiated. This will enable readers who are not experts in a particular field to grasp the significance and impact of work performed in other fields. It is our hope and expectation that Thematic Synopses will help readers to gain a broader awareness and a deeper understanding of the status of research across the vast landscape of cardiovascular research. —The Editors

Circulation Research Thematic Synopsis
Cardiac Arrhythmias

The Editors

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 United States 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. In a minority of the patients, SCD 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.

Elucidation of the molecular genetic basis of monogenic cardiac rhythm disorders in conjunction with characterization of ion channel 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 antiarrhythmic pharmacological agents that have proven clinical success and are effective without proarrhythmic 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 2 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 posttranslational regulation of the channels. The diversity is best reflected by the fact that human genome contains about 3000 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 the ion channels 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 posttranslational 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 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.

Role of KATP Channels in the Maintenance of Ventricular Fibrillation in Cardiomyopathic Human Hearts; Farid et al8

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 refractoriness across the left ventricular myocardium.
- Blockade of KATP channels by glibenclamide attenuates spatio-temporal heterogeneity in refractoriness, causing spontaneous termination of VF.

Conclusions
KATP 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 refractoriness during early VF.

Hydrogen Sulfide as Endothelium-Derived Hyperpolarizing Factor Sulfhydrates Potassium Channels; Mustafa et al9

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 al10

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. Downregulation 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 Arrhythmogeneicity by Pharmacological Ablation of α-Smooth Muscle Actin Containing Stress Fibers; Rosker et al.11

What Is Known?
• Following insults to the heart such as 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 arrhythmogeneicity 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 arrhythmogeneicity 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 arrhythmogeneicity. After ablation of this cytoskeletal component, cells lose their arrhythmogenic effects on cardiomyocytes, even if heterocellular electronic 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 Goq/11-Mediated Signaling to Ca2+ Sensitization of Vascular Smooth Muscle Contractility; Momotani et al.12

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 Goq/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 Goq/11 in blood vessels and maintain normotensive blood pressure.
• Knockdown 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 Goq/11 but not Gα12/13 to RhoA activation in blood vessels and cultured cells and thus mediates the physiologically important Ca2+ sensitization of force induced with Goq/11-coupled agonists. Our results suggest that signaling through p63RhoGEF provides a novel mechanism for selective regulation of blood pressure.

Human Atrial Action Potential and Ca2+ Model: Sinus Rhythm and Chronic Atrial Fibrillation; Grandi et al.13

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), electric 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) homeostases critically mediate abnormal repolarization in AF.

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**Phosphodiesterase 4D Regulates Baseline Sarcoplasmic Reticulum Ca\(^{2+}\) Release and Cardiac Contractility, Independently of L-Type Ca\(^{2+}\) Current; Beca et al\(^{15}\)**

**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) Ca\(^{2+}\) ATPase type 2a (SERCA2a), thereby suppressing baseline cAMP/protein kinase A–dependent Ca\(^{2+}\) cycling.

**Conclusions**

PDE4D regulates basal cAMP levels in SR microdomains containing SERCA2a-PLN but not L-type Ca\(^{2+}\) channels or ryanodine receptor. Because whole-cell Ca\(^{2+}\) transient amplitudes are reduced in failing human myocardium, these observations may have therapeutic implications for patients with heart failure.

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**HCN3 Contributes to the Ventricular Action Potential Waveform in the Murine Heart; Fenske et al\(^{16}\)**

**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 4 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 al**17

**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 the predominant IP3R isoform 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 al**18

**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.

**Fibroblast Growth Factor Homologous Factor 13 Regulates Na+ Channels and Conduction Velocity in Murine Hearts; Wang et al**19

**What Is Known?**

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

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

- FGF13 (FGF2) is the dominant FHF present in murine ventricular myocytes.
- 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 FHF s are potent regulators of Na+ channels in adult ventricular myocytes and suggest that loss-of-function mutations in FHF s 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**20

**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.

**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.

**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 arrhythmicogenic 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 RyR2R496C\(+/−\) myocytes.

**Conclusions**

Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in RyR2R496C\(+/−\) mice; however, at variance with previous reports, we observed minimal effects on intracellular Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 Ca\(^{2+}\) 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.

**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 and Horie**

**Abstract**

Since 1995, when a potassium channel gene, hERG (human ether-à-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 Ca\(^{2+}\) Cycling: Rate Dependence, Triggered Activity, and Comparison to Ventricular Myocytes; Li and 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?

Small Heat Shock Protein 20 Interacts With Protein Phosphatase-1 and Enhances Sarcoplasmic Reticulum Calcium Cycling; Qian et al

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.

Conclusions

Hsp20 is a novel regulator of sarcoplasmic reticulum calcium 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 Ca^{2+} Channels: New Mechanism for Depolarization-Evoked Mammalian Arterial Contraction; Fernandez-Tenorio et al

What Is Known?

- L-type Ca^{2+} channels constitute an important pathway for extracellular Ca^{2+} influx and vascular smooth muscle contraction.
- In the absence of extracellular Ca^{2+}, Ca^{2+} channels in vascular myocytes act as voltage sensors that couple membrane depolarization to G-protein/PLC/InsP3 synthesis and Ca^{2+} release from the sarcoplasmic reticulum (SR) (calcium channel-induced calcium release [CCICR]). Both agonist stimulation and membrane depolarization can evoke Ca^{2+}-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 Ca^{2+} release from the SR.
- L-type Ca^{2+} channel activation and metabotropic Ca^{2+} channel-induced Ca^{2+} 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 Ca^{2+} 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 Ca\(^{2+}\) release.

**Conclusions**

These findings reveal that calcium channel-induced Ca\(^{2+}\) release has a major role in tonic vascular smooth muscle contractility because it links membrane depolarization and Ca\(^{2+}\) channel activation with metabotropic Ca\(^{2+}\) release and sensitization (RhoA/ROCK stimulation).

**Chronic Electric Neuronal Stimulation Increases Cardiac Parasympathetic Tone by Eliciting Neurotrophic Effects; Rana et al**

**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 (VAChT) expression, and acetylcholine (ACh) excretion.

**Conclusions**

HFES of cardiac neurons in vivo and in vitro causes neuronal cellular hypertrophy, which is mediated by NGF and boosters cellular function by NT-3–mediated acetylcholine upregulation. This knowledge may contribute to develop HFES techniques to augment cardiac parasympathetic tone.

**FKBP12 Is a Critical Regulator of the Heart Rhythm and the Cardiac Voltage-Gated Sodium Current in Mice; Maruyama et al**

**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 electrical 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 al**

**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 Ca\(^{2+}\)-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 Ca\(^{2+}\), 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 Ca\(^{2+}\), leading to upregulation of IKAS, postshock APD shortening, and recurrent SVF.
Phenotypic Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel [Review]; Wilde and 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 have witnessed an explosion of knowledge about these disease entities.

Inherited Dysfunction of Sarcoplasmic Reticulum Ca2+ Handling and Arrhythmogenesis [Review]; Priori and 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 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 mimicetic 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**

αCT1 increases Cx43-pS368 in vitro in a PKC-ε-dependent manner and in the IBZ in vivo acutely following ventricular injury. αCT1-mediated increase in Cx43-pS368 phosphorylation may contribute to reductions in inducible-arrhythmia following injury.

**Phenotypic Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Voltage–Dependent L-Type Calcium Channel** [Review]; Napolitano and 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.

**Reactive Oxygen Species–Activated Ca/CaMKII Kinase II 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 Ca$^{2+}$/calmodulin-dependent protein kinase (CaMKII) 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?**

- Ca$^{2+}$ 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 Ca$^{2+}$ overload.
- ROS-activated CaMKII is arrhythmogenic.

**Conclusions**

Free [Ca]$^{2+}$ and a functional SR are required for ROS activation of CaMKII. ROS-activated CaMKIIa enhances late INa, which may lead to cellular Na and Ca overload. This may be of relevance in hear 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 β-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 al**

**What Is Known?**

- The human ether-a-gogo-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.
SAP97 and Dystrophin Macromolecular Complexes Determine Two Pools of Cardiac Sodium Channels Nav1.5 in Cardiomyocytes; Petitprez et al

**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: 1 localized at lateral membranes with the dystrophin complex and 1 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: 1 targeted at lateral membranes by the syntrophin-dystrophin complex, and 1 at intercalated discs by SAP97.

**Quirky Calcium Release in the Heart: Brochet et al**

**What Is Known?**
- Ca\(^{2+}\) sparks represent the elemental units of Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR) of the cardiac myocyte.
- Ca\(^{2+}\) depletion from the junctional (j)SR during a Ca\(^{2+}\) spark can be measured and has been termed "Ca\(^{2+}\) 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 cytosolic Ca\(^{2+}\) simultaneously enables the detection of subtle Ca\(^{2+}\) release events that otherwise would be difficult to discriminate from noise.
- Using this method, we detected low amplitude, solitary Ca\(^{2+}\) release events, referred to as quirky Ca\(^{2+}\) release (QCR), which occurred either independently or during the declining phase of a full amplitude Ca\(^{2+}\) spark.
- QCR events, but not the primary spark-mediated Ca\(^{2+}\) release, were suppressed by the slow Ca\(^{2+}\) buffer EGTA, indicating that they were triggered by a Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) mechanism. The stochastic recruitment of QCR events spawned by the Ca\(^{2+}\) spark plausibly explains the variability of spark duration.
- In paced myocytes, QCR events were so frequent that the SR Ca\(^{2+}\) leak from these events could be equal to that through Ca\(^{2+}\) sparks.
- QCR events not associated Ca\(^{2+}\) sparks could contribute to "invisible" Ca\(^{2+}\) leak in health and disease.

**Conclusions**
QCR events play an important role in shaping elemental Ca\(^{2+}\) release characteristics and the nonspark QCR events contribute to "invisible" Ca\(^{2+}\) leak in health and disease.

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

**What Is Known?**
- The rennin-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.
- Ang II/AT1 receptor–specific downregulation of the inhibitory Smad7 plays a key causal role in AF/Ang II–induced atrial fibrosis.

**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 sarcosomal membrane adjoin ryanodine receptors in the sarcoplasmic reticulum membrane, we show how a network of couplings 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 and 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.

A Major Role for hERG in Determining Frequency of Reentry in Neonatal Rat Ventricular Myocyte Monolayer; Hou et al

**What Is Known?**

- The human ether-a-go-go-related (hERG) potassium channel responsible for the rapid component (IKr) of the delayed rectifier current IK is important in determining the shape and duration of the human cardiac action potential.
- Alterations in the density of IKr change the action potential duration (APD) and may result in cardiac arrhythmias and sudden cardiac death.
- Gain-of-function mutation in hERG (N588K) leads to short-QT syndrome, indicating that an increase in IKr could be arrhythmogenic.

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

- Overexpression of IKr significantly alters the frequency and stability of functional reentry: the mechanism underlying ventricular tachyarrhythmias including fibrillation, the most dangerous type of cardiac arrhythmia.
- The increase in reentry frequency depends on APD abbreviation and wavelength shortening, as well as on transient hyperpolarization of the resting membrane potential.
- Transient hyperpolarization increases the sodium channel availability and cellular excitability and therefore contributes significantly to an increase in reentry frequency.

**Conclusions**

hERG overexpression dramatically accelerates reentry frequency in NRVM monolayers. Both APD and WL shortening, together with transient hyperpolarization, underlie the increased rotor frequency and stability.

In the RyR2R4496C Mouse Model of CPVT, β-Adrenergic Stimulation Induces Ca Waves by Increasing SR Ca Content and Not by Decreasing the Threshold for Ca Waves; Kashimura et al

**What Is Known?**

- Patients with mutations in the sarcoplasmic reticulum (SR) Ca release channel (ryanodine receptor [RyR]) are predisposed to a lethal arrhythmia (catecholaminergic polymorphic ventricular tachycardia [CPVT]) during exercise.
- This arrhythmia is a consequence of intracellular Ca waves resulting from spontaneous SR Ca release that produce delayed afterdepolarizations (DADs).
- Ca waves occur when the SR Ca content exceeds a threshold level.

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

- The threshold SR Ca content for Ca wave production is lower in mice expressing the RyR mutation than in wild-type littermates.
- The decreased threshold explains why mice expressing the RyR mutation are more likely to develop Ca waves, DADs, and arrhythmias.
- The inducibility of Ca waves by β-adrenergic stimulation is attributable to an increase of SR Ca content.
- In contrast to previous suggestions, β-adrenergic stimulation increases the SR threshold for Ca waves.

**Conclusions**

In the R4496C CPVT model, the RyR is leaky, and this lowers both SR Ca content and the threshold for waves. β-Adrenergic stimulation produces Ca waves by increasing SR Ca content and not by lowering threshold.

RGS6/Gβ5 Complex Accelerates IKACH Gating Kinetics in Atrial Myocytes and Modulates Parasympathetic Regulation of Heart Rate; Posokhova et al

**What Is Known?**

- Activation of the parasympathetic branch of the autonomic nervous system decreases heart rate via the neurotransmitter acetylcholine.
- Acetylcholine stimulates m2 muscarinic receptors (m2Rs) on sinoatrial nodal cells and atrial myocytes, leading to the G protein–dependent activation of the potassium channel IKACH.
- Modulating m2R-IKACH signaling can impact heart rate.

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

- The Rgs6/Gβ5 protein complex is an essential modulator of m2R-IKACH signaling in cardiac myocytes and sinoatrial cells.
- Inactivation of the Rgs6 gene in mice results in a mild bradycardia and an enhanced effect of drug-induced parasympathetic stimulation.

**Conclusions**

The cardiac Rgs6/Gβ5 complex modulates the timing of parasympathetic influence on atrial myocytes and heart rate in mice.
RGS6, a Modulator of Parasympathetic Activation in Heart; Yang et al

**What Is Known?**
- Parasympathetic stimulation of the heart is achieved through acetylcholine (ACh) release from the vagus, which binds to G protein–coupled muscarinic M2 receptors (M2Rs) located at pacemaking nodes and electrically conducting portions of the heart.
- Stimulation of M2Rs by ACh results in release of the Gβγ component of the heterotrimeric G protein complex associated with the receptor which promotes activation of G protein–coupled inwardly rectifying K⁺ (GIRK) channels, hyperpolarization of the membrane, inhibition of cell firing, and a net decrease in heart rate.
- Regulator of G protein signaling (RGS) proteins determine the magnitude and duration of the cellular response to G protein–coupled receptor stimulation through inactivation of G proteins and are essential for proper GIRK channel gating kinetics and normal parasympathetic control of the heart.

**What New Information Does This Article Contribute?**
- RGS6 is expressed robustly in heart, particularly in the sinoatrial node (SAN) and atrioventricular node (AVN), which are known to control heart rate and cardiac contractility.
- RGS6 is required for desensitization and rapid deactivation of M2R GIRK-mediated IK-Ach current and suppression of atrial myocyte membrane excitability.
- Loss of RGS6 is associated with severely exaggerated bradycardia and atrioventricular block in response to parasympathetic stimulation, demonstrating that RGS6 is essential for modulating M2R signaling in heart to prevent parasympathetic override.

**Conclusions**
RGS6 is a previously unrecognized but essential regulator of parasympathetic activation in heart, functioning to prevent parasympathetic override and severe bradycardia. These effects likely result from actions of RGS6 as a negative regulator of G protein activation of GIRK channels.

Variability in Timing of Spontaneous Calcium Release in the Intact Rat Heart Is Determined by the Time Course of Sarcoplasmic Reticulum Calcium Load; Wasserstrom et al

**What Is Known?**
- Spontaneous Ca release (SCR), in the form of intracellular Ca waves, occurs as a result of Ca overload of the sarcoplasmic reticulum (SR).
- SCR is responsible for delayed afterdepolarizations (DADs) that can reach voltage threshold and cause triggered beats and arrhythmias.
- SCR events and DADs occur earlier with increasing degree of SR Ca overload.

**What New Information Does This Article Contribute?**
- Increasing SR Ca overload causes progressively earlier SCR because initiation occurs from more intracellular sites within each myocyte.
- Increasing SR Ca overload decreases both the average latency and the variability in latencies for SCR, which are both responsible for the early coordination of SCR events among many cells and determine DAD timing and magnitude.
- Earlier and less variable SCR between cells occurs as the result of accelerated SR Ca reuptake during Ca overload and is an intrinsic property of SR Ca cycling.

**Conclusions**
Our results demonstrate that the variability of the timing of SCR in a population of cells in tissue decreases with SR load and is dictated by the time course of the SR Ca content.

Local Regulation of Arterial L-Type Calcium Channels by Reactive Oxygen Species; Amberg et al

**What Is Known?**
- Reactive oxygen species (ROS) are important signaling molecules in cardiovascular cells.
- Because of their reactive nature and as a mechanism conferring specificity, ROS production is thought to be localized for them to function effectively.
- Highly localized L-type calcium channel activity has been observed in arterial smooth muscle cells and shown to contribute to arterial contraction.

**What New Information Does This Article Contribute?**
- Exogenous ROS increase localized protein kinase C–dependent, L-type calcium channel activity in isolated arterial smooth muscle cells and constrict intact arteries in an L-type, calcium channel–dependent manner.
- Generation of endogenous ROS by NADPH oxidase is necessary for stimulation of L-type calcium channels and arterial contraction in response to the vasoconstrictor angiotensin II.
- Angiotensin II induces punctate sites of ROS generation that precede and colocalize with L-type calcium channel activity in isolated arterial smooth muscle cells.

**Conclusions**
Our data support a novel model of local oxidative regulation of Ca²⁺ influx where vasoconstrictors coupled to NAPDH oxidase (eg, angiotensin II) induce discrete sites of ROS generation resulting in oxidative activation of adjacent protein kinase Ca molecules that in turn promote local sites of enhanced L-type Ca²⁺ channel activity, resulting in increased Ca²⁺ influx and contraction.

Cardiac Small Conductance Ca²⁺-Activated K⁺ Channel Subunits Form Heteromultimers via the Coiled-Coil Domains in the C Termini of the Channels; Tuteja et al

**What Is Known?**
- Small conductance Ca²⁺-activated K⁺ (SK) channels represent a highly unique family of K⁺ channels because they
are gated solely by changes in intracellular Ca²⁺ concentration and consequently function to integrate changes in Ca²⁺ concentration with changes in K⁺ conductance and membrane potentials.

- Several isoforms of SK channel subunits including SK1, SK2, and SK3 have been documented to be expressed in the heart and play important functional roles in atrial myocytes and pacemaking tissues compared with the ventricle.

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

- The present work provides evidence for the formation of heteromultimers of the 3 SK channel subunits in human and mouse atrial myocytes.
- In vitro interaction assay provides evidence for a direct physical interaction of the C-terminal regions among the 3 SK channel subunits.
- Using functional analyses, the present study demonstrates that disruption of the subunit interaction via the coiled-coil domain in the carboxyl termini of the channel subunits results in a significant inhibition of Ca²⁺-activated K⁺ current in atrial myocytes.

**Conclusions**

The data provide evidence for the formation of heteromultimeric complexes among different SK channel subunits in atrial myocytes. Because SK channels are predominantly expressed in atrial myocytes, specific ligands of the different isoforms of SK channel subunits may offer a unique therapeutic opportunity to directly modify atrial cells without interfering with ventricular myocytes.

**Sarcoplasmic Reticulum Ca²⁺ Pumping Kinetics Regulates Timing of Local Ca²⁺ Releases and Spontaneous Beating Rate of Rabbit Sinoatrial Node Pacemaker Cells; Vinogradova et al**

**What Is Known?**

- For more than 60 years, a prevailing view has been that the physiological timekeeping mechanism of the heart’s pacemaker resides primarily in an ensemble of surface membrane ion transport proteins.
- More recently, the view that the sarcoplasmic reticulum (SR) of sinoatrial nodal cells (SANCs) functions as a “Ca²⁺ clock” by generating rhythmic local Ca²⁺ releases (LCRs) beneath the cell membrane has emerged as an important player in pacemaker timing. Thus, the timekeeping mechanism of the heart’s pacemaker cells is regulated by a robust, coupled-clock system involving surface membrane and intracellular oscillators.

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

- By directly measuring local SR Ca²⁺ depletion and refilling time, the rate at which the SR refills with Ca²⁺ following the prior AP-triggered Ca²⁺ release is shown to regulate the LCR period.
- The Ca²⁺ refilling time of the SR can be inferred from the decay kinetics of the cytosolic Ca²⁺ transient to predict changes in LCR period and spontaneous AP cycle length in response to β-adrenergic receptor stimulation (β-ARs).

**Conclusions**

The LCR period, a critical determinant of the spontaneous SANC cycle length, is defined by the rate of SR Ca²⁺ replenishment, which is critically dependent on SR pumping rate, Ca²⁺ available for pumping, supplied by L-type Ca²⁺ channel, and ryanodine receptor Ca²⁺ release flux, each of which is modulated by cAMP-mediated protein kinase A–dependent phosphorylation.

**Sympathetic Stimulation of Adult Cardiomyocytes Requires Association of AKAP5 With a Subpopulation of L-Type Calcium Channels; Nichols et al**

**What Is Known?**

- β-Adrenergic stimulation of heart cells produces a highly compartmentalized increase in cAMP and PKA activity that culminates in increased contractility.
- Scaffolding proteins like A kinase anchoring proteins (AKAPs) are implicated in controlling this process and contribute to specificity and subcellular localization of responses.
- Protein kinase (PK)A-dependent phosphorylation of both the L-type Ca²⁺ channel (LTCC) and phospholamban (PLN) are believed to regulate the amplitude and kinetics of the calcium transient evoked by an action potential during excitation–contraction coupling.

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

- AKAP5 (also known as AKAP150 in rodents and AKAP79 in humans) is essential for ventricular myocytes to increase the amplitude and decay rate of the cytosolic Ca²⁺ transient in response to β-adrenergic stimulation, yet the whole-cell LTCC current remains intact in AKAP5 knockout myocytes.
- The role of AKAP5 is to recruit adenylyl cyclase to a membrane-associated complex of signaling molecules which directs the PKA phosphorylation of PLN, ryanodine receptor (RyR), and a subpopulation of LTCCs.
- The increase in LTCC current in AKAP5 knockout myocytes is caused by phosphorylation of a different subpopulation of LTCCs than in wild-type myocytes.
- Ventricular myocytes compartmentalize LTCCs such that only ≈50% are responsive to physiological levels of β-adrenergic stimulation.

**Conclusions**

These findings identify an AKAP5-organized signaling module that is associated with caveolin 3 and is essential for sympathetic stimulation of the calcium transient in adult heart cells.

**Molecular Mechanisms, and Selective Pharmacological Rescue, of Rem-Inhibited CaV1.2 Channels in Heart; Xu et al**

**What Is Known?**

- RGK GTases use multiple mechanisms to inhibit L-type calcium current (ICa,L) in reconstituted systems.
- Overexpressing RGK GTases in heart cells inhibits ICa,L.
Purkinje Cells From RyR2 Mutant Mice Are Highly Arrhythmogenic But Responsive to Targeted Therapy; Kang et al⁵¹

What Is Known?
- Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia that is characterized by aberrant regulation of intracellular calcium handling.
- Studies at the organ or organismal level of resolution suggest that the Purkinje fiber network may serve as the arrhythmic trigger in CPVT.

What New Information Does This Article Contribute?
- We determined the kinetic properties of intracellular calcium transients in adult ventricular myocytes and Purkinje cells from both wild-type and RyR2R4496C/+ mutant mice.
- We found that both wild-type and RyR2R4496C/+ mutant Purkinje cells have a great propensity to develop unstimulated spontaneous calcium release events and triggered action potentials compared with working ventricular myocytes of the same genotype.
- We determined that flecainide and tetracaine both potently suppress spontaneous calcium release events in Purkinje cells.

Conclusions
Purkinje cells display a greater propensity to develop abnormalities in intracellular Ca²⁺ handling than ventricular myocytes. This proarrhythmic behavior is enhanced by disease-causing mutations in the RyR2 Ca²⁺ release channel and greatly exacerbated by catecholaminergic stimulation, with the development of arrhythmogenic triggered beats. These data support the concept that Purkinje cells are critical contributors to arrhythmogenic triggers in animal models and humans with CPVT and suggest a broader role for the Purkinje fiber network in the genesis of ventricular arrhythmias.

Defining a New Paradigm for Human Arrhythmia Syndromes: Phenotypic Manifestations of Gene Mutations in Ion Channel– and Transporter-Associated Proteins [Review]; Ackerman and Mohler⁶

Abstract
Over the past 15 years, gene mutations in cardiac ion channels have been linked to a host of potentially fatal human arrhythmias including long-QT syndrome, short-QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia. More recently, a new paradigm for human arrhythmia has emerged based on gene mutations that affect the activity of cardiac ion channel- and transporter-associated proteins. As part of the Circulation Research thematic series on inherited arrhythmias, this review focuses on the emerging field of human arrhythmias caused by dysfunction in cytosolic gene products (including ankryins, yotiao, syntrophin, and caveolin-3) that regulate the activities of key membrane ion channels and transporters.

The Fifteen Years of Discoveries That Shaped Molecular Electrophysiology: Time for Appraisal [Review]; Priori²

Abstract
This article serves as an introductory overview to a thematic review series that will present the latest advancements in the field of inherited arrhythmias. This area of cardiac electrophysiology started approximately 15 years ago, thanks to the contribution of Mark Keating and coworkers, who discovered the molecular basis of long-QT syndrome. The field rapidly expanded when clinicians, molecular biologists, geneticists, and cellular electrophysiologists undertook an impressive collaborative effort to clarify the genetic basis of “cardiac channelopathies.” As a result of this hard work, the paradigms for diagnosis and management of patients with inherited arrhythmogenic diseases were substantially modified, demonstrating once more the value of “translational research.” As more and more genes have been implicated in the genesis of inherited arrhythmias, we keep broadening our understanding of the complexity of ion channels and their multifaceted regulatory processes. Despite the fact that several discoveries have already been made, the field is facing new challenges that are attracting young investigators who share with the pioneers the ambitious goal of finding new therapies and even a cure for these conditions.

Alterations of L-Type Calcium Current and Cardiac Function in CaMKIIδ Knockout Mice; Xu et al³³

What Is Known?
- CaMKIIδ is an important regulator of Ca²⁺ handling and excitation-contraction coupling in ventricular myocytes via modulating L-type calcium current (ICa) and sarcoplasmic reticulum function.
In common cardiomyopathies, CaMKII activity is excessively increased. Inhibition of CaMKII reduces ICa and SR Ca\(^{2+}\) leak and protects against the development of structural heart disease and ventricular arrhythmias.

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

- Physiological CaMKII activity is important in maintaining normal heart rate adaptation to excessive workload and \(\beta\)-adrenergic stimulation.
- Chronic inhibition of CaMKII causes an increase in ICa density but significantly decreased ICa response to \(\beta\)-adrenergic activation, leading to a potentiated basal ventricular contractility but a reduction of cardiac reserve to workload and \(\beta\)-adrenergic stimulation.

**Conclusions**

Our results implicate physiological CaMKII activity in maintaining normal ICa, Ca\(^{2+}\) handling, excitation-contraction coupling, and the in vivo heart function in response to cardiac stress.

**Effects of Chronic Atrial Fibrillation on Active and Passive Force Generation in Human Atrial Myofibrils; Belus et al\(^{54}\)**

**What Is Known?**

- Chronic atrial fibrillation (cAF) is associated with persistent atrial contractile dysfunction, a major contributor to atrial thrombogenesis.
- Mechanisms responsible for impaired contractility are poorly defined and available therapies do not address this dysfunction.
- Most studies focus on alterations in atrial myocyte Ca\(^{2+}\) handling, but we and others emphasize the role of myofilament protein remodeling.

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

- We demonstrate that diastolic and systolic sarcomere mechanics and myofilament Ca\(^{2+}\) sensitivity are altered in atrial myofibrils from cAF patients.
- These mechanical changes are explained by shifts in protein isoforms and by increased phosphorylation of multiple myofilament proteins.
- Myofilament remodeling is part of atrial contractile dysfunction in human cAF and probably contributes to the progressive and self-perpetuating nature of the arrhythmia.

**Conclusions**

Alterations in active and passive tension generation at the sarcomere level, explained by translational and posttranslational changes of multiple myofilament proteins, are part of the contractile dysfunction of human cAF and may contribute to the self-perpetuation of the arrhythmia and the development of atrial dilatation.

**Mechanistic Links Between Na\(^{+}\) Channel (SCN5A) Mutations and Impaired Cardiac Pacemaking in Sick Sinus Syndrome; Butters et al\(^{55}\)**

**What Is Known?**

- Sick sinus syndrome (SSS) is a collection of cardiac arrhythmias associated with dysfunction of the cardiac primary pacemaker: the sinoatrial node (SAN).
- Recent studies have identified several gene mutations in congenital SSS patients. Among them are mutations of the SCN5A cardiac Na\(^{+}\) channel.
- It is still unclear how SCN5A mutations compromise the ability of the SAN to pace and drive the surrounding atrial muscle in SSS patients.

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

- At the single-cell level, SCN5A mutations slow down pacemaking rates in peripheral but not in central SAN cells that control the heart rhythm.
- In the SAN-atrial tissue, the mutations not only slow down pacemaking, but also slow down the conduction across the SAN-atrium, leading to a possible SAN conduction exit block or sinus arrest, the major features of SSS.
- Vagal nerve activity amplifies the bradycardiac effects of the SCN5A mutations; it also compromises the ability of the SAN to pace and drive the atrium, leading to a higher probability of sinus arrest or SAN exit block than with the mutations alone.

**Conclusions**

Our study substantiates the causative link between SCN5A gene mutations and SSS and illustrates mechanisms by which the mutations impair the driving ability of the SAN.

**Competing Oscillators in Cardiac Pacemaking: Historical Background [Review]; Noble and Fink\(^{56}\)**

**Abstract**

Interaction between a membrane oscillator generated by voltage-dependent ion channels and an intracellular calcium signal oscillator was present in the earliest models (1984–1985) using representations of the sarcoplasmic reticulum. Oscillatory release of calcium is inherent in the calcium-induced calcium release process. Those historical results fully support the synthesis proposed in the articles in this review series. The oscillator mechanisms do not primarily compete with each; they entrain each other. However, there is some asymmetry: the membrane oscillator can continue indefinitely in the absence of the calcium oscillator. The reverse seems to be true only in pathological conditions. Studies from tissue-level work and on the development of the heart also provide valuable insights into the integrative action of the cardiac pacemaker.

**Silencing Nox4 in the Paraventricular Nucleus Improves Myocardial Infarction–Induced Cardiac Dysfunction by Attenuating Sympathoexcitation and Periinfarct Apoptosis; Infanger et al\(^{57}\)**

**What Is Known?**

- Overactivation of the sympathetic nervous system and declining cardiac function are hallmarks of myocardial infarction (MI)-induced heart failure.
- The paraventricular nucleus (PVN) of the hypothalamus in the brain is involved in sympathoexcitation during heart failure.
- Oxidative stress in the PVN and other brain sites may be involved in the pathogenesis of certain cardiovascular diseases.
What New Information Does This Article Contribute?

- There are direct causal links between oxidative stress in the PVN, sympathetic overactivity, and declining cardiac function after myocardial infarction (MI).
- Nox4-containing NADPH oxidase is the primary source of free radicals in the PVN after MI.
- Selective silencing of Nox4 in the PVN improves MI-induced cardiac dysfunction by diminishing sympathoexcitation and apoptosis in the heart.

Conclusions

These results suggest that MI causes dysregulation of Nox4-mediated redox signaling in the PVN, which leads to sympathetic overactivation and a decline in cardiac function. Targeted inhibition of oxidant signaling in the PVN could provide a novel treatment for MI-induced heart failure.

Kinetics of FKBP12.6 Binding to Ryanodine Receptors in Permeabilized Cardiac Myocytes and Effects on Ca Sparks; Guo et al58

What Is Known?

- Small intracellular immunophilins FKBP12 and FKBP12.6 can bind to the cardiac ryanodine receptor (RyR2) and are thought to modulate RyR2 function as the SR Ca release channel.
- In some studies, protein kinase (PK)A-dependent RyR2 phosphorylation (especially in heart failure) has been reported to cause the dissociation of FKBP12.6 and consequent RyR2 leak, which could contribute to both reduced SR Ca content and arrhythmogenesis. Importantly, other studies have found opposite results, and the field remains controversial.
- FKBP12.6 is known to bind RyR2 with higher affinity than FKBP12, but there are no direct measurements of binding in the physiological cardiac myocyte environment.

What New Information Does This Article Contribute?

- The first direct measurements of FKBP12/12.6 binding kinetics with RyR2 in the adult cardiac myocytes using fluorescent FKBP and confocal microscopy. The Kd for FKBP12.6 is \(1 \text{ nmol/L}\) and for FKBP12 is \(200 \text{ nmol/L}\).
- Simultaneous measurements of RyR2 function (Ca sparks) and FKBP binding. FKBP12 had no functional effect, whereas FKBP12.6 inhibited Ca sparks and enhanced SR Ca content.
- Measurements of the in situ effects of PKA-driven RyR2 phosphorylation on FKBP association and RyR2 function (there was no detectable effect).
- The endogenous myocyte concentrations of FKBP12 is \(1 \mu\text{mol/L}\) and that of FKBP12.6 \(<100 \mu\text{mol/L}\), whereas the concentration of RyR2 is \(1 \mu\text{mol/L}\).

Conclusions

Only 10% to 20% of endogenous myocyte RyR2s have FKBP12.6 associated, but virtually all myocyte FKBP12.6 is RyR2-bound (because of very high affinity). FKBP12.6 but not FKBP12 inhibits basal RyR2 activity. PKA-dependent RyR2 phosphorylation has no significant effect on binding of either FKBP12 or 12.6 to RyR2 in myocytes.

Isoform-Selective Physical Coupling of TRPC3 Channels to IP3 Receptors in Smooth Muscle Cells Regulates Arterial Contractility; Adebiyi et al59

What Is Known?

- Phospholipase C–coupled receptor agonists elevate inositol 1,4,5-trisphosphate (IP3) in arterial smooth muscle cells, leading to the activation of sarcoplasmic reticulum (SR) type 1 IP3 receptors (IP3Rs), an increase in intracellular calcium ([Ca\(^{2+}\)]i) concentration, and vasoconstriction.
- Canonical transient receptor potential (TRPC) channels are molecularly and functionally diverse proteins that regulate smooth muscle cell plasma membrane cation influx and vascular contractility.
- The conventional view has been that IP3Rs stimulate vasoconstriction by releasing SR Ca\(^{2+}\), although recent evidence indicates that IP3Rs also induce vasoconstriction via an SR Ca\(^{2+}\) release-independent mechanism that involves TRPC channel activation; the mechanism is unidentified.

What New Information Does This Article Contribute?

- Type 1 IP3 receptors are located in very close spatial proximity to plasma membrane TRPC3 channels in arterial smooth muscle cells.
- Vasoconstrictor agonists and IP3 induce physical interaction between the IP3R1 N terminus and the TRPC3 channel C-terminal calmodulin and IP3R binding domain, leading to TRPC3 channel activation and vasoconstriction.
- In arterial smooth muscle cells, IP3R1 coupling to TRPC3 channels is isoform-selective because IP3R1 does not activate spatially separated TRPC6 channels.

Conclusions

IP3 stimulates direct coupling between IP3R1 and membrane-resident TRPC3 channels in arterial myocytes, leading to ICat activation and vasoconstriction. Close spatial proximity between IP3R1 and TRPC3 establishes this isoform-selective functional interaction.

Spark-Induced Sparks as a Mechanism of Intracellular Calcium Alternans in Cardiac Myocytes; Rovetti et al60

What Is Known?

- Pulsus alternans and T-wave alternans are associated with cardiac arrhythmias and sudden death, and calcium alternans has been assumed to be one type of cellular alternans responsible for T-wave alternans and pulsus alternans of the heart.
- Calcium alternans tends to occur under conditions of calcium overload and fast heart rates in normal cells and is exacerbated under diseased conditions such as heart failure and ischemia.
What New Information Does This Article Contribute?

- A computer model that simulates spatially distributed Ca sparks and whole-cell Ca alternans.
- A novel mechanism of Ca alternans that links whole-cell Ca alternans to the properties of Ca sparks.

Conclusions

We present a general theory for the mechanisms of intracellular Ca alternans, which mechanistically links Ca sparks to whole-cell Ca alternans, and is applicable to Ca alternans in both physiological and pathophysiological conditions.

Catecholaminergic Polymorphic Ventricular Tachycardia Is Caused by Mutation-Linked Defective Conformational Regulation of the Ryanodine Receptor; Uchinoumi et al61

What Is Known?

- Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by single point mutation in cardiac, type 2, ryanodine receptor (RyR2).
- Aberrant Ca\(^{2+}\) release occurs in CPVT-type mutant RyR2.

What New Information Does This Article Contribute?

- Defective interdomain interaction (namely, domain unzipping) within the RyR2 is a source mechanism of catecholamine-induced aberrant Ca\(^{2+}\) release in CPVT.
- Correction of the defective interdomain interaction could be a new strategy against CPVT.

Conclusions

A single point mutation within the RyR2 sensitizes the channel to agonists and reduces the threshold of luminal [Ca\(^{2+}\)] for activation, primarily mediated by defective interdomain interaction within the RyR2.

Trafficking Defects and Gating Abnormalities of a Novel SCN5A Mutation Question Gene-Specific Therapy in Long-QT Syndrome Type 3; Ruan et al62

What Is Known?

- Long-QT syndrome type 3 (LQT3) is caused by SCN5A mutations characterized by “gain-of-function” of the Nav 1.5 channel.
- In clinical practice, sodium channel blockers are used to reduce sodium current and are considered a gene-specific therapy for LQT3.
- SCN5A mutations characterized by “loss-of-function” are often caused by trafficking defect and can be rescued by sodium channel blockers.

What New Information Does This Article Contribute?

- Sodium channel blockers can prolong QT interval and worsen arrhythmias in carriers of selected SCN5A mutations.
- Bench study may be used to personalize therapy for LQT3 patients.

Conclusions

Sodium channel blockers are largely used to shorten QT intervals in carriers of SCN5A mutations. We provided evidence that these agents may facilitate trafficking of mutant proteins, thus exacerbating QT prolongation. These data suggest that caution should be used when recommending this class of drugs to carriers of mutations with undefined electrophysiological properties.

Voltage-Gated Sodium Channels Are Required for Heart Development in Zebrafish; Chopra et al63

What Is Known?

- Activation of voltage-gated sodium channels leads to the depolarization of excitatory membranes in mature myocardium, skeletal muscle, and nerve.
- Mutations in Scn5a, the predominant cardiac sodium channel gene, are associated with multiple heritable disorders of heart rhythm.
- Whereas Scn5a+/− mice develop myocardial fibrosis in senescence, Scn5a−/− mice develop marked abnormalities of ventricular morphogenesis by embryonic day 10.5 and die by day 11.5.

What New Information Does This Article Contribute?

- In zebrafish, scn5a orthologs are expressed in the gastrulating embryo, before the differentiation of excitable tissues.
- Antisense knockdown of either zebrafish cardiac sodium channel resulted in reduced expression of early markers of cardiomyocyte fate and marked chamber dysmorphogenesis.
- These findings suggest that the zebrafish cardiac sodium channels affect heart development via a nonelectrogenic mechanism.

Conclusions

These findings identify a novel and possibly nonelectrogenic role for cardiac sodium channels in heart development.

Genetic Ablation of L-Type Ca\(^{2+}\) Channels Abolishes Depolarization-Induced Ca\(^{2+}\) Release in Arterial Smooth Muscle; Fernandez-Tenorio et al64

What Is Known?

- Contraction of vascular smooth muscle depends on cytosolic [Ca\(^{2+}\)].
- The best-known mechanism for the rise in cytosolic [Ca\(^{2+}\)] is the influx of the cation through Ca\(^{2+}\) channels of the plasma membrane.

What New Information Does This Article Contribute?

- Ca\(^{2+}\) channels of arterial myocytes are voltage sensors that, in the absence of transmembrane Ca\(^{2+}\) influx, couple membrane depolarization to Ca\(^{2+}\) release from the sarcoplasmic reticulum.
- This ion-independent function of Ca\(^{2+}\) channels explains how the cell membrane potential influences intracellular functions.
**Conclusions**

These data suggest that Cav1.2 channels are indeed voltage sensors coupled to the metabolic cascade, leading to SR Ca$^{2+}$ release. These findings support a novel, ion-independent, functional role of L-type Ca$^{2+}$ channels linked to intracellular signaling pathways in vascular myocytes.

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

- Amino acid residues in the channel permeation pathway are involved in the extracellular K$^+$ dependence of HERG function and membrane stability.

**Conclusions**

Extracellular K$^+$ is a prerequisite for HERG function and membrane stability.

**TRPM7-Mediated Ca$^{2+}$ Signals Confer Fibrogenesis in Human Atrial Fibrillation; Du et al**

**What Is Known?**

- Atrial fibrosis is a hallmark feature of structural remodeling in atrial fibrillation (AF).
- Previous studies have suggested that Ca$^{2+}$ entry is essential for fibroblast function. However, the Ca$^{2+}$ signaling mechanisms in cardiac fibrogenesis have not elucidated.

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

- Here, we report that the Ca$^{2+}$-permeable cation channel TRPM7 is the major Ca$^{2+}$-permeable channel in human atrial fibroblasts.
- TRPM7-mediated Ca$^{2+}$ entry is essential for TGF-β1-induced fibroblast differentiation.
- TRPM7 is significantly upregulated in AF fibroblasts and plays a key role in enhanced fibroblast proliferation, differentiation, and collagen production during AF.

**Conclusions**

Our results establish that TRPM7 is the major Ca$^{2+}$-permeable channel in human atrial fibroblasts and likely plays an essential role in TGF-β1-elicted fibrogenesis in human AF.

**Increased Coupled Gating of L-Type Ca$^{2+}$ Channels During Hypertension and Timothy Syndrome; Navedo et al**

**What Is Known?**

- Increased calcium influx via dihydropyridine-sensitive L-type calcium channels has been identified in the pathogenesis of diseases such as cardiac arrhythmias, autism, and hypertension.
- Blockade of L-type calcium channels is currently widely used in the treatment of arrhythmias, angina, and hypertension.
- Not all L-type calcium channels are equal; some sites of channel activity allow more calcium influx than others.

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

- Instead of operating independently, clusters of L-type calcium channels can be formed spontaneously and transiently, to allow these clusters of channels to open concertedly in a “coupled” manner.
- The observed frequency of these “coupled” L-type calcium channel openings is significantly increased in diseases such as Timothy syndrome (LQT8) and hypertension.
- When calmodulin has reduced affinity for the L-type calcium channel, L-type calcium channels couple more frequently in the presence of AKAP150.

**Conclusions**

Coupled gating of Cav1.2 channels may represent a novel mechanism for the regulation of Ca$^{2+}$ influx and excitability.
in neurons, cardiac, and arterial smooth muscle under physiological and pathological conditions.

**Intracellular Ca\(^{2+}\) Silences L-Type Ca\(^{2+}\) Channels in Mesenteric Veins: Mechanism of Venous Smooth Muscle Resistance to Calcium Channel Blockers; Thakali et al**\(^{69}\)

**What Is Known?**

- Calcium channel blockers block Ca\(^{2+}\) entry through voltage-gated L-type Ca\(^{2+}\) channels and are widely prescribed as antihypertensive medications to lower high blood pressure.
- These drugs block Ca\(^{2+}\) influx into arteries but not veins to increase vessel diameter and lower blood pressure, but the reason why veins fail to dilate to calcium channel blockers is unclear.

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

- The present study shows that Ca\(^{2+}\) channel blockers prevent the constriction of small arteries but not veins from the rat mesenteric circulation; the mesenteric circulation is a key vascular bed that controls blood pressure in animals and humans.
- Next, we show that L-type Ca\(^{2+}\) channels are present in similar numbers in mesenteric arteries and veins, but the Ca\(^{2+}\) channels in veins are not functional.
- Finally, we provide evidence that in veins, Ca\(^{2+}\) from intracellular stores “silences” L-type Ca\(^{2+}\) channels.

**Conclusions**

We report that intracellular Ca\(^{2+}\) inactivates LTCCs in venous SMCs to confer venous resistance to CCB-induced dilation, a fundamental drug property that was previously unexplained.

**Biological Therapies for Cardiac Arrhythmias: Can Genes and Cells Replace Drugs and Devices? [Review]; Cho and Marban**\(^{70}\)

**Abstract**

Cardiac rhythm disorders reflect failures of impulse generation and/or conduction. With the exception of ablation methods that yield selective endocardial destruction, present therapies are nonspecific and/or palliative. Progress in understanding the underlying biology opens up prospects for new alternatives. This article reviews the present state of the art in gene- and cell-based therapies to correct cardiac rhythm disturbances. We begin with the rationale for such approaches, briefly discuss efforts to address aspects of tachyarrhythmia, and review advances in creating a biological pacemaker to cure bradyarrhythmia. Insights gained bring the field closer to a paradigm shift away from devices and drugs, and toward biologics, in the treatment of rhythm disorders.

**A Coupled SYSTEM of Intracellular Ca\(^{2+}\) Clocks and Surface Membrane Voltage Clocks Controls the Timekeeping Mechanism of the Heart’s Pacemaker [Review]; Lakatta et al**\(^{71}\)

**Abstract**

Ion channels on the surface membrane of sinoatrial nodal pacemaker cells (SANCs) are the proximal cause of an action potential. Each individual channel type has been thoroughly characterized under voltage clamp, and the ensemble of the ion channel currents reconstructed in silico generates rhythmic action potentials. Thus, this ensemble can be envisioned as a surface “membrane clock” (M clock). Localized subsarcolemmal Ca\(^{2+}\) releases are generated by the sarcoplasmic reticulum via ryanodine receptors during late diastolic depolarization and are referred to as an intracellular “Ca\(^{2+}\) clock,” because their spontaneous occurrence is periodic during voltage clamp or in detergent-permeabilized SANCs and in silico as well. In spontaneously firing SANCs, the M and Ca\(^{2+}\) clocks do not operate in isolation but work together via numerous interactions modulated by membrane voltage, subsarcolemmal Ca\(^{2+}\), and protein kinase A and CaMKII-dependent protein phosphorylation. Through these interactions, the 2 subsystem clocks become mutually entrained to form a robust, stable, coupled-clock system that drives normal cardiac pacemaker cell automaticity. G protein–coupled receptors signaling creates pacemaker flexibility, for example, effects changes in the rhythmic action potential firing rate by impacting on these very same factors that regulate robust basal coupled-clock system function. This review examines evidence that forms the basis of this coupled-clock system concept in cardiac SANCs.

**S-Nitrosylation in Cardiovascular Signaling [Review]; Lima et al**\(^{72}\)

**Abstract**

Well over 2 decades have passed since the endothelium-derived relaxation factor was reported to be the gaseous molecule nitric oxide (NO). Although soluble guanylyl cyclase (which generates cyclic guanosine monophosphate, cGMP) was the first identified receptor for NO, it has become increasingly clear that NO exerts a ubiquitous influence in a cGMP-independent manner. In particular, many, if not most, effects of NO are mediated by S-nitrosylation, the covalent modification of a protein cysteine thiol by an NO group to generate an S-nitrosothiol (SNO). Moreover, within the current framework of NO biology, endothelium-derived relaxation factor activity (ie, G protein–coupled receptor–mediated, or shear-induced endothelium-derived NO bioactivity) is understood to involve a central role for SNOs, acting both as second messengers and signal effectors. Furthermore, essential roles for S-nitrosylation have been implicated in virtually all major functions of NO in the cardiovascular system. Here, we review the basic biochemistry of S-nitrosylation (and denitrosylation), discuss the role of S-nitrosylation in the vascular and cardiac functions of NO, and identify current and potential clinical applications.

**The Role of the Funny Current in Pacemaker Activity [Review]; DiFrancesco**\(^{73}\)

**Abstract**

Pacemaking is a basic physiological process, and the cellular mechanisms involved in this function have always attracted the keen attention of investigators. The “funny” (If) current, originally described in sinoatrial node myocytes as an inward current activated on hyperpolarization to the diastolic range...
of voltages, has properties suitable for generating repetitive activity and for modulating spontaneous rate. The degree of activation of the funny current determines, at the end of an action potential, the steepness of phase 4 depolarization; hence, the frequency of action potential firing. Because If is controlled by intracellular cAMP and is thus activated and inhibited by β-adrenergic and muscarinic M2 receptor stimulation, respectively, it represents a basic physiological mechanism mediating autonomic regulation of heart rate. Given the complexity of the cellular processes involved in rhythmic activity, an exact quantification of the extent to which If and other mechanisms contribute to pacemaking is still a debated issue; nonetheless, a wealth of information collected since the current was first described more than 30 years ago clearly agrees to identify If as a major player in both generation of spontaneous activity and rate control. If-dependent pacemaking has recently advanced from a basic, physiologically relevant concept, as originally described, to a practical concept that has several potentially useful clinical applications and can be valuable in therapeutically relevant conditions. Typically, given their exclusive role in pacemaking, f-channels are ideal targets of drugs aiming to pharmacological control of cardiac rate. Molecules able to bind specifically to and block f-channels can thus be used as pharmacological tools for heart rate reduction with little or no adverse cardiovascular side effects. Indeed, a selective f-channel inhibitor, ivabradine, is today commercially available as a tool in the treatment of stable chronic angina. Also, several loss-of-function mutations of HCN4 (hyperpolarization-activated, cyclic-nucleotide gated 4), the major constitutive subunit of f-channels in pacemaker cells, are known today to cause rhythm disturbances, such as for example inherited sinus bradycardia. Finally, gene- or cell-based methods for in situ delivery of f-channels to silent or defective cardiac muscle represent novel approaches for the development of biological pacemakers eventually able to replace electronic devices.

Diastolic Intracellular Calcium-Membrane Voltage Coupling Gain and Postshock Arrhythmias: Role of Purkinje Fibers and Triggered Activity; Maruyama et al⁷⁴

Rationale
Recurrent ventricular arrhythmias after initial successful defibrillation are associated with poor clinical outcome.

Objective
We tested the hypothesis that postshock arrhythmias occur because of spontaneous sarcoplasmic reticulum Ca release, delayed afterdepolarization (DAD), and triggered activity (TA) from tissues with high sensitivity of resting membrane voltage (Vm) to elevated intracellular calcium (Cai) (high diastolic Cai–voltage coupling gains).

Methods and Results
We simultaneously mapped Cai and Vm on epicardial (n=14) or endocardial (n=14) surfaces of Langendorff-perfused rabbit ventricles. Spontaneous Cai elevation (SCaE) was noted after defibrillation in 32% of ventricular tachycardia/ventricular fibrillation at baseline and in 81% during isoproterenol infusion (0.01–1 μmol/L). SCaE was reproducibly induced by rapid ventricular pacing and inhibited by 3 μmol/L of ryanodine. The SCaE amplitude and slope increased with increasing pacing rate, duration, and dose of isoproterenol. We found TAs originating from 6 of 14 endocardial surfaces but none from epicardial surfaces, despite similar amplitudes and slopes of SCaEs between epicardial and endocardial surfaces. This was because DADs were larger on endocardial surfaces as a result of higher diastolic Cai–voltage coupling gain, compared with those of epicardial surfaces. Purkinje-like potentials preceded TAs in all hearts studied (n=7). IK1 suppression with CsCl (5 mmol/L, n=3), BaCl2 (3 μmol/L, n=3), and low extracellular potassium (1 mmol/L, n=2) enhanced diastolic Cai–voltage coupling gain and enabled epicardium to also generate TAs.

Conclusions
Higher diastolic Cai–voltage coupling gain is essential for genesis of TAs and may underlie postshock arrhythmias arising from Purkinje fibers. IK1 is a major factor that determines the diastolic Cai–voltage coupling gain.

Rad as a Novel Regulator of Excitation-Contraction Coupling and β-Adrenergic Signaling in Heart; Wang et al⁷⁵

Rationale
Rad (Ras associated with diabetes) GTPase, a monomeric small G protein, binds to Cavβ subunit of the L-type Ca2+ channel (LCC) and thereby regulates LCC trafficking and activity. Emerging evidence suggests that Rad is an important player in cardiac arrhythmogenesis and hypertrophic remodeling. However, whether and how Rad involves in the regulation of excitation-contraction (EC) coupling is unknown.

Objective
This study aimed to investigate possible role of Rad in cardiac EC coupling and β-adrenergic receptor (βAR) inotropic mechanism.

Methods and Results
Adenoviral overexpression of Rad by 3-fold in rat cardiomyocytes suppressed LCC current (ICa), [Ca2+]i transients, and contractility by 60%, 42%, and 38%, respectively, whereas the “gain” function of EC coupling was significantly increased, due perhaps to reduced “redundancy” of LCC in triggering sarcoplasmic reticulum release. Conversely, ~70% Rad knockdown by RNA interference increased ICa (50%), [Ca2+]i transients (52%) and contractility (58%) without altering EC coupling efficiency; and the dominant negative mutant RadS105N exerted a similar effect on ICa. Rad upregulation caused depolarizing shift of LCC activation and hastened time-dependent LCC inactivation; Rad downregulation, however, failed to alter these attributes. The Na+/Ca2+ exchange activity, sarcoplasmic reticulum Ca2+ content, properties of Ca2+ sparks, and propensity for Ca2+ waves all remained unperturbed regardless of Rad manipulation. Rad overexpression, but not knockdown, negated βAR effects on ICa and Ca2+ transients.
Conclusions
These results establish Rad as a novel endogenous regulator of cardiac EC coupling and βAR signaling and support a parsimonious model in which Rad buffers Cavβ to modulate LCC activity, EC coupling, and βAR responsiveness.

Development of the Pacemaker Tissues of the Heart [Review]; Christoffels et al

Abstract
Pacemaker and conduction system myocytes play crucial roles in initiating and regulating the contraction of the cardiac chambers. Genetic defects, acquired diseases, and aging cause dysfunction of the pacemaker and conduction tissues, emphasizing the clinical necessity to understand the molecular and cellular mechanisms of their development and homeostasis. Although all cardiac myocytes of the developing heart initially possess pacemaker properties, the majority differentiate into working myocardium. Only small populations of embryonic myocytes will form the sinus node and the atrioventricular node and bundle. Recent efforts have revealed that the development of these nodal regions is achieved by highly localized suppression of working muscle differentiation and have identified transcriptional repressors that mediate this process. This review will summarize and reflect new experimental findings on the cellular origin and the molecular control of differentiation and morphogenesis of the pacemaker tissues of the heart. It will also shed light on the etiology of inborn and acquired errors of nodal tissues.

Be Still, My Beating Heart: Never! [Review]; O’Rourke

Abstract
For me, it takes only a short bout of insomnia to trigger an acute awareness and reflection on the pacemaker mechanisms of the heart. In my quiet wakefulness, attempting to suppress vexing thoughts of grant deadlines, perplexing experimental results, or various other items, I often focus on counting heartbeats rather than sheep. How can the pacemaker deliver more than 90 thousand beats per day, 365 days a year for more (hopefully many more) than 70 years straight? What was that stray irregularity in the rhythm I just noticed? Can I consciously change the rate? Undoubtedly, the heartbeat is the preeminent biological oscillator that pervades our thoughts, inspires our literature, and even defines our perception of time. Thus, 100 years after the anatomic and functional descriptions of the pacemaker regions of the heart, we consciously change the rate? Undoubtedly, the heartbeat is the preeminent biological oscillator that pervades our thoughts, inspires our literature, and even defines our perception of time. Thus, 100 years after the anatomic and functional descriptions of the pacemaker regions of the heart, we continue to be fascinated by the mechanism of spontaneous self-organization, occurring in both time and space, of these robust cardiac timekeepers.

Mechanisms of Mechanically Induced Spontaneous Arrhythmias in Acute Regional Ischemia; Jie et al

Rationale
Although ventricular premature beats (VPBs) during acute regional ischemia have been linked to mechanical stretch of ischemic tissue, whether and how ischemia-induced mechanical dysfunction can induce VPBs and facilitate their degradation into reentrant arrhythmias has not been yet addressed.

Objective
This study used a novel multiscale electromechanical model of the rabbit ventricles to investigate the origin of and the substrate for spontaneous arrhythmias arising from ischemia-induced electrophysiological and mechanical changes.

Methods and Results
Two stages of ischemia were simulated. Dynamic mechanoelectrical feedback was modeled as spatially and temporally nonuniform membrane currents through mechanosensitive channels, the conductances of which depended on local strain rate. Our results reveal that both strains and strain rates were significantly larger in the central ischemic zone than in the border zone. However, in both ischemia stages, a VPB originated from the ischemic border in the left ventricular apical endocardium because of mechanically induced suprathreshold depolarizations. It then traveled fully intramurally until emerging from the ischemic border on the anterior epicardium. Reentry was formed only in the advanced ischemia stage as the result of a widened temporal excitable gap. Mechanically induced, delayed afterdepolarization–like events contributed to the formation of reentry by further decreasing the already reduced-by-hyperkalemia local excitability, causing extended conduction block lines and slowed conduction in the ischemic region.

Conclusions
Mechanically induced membrane depolarizations in the ischemic region are the mechanism by which mechanical activity contributes to both the origin of and substrate for spontaneous arrhythmias under the conditions of acute regional ischemia.

Structural Heterogeneity in the Ventricular Wall Plays a Significant Role in the Initiation of Stretch-Induced Arrhythmias in Perfused Rabbit Right Ventricular Tissues and Whole Heart Preparations; Seo et al

Rationale
Mechanical stress is known to alter the electrophysiological properties of the myocardium and may trigger fatal arrhythmias when an abnormal load is applied to the heart.

Objective
We tested the hypothesis that the structural heterogeneity of the ventricular wall modulates globally applied stretches to create heterogeneous strain distributions that lead to the initiation of arrhythmias.

Methods and Results
We applied global stretches to arterially perfused rabbit right ventricular tissue preparations. The distribution of strain (determined by marker tracking) and the transmembrane potential (measured by optical mapping) were simultaneously recorded while accounting for motion artifacts. The 3D structure of the preparations was also examined using a laser
displacement meter. To examine whether such observations can be translated to the physiological condition, we performed similar measurements in whole heart preparations while applying volume pulses to the right ventricle. At the tissue level, larger stretches (≥20%) caused synchronous excitation of the entire preparation, whereas medium stretches (10% and 15%) induced focal excitation. We found a significant correlation between the local strain and the local thickness and the probability for focal excitation was highest for medium stretches. In the whole-heart preparations, we observed that such focal excitations developed into reentrant arrhythmias.

Conclusions
Global stretches of intermediate strength, rather than intense stretches, created heterogeneous strain (excitation) distributions in the ventricular wall, which can trigger fatal arrhythmias.

References


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