Introduction to Cardiac Mechanosensitivity

History and Scope

The heart’s propensity to respond to mechanical stimuli with acute changes in its activity has been known for centuries. Early reports in the European medical literature describing mechanical effects on human heart rhythm date back to the 19th century, such as the communications by Nélaton and Meola on sudden death caused by precordial impact. At about the same time, Langendorff developed his isolated perfused heart model which, by the way, offers vivid illustrations of mechanosensitivity (eg, touch-induced ectopy). Building on the Langendorff-method, physiologists like Henry Bowditch, Joseph Coats, and Elias Cyon described effects of cardiac volume loading on contractility, nowadays commonly credited to subsequent defining work by Frank and Starling. Astonishingly, given the long history and vast importance of this mechano-mechanical feedback for autoregulation of cardiac output, the mechanisms underlying the Frank-Starling effect are still subject of debate.

Abstract: Mechanical forces will have been omnipresent since the origin of life, and living organisms have evolved mechanisms to sense, interpret, and respond to mechanical stimuli. The cardiovascular system in general, and the heart in particular, is exposed to constantly changing mechanical signals, including stretch, compression, bending, and shear. The heart adjusts its performance to the mechanical environment, modifying electrical, mechanical, metabolic, and structural properties over a range of time scales. Many of the underlying regulatory processes are encoded intracardially and are, thus, maintained even in heart transplant recipients. Although mechanosensitivity of heart rhythm has been described in the medical literature for over a century, its molecular mechanisms are incompletely understood. Thanks to modern biophysical and molecular technologies, the roles of mechanical forces in cardiac biology are being explored in more detail, and detailed mechanisms of mechanotransduction have started to emerge. Mechano-gated ion channels are cardiac mechanoreceptors. They give rise to mechano-electric feedback, thought to contribute to normal function, disease development, and, potentially, therapeutic interventions. In this review, we focus on acute mechanical effects on cardiac electrophysiology, explore molecular candidates underlying observed responses, and discuss their pharmaceutical regulation. From this, we identify open research questions and highlight emerging technologies that may help in addressing them. (Circ Res. 2016;118:311-329. DOI: 10.1161/CIRCRESAHA.115.305043.)

Key Words: cardiac electrophysiology ■ heart rhythm ■ mechanotransduction

Cardiac Mechano-Gated Ion Channels and Arrhythmias

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This Review is in a thematic series on Mechanotransduction, which includes the following articles:

The Hippo Pathway in Heart Development, Regeneration, and Diseases [Circ Res. 2015;116:1431–1447]
Role of Mechanotransduction in Vascular Biology: Focus on Thoracic Aortic Aneurysms and Dissections [Circ Res. 2015;116:1448–1461]
Mechanotransduction in Cardiac Hypertrophy and Failure [Circ Res. 2015;116:1462–1476]
Mechanical Forces Reshape Differentiation Cues that Guide Cardiomyogenesis

Cardiac Mechano-Gated Ion Channels and Arrhythmias

Mechanotransduction in Myocyte Biology

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MGC may serve both as sensors and as effectors of MEF responses. Embedded in membranes, they convert mechanical stimuli, putatively including in-plane membrane tension, membrane thickness and curvature, as well as matrix–protein interactions, into electrical and biochemical signals. MGC can affect, therefore, a wide range of cellular processes, with response times in the millisecond domain relevant for acute cardiac MEF.

MGC were discovered in 1984 by Guharay and Sachs in embryonic chick skeletal myocytes. Four years later, Craelius et al published the first MGC recordings from mammalian cardiomyocytes. Since then, in addition to stretch-activated whole-cell currents, single-channel activity has been identified in a wide range of cardiac cells, including atrial myocytes, and (for potassium-selective MGC at least) adult ventricular myocytes, as well as cardiac nonmyocytes.

In the 1990s, the first MGC was cloned from Escherichia coli (mechanosensitive channel of large conductance [MscL]), and the molecular nature of the first mammalian MGC was reported. Since then, an increasing number of MGC has been identified and a large proportion of them are expressed and functional in the heart (Figure 1).

Because block of MGC in the mammalian heart can prevent certain forms of mechanically induced heart rhythm disturbances in the experimental setting, they form a putative therapeutic target. This has motivated the present assessment of what we know about them so far.

Channel Activation: Mechanical Modulation Versus Mechanical Gating

Mechanically Modulated Ion Channels Versus Mechanically Gated Ion Channels

Nomenclature

Ion channels, relevant for MEF, are characterized by their ability to change open probability in direct response to mechanical stimulation. Traditionally, mechanically gated ion channels have been classed according to the stimulus by which they were activated (eg, cell volume-activated channels [VAC], stretch-activated channels [SAC]; Figure 2). However, it is difficult to apply perturbations in a way that alters only one mechanical parameter, even if techniques for controlled mechanical stimulation of membrane patches have improved. In this paper, we refer to MGC as channels that can be activated by a mechanical stimulus alone. Channels that are normally activated by a different type of stimulus but with a gain that is affected by the mechanical environment, or those that require coactivation by nonmechanical stimuli, will be referred to as mechanically modulated channels (MMC).

Voltage-Gated MMC

MMC normally classed as voltage-gated include potassium, calcium, and sodium channels. Mechanical modulation of Kv channels (K+ channel, voltage-gated) ranges from mechanically induced redistribution (eg, integration of Kv1.5 channels into the sarcolemma of rat atrial myocytes, to direct stretch-induced gating (eg, of Kir channels [K+ inwardly rectifying channel] in murine ventricular myocytes).

Similarly, voltage-sensitive sodium channels can be affected by the mechanical environment (such as Na1.5 [Na+ channel, voltage-gated] in human embryonic kidney cells).
Modification of the channel stability at the membrane is another type of modulation and has recently been exemplified for the L-type calcium channel: polycystin-1, well-known to act as a mechanosensor in several cell types, can stabilize the entire pool of L-type channel proteins in rat cardiomyocytes. Mechanical modulation of voltage-sensitive channel gating is perhaps less surprising than often assumed, given that voltage sensing requires conformational rearrangements of the channel protein. If channel opening is associated with an increase in protein dimensions in the membrane plane, then the open state should be favored by increased membrane tension. That said, the precise conformational changes of many ion channels are not known, and it is clear that not all channels are mechanosensitive in standard experimental conditions (e.g., TWIK-related acid-sensitive K+ channels with TWIK standing for tandem of 2-pore K+ domains in a weak inwardly rectifying K+ channel).

Ligand-Activated MMC

Both γ-aminobutyric acid and purinergic (P2X) receptors (P2X2 and P2X4 subtypes in particular) are expressed in the heart, although not in cardiomyocytes but in neurons and smooth muscle cells, respectively. They were suggested to participate in mechanotransduction processes, but their direct mechanosensitivity remains to be established. P2X4 is not activated by shear stress alone, and their role in mechanotransduction is suggested to stem from ATP release that can be mechanically induced by them, as shown in endothelial cells.

Sarcolemmal K+ATP channels (K+ channel, ATP inactivated), discovered in cardiac myocytes in the early 1980s, understood to be activated by transmembrane voltage, ligands, stretch (stretch-activated channel, SAC) or intracellular volume change (cell-volume-activated channel, VAC). In red: channels expressed in the heart; in blue: channels with no known mammalian homologues. Only a selection of the more well-known channels and receptors for mammals are explained in Cardiac SAC: Molecular Candidates section of this article. AchR, acetylcholine receptor; ASIC, acid-sensing ion channel; BK, big K+ channels; NMDA, N-methyl-D-aspartate; TREK, TWIK-related K+ channel; TRPA, C, M, and V, transient receptor potential ankyrin, melastatin, NOMP, polycystin, and vanilloid; TPK, 2-pore domain K+ channels; OSM, OSMotic avoidance abnormal family.

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Figure 2. Mechanically modulated channels (MMC) versus mechanically gated channels (MGC). Presentation includes channels understood to be activated by transmembrane voltage, ligands, stretch (stretch-activated channel, SAC) or intracellular volume change (cell-volume-activated channel, VAC). In red: channels expressed in the heart; in blue: channels with no known mammalian homologues. Only a selection of the more well-known channels and receptors for mammals are explained in Cardiac SAC: Molecular Candidates section of this article. AchR, acetylcholine receptor; ASIC, acid-sensing ion channel; BK, big K+ channels; NMDA, N-methyl-D-aspartate; TREK, TWIK-related K+ channel; TRAAK, TWIK-related arachidonic acid-activated K+ channel; TRPA, C, M, and V, transient receptor potential ankyrin, melastatin, and vanilloid.
are sensitive to their mechanical environment. These $\kappa_{\text{ATP}}$ channels are hetero-octamers comprising 2 subunits: the pore-forming subunit with 2 membrane-spanning regions ($K^+$ inwardly rectifying channel [Kir6.1 or Kir6.2]) and the regulatory subunit sulfonylurea receptor (SUR1, SUR2A, or SUR2B). K$_{\text{ATP}}$ channels are highly expressed in atrial and ventricular cardiomyocytes of murine models and in human heart.

In normal metabolic conditions, K$_{\text{ATP}}$ channels are inactivated. If ATP levels fall, K$_{\text{ATP}}$ open probability increases. In the presence of stretch, this increase occurs at less reduced ATP levels. This may explain the difference between in vitro studies (where ATP levels have to be severely reduced to open K$_{\text{ATP}}$) and in vivo setting (where stretch of cardiac tissue is present at all times and presumably elevated in regions with reduced ATP). It is thought that K$_{\text{ATP}}$ channels are gated by local bilayer tension, and that this is affected by the cytoskeleton.

K$_{\text{ATP}}$ channels may have a protective role in ischaemia. Interestingly, stretch preconditioning, known to reduce ischaemia-reperfusion injury, is abolished by blocking K$_{\text{ATP}}$ channels. Of note, cardiac K$_{\text{ATP}}$ channels are also present and active in fibroblasts, suggesting that one must consider cardiac pre/postconditioning effects on cells other than just cardiomyocytes. As with other $K^+$ channels, K$_{\text{ATP}}$ opening favors re/hyperpolarization. Although beneficial in preventing spurious excitation of resting cells, this also shortens action potential duration and reduces the refractory period. The latter could help to establish an arrhythmogenic substrate and support re-entry.

Channel Activation: Cell Volume Versus Stretch

VAC are generally regarded to be MGC. That said, their mechanism of activation in the heart is poorly understood. What is known is that increases in cell volume, whether by swelling or pipette-based cell inflation, tend to activate chloride or potassium conductances. Although cell volume changes undoubtedly cause mechanical deformation, VAC-activation tends to occur with significant lag times (tens of seconds to minutes) after the onset of cell volume changes. This has put into question the role of direct mechanical stimuli as drivers of VAC gating, and it has been suggested that swelling-induced changes in cytoskeletal structures must take place before mechanosensitive electrophysiological responses are seen.

In terms of pathophysiological settings, cell swelling can be observed in ischaemia, particularly upon reperfusion, and VAC are understood to affect cardiac electrical behavior in these conditions. Interestingly, VAC-like Cl$^-$ conductances are constitutively activated in hypertrophied cardiomyocytes, lending credence to the notion that structural aspects of cardiomyocyte organization matter. Recently, LRRCSA (a leucine-rich repeat containing protein 8A, aka SWELL1) has been identified by two independent groups as an essential component of the ubiquitous volume-regulated anion channels. This discovery has been a result of genome-wide RNAi screens and provided a new molecular candidate to better understand cell volume regulation.

At the same time, the normal cycle of cardiomyocyte contraction and relaxation is not generally assumed to be associated with pronounced changes in cell volume. This, and the lag time for VAC activation, makes it unlikely that these channels are main contributors to acute, beat-by-beat MEF (for more information on cardiac VAC see review by Baumgarten and Clemo). SAC—the quintessential MGC—increase their open probability in direct response to membrane deformation. Evidence demonstrating that lipid bilayer forces are sufficient to gate SAC was obtained for several bacterial, fungal, and two vertebrate channels, TWIK-related K$^+$ channels (TREK-1) and TWIK-related arachidonic acid-activated K$^+$ channels (TRAAK). The small number of channels tested in this way is caused in part by technical difficulties to purify or produce functional channel reconstitutes in pure lipid bilayers. Also, several SAC are likely to require cytoskeletal and linker proteins, and/or possibly soluble factors or messengers for activation. Interestingly, mutations in cytoskeletal proteins have been linked to cardiac pathologies, including rhythm disturbances, though, thus far, this would not seem to act via effects on MGC but rather through effects on voltage-dependent channels and transporters that indirectly affect cardiac excitation–contraction coupling. Several biophysical models have been proposed to address energetic interactions at the membrane–protein interface and contributions of lipid organization or, in addition to in-plane stress, changes such as membrane thinning have been suggested as relevant atomistic-level stimuli. These models, mainly obtained from bacterial channels reconstituted in liposomes, suggest that MGC can be gated by forces from lipids in the range of hundreds of piconewton (eg, 220 pN for MscL). More broadly, including eukaryotic channels in the cellular context (ie, in the presence of the cytoskeleton), it seems that MGC are sensitive to a wide range of force intensities characteristic for living cells, from 2 to 10 mN/m (data acquired on cultured cells). To understand the mechanical gating of SAC, structural data are needed. So far, the structure of MscL and mechanosensitive channel of small conductance (MscS), as well as of mammalian TRAAK, Piezo1, and Big K$^+$ (BK) channels, has been resolved at atomic resolution.

Channel Location: Sarcolemmal Versus Nonsarcolemmal

Sarcolemmal MGC

Single-channel patch clamp investigations require direct access of the pipette tip to the membrane containing channels, such as MGC. As the outer surface of the sarcolemma is easily accessible, it is the membrane from which most electrophysiological MGC data have been reported, so much so, that the notion of MGC seems synonymous with sarcolemmal ion channel. However, not all sarcolemmal channels are present at the accessible cell surface, as “hidden” cell surface membrane, such as in transverse tubules (T-tub) and caveolae, contains ion channels. In addition, MGC are present also on endomembranes of organisms such as plants and yeast, and there is evidence to suggest that the same may hold true for mammalian heart cells. Apart from BK channels, which appear in a range of endomembranes and whose mechanogating is discussed controversially (see section on BK channels, below), the following MGC are interesting examples that warrant further investigation.

SARCOLEMMA
**Nonsarcolemmal MGC: Sarcoplasmic Reticulum**

Calcium handling in cardiac cells is mechanosensitive, involving mechanisms from changes in Ca\(^{2+}\) buffering and troponin-C binding, to Ca\(^{2+}\) fluxes.\(^{105,106}\) This includes Ca\(^{2+}\) releasability from the sarcoplasmic reticulum (SR), such as evident in an increased frequency of SR Ca\(^{2+}\) release events (sparks) upon acute mechanical stimulation, whether using axial cell stretch or local application of fluid puffs to deform isolated cells.\(^{21,104}\) Mechanisms underlying the stretch-induced increase in spark rate continue to be investigated and may include mechanical modulation of ryanodine receptor Ca\(^{2+}\)-release channels of the SR.

This could have functional relevance not only for priming SR Ca\(^{2+}\) release and/or terminating it upon successful cell shortening, but it would also have the potential of affecting cardiac electrophysiology. If stretch promoted Ca\(^{2+}\) release from the SR, this could affect trans-sarcolemmal Na\(^{+}/Ca\(^{2+}\) exchange and—in particular, in cells that are already Ca\(^{2+}\) overloaded (eg, in ischaemic conditions)—trigger ectopic excitation.\(^{34}\)

**Nonsarcolemmal MGC: Other Organelles**

Mitochondrial K\(_{\text{ATP}}\) channels contribute to ischemic pre-\(^{107}\) and postconditioning,\(^{108,109}\) potentially protecting cells by maintaining ATP production during hypoxic episodes.\(^{110}\)

Although the molecular identity of the cardiac mitochondrial K\(_{\text{ATP}}\) is still debated, it is possible that it shares mechanical modulation with its sarcolemmal counterpart (discussed in Ligand-Activated MMC section of this article).

The nuclear envelope shows significant deformation during application of mechanical forces to a cell.\(^{111–113}\) Nuclear mechanosensing is an underappreciated research area with significant importance for the understanding of mechanically induced changes in cell behavior.\(^{114}\) In terms of ion channels, TRPV4 (transient receptor potential channel, vallinoid, #4) was localized in cultured neonatal rat ventricular myocytes in the nucleus only,\(^{115}\) although functional data confirming actual ion channel activity of this protein are still outstanding.

Of course, for MEF to affect heart rhythm, transmembrane potential changes must occur, and the focus on sarcolemmal MGC is, therefore, not merely a consequence of conceptual restrictions or technical constraints (in terms of channel accessibility by recording tools) but related to the focus on functional responses of interest and, thus, the topic of this review.

Beyond cardiomyocytes: another questionable preconception is that the normal heart consists chiefly of cardiomyocytes. Although true in terms of volume fraction, nonmyocytes, such as endothelial and interstitial cells, outnumber muscle cells in the heart. Endothelial cells of the cardiovascular system possess MGC,\(^{116–118}\) and so do fibroblasts.\(^{116–121}\) Although presumably required for functions, such as shear sensing and directional extracellular matrix protein deposition in response to mechanical clues,\(^{122}\) ion channels in nonmyocytes may affect cardiac electrophysiology in the presence of heterotypic cell coupling in the heart. Such coupling seems to exist in native myocardium, both in normal\(^{123–125}\) and fibrotic/scared tissue,\(^{126,127}\) as reviewed in detail elsewhere.\(^{128}\)

**Cardiac SAC: Molecular Candidates**

**Criteria and Terminology**

Knowledge about molecular candidates for cardiac MGC has seen significant improvements over the past decade. Candidate proteins and protein families have emerged, and new, often complex regulatory contributions have been proposed. At the same time, specific information about mechanotransduction pathways remains relatively limited. To address present controversies, Arnadóttir and Chalfie\(^{129}\) suggested 4 criteria to establish whether or not a protein forms an ion channel that transduces mechanical forces and is relevant for organ function: 1) the protein must be expressed and localized in the mechanosensory organ; 2) the channel is required for mechanosensitive responses but not merely for normal development of the mechanoreceptor cell or signaling downstream of the stimulus; 3) alteration of channel properties (conductance, kinetics, sensitivity, and selectivity) alter the properties of mechanical responses; and 4) the channel should be gated mechanically in heterologous expression system. In the mammalian heart, proteins have not generally been assessed as yet for these 4 criteria, highlighting avenues for further investigation.

In this review, SAC are subdivided by their ion selectivity into K\(^{+}\)-selective (SAC\(_{K}\)) and cation nonselective channels (SAC\(_{\text{NS}}\)).

**Stretch-Activated Channels, K\(^{+}\)-Selective**

Kim\(^{32}\) described first whole-cell SAC\(_{K}\) currents (I\(_{\text{SAC,K}}\)) in cardiac cells. SAC\(_{K}\) are outwardly rectifying and allowing potassium ions to move more easily out of the cell than into it. They have large single-channel conductances and inactivate in a time-dependent manner. Their activation causes membrane re- or hyperpolarization.\(^{64}\) Single-channel recordings of I\(_{\text{SAC,K}}\) in adult mammalian cardiac myocytes have been obtained from atrial\(^{32}\) and ventricular myocytes.\(^{33,130,131}\) I\(_{\text{SAC,K}}\) is thought to be carried by K\(_{\text{2p}}\) (K\(^{+}\); 2 P domain; a P domain), although functional data confirming actual ion channel activity of this protein are still outstanding.

**TREK and TREK-Like Channels**

TREK-1 is active over a range of physiological membrane voltages. Channel gating is polymodal, activated by an impressive number of stimuli including intra- and extracellular pH, temperature, fatty acids, anesthetics, and crucially, membrane deformation (curvature) or stretch.\(^{135}\)

TREK-1 expression appears distinctly heterogeneous in the heart, with a gradient of mRNA expression that increases transmurally, from subepicardial to subendocardial myocytes.\(^{134,135}\) This heterogeneity seems to correlate with transmural changes in MEF sensitivity, whereas stretch causes the most pronounced AP shortening in the subendocardium.\(^{136,137}\) However, although TREK-1 mRNA expression was observed in murine atria and ventricles,\(^{132,134,138,139}\) it has not, to our knowledge, been found in the human heart.\(^{140,141}\) That said, whole-tissue mRNA, and even protein assays, are not necessarily true reflections of presence, let alone relevance, of a target, which may derive importance from high expression...
in a minority cell population (cardiac Purkinje fibers would be an example).

Where observed, TREK-1 protein appears to be arranged in longitudinal stripes on the surface of cardiomyocytes: a pattern that could support directional stretch sensing. Whole-cell currents exhibiting the characteristics of recombinant TREK-1 (including sensitivity to volatile anesthetics, arachidonic acid, pH, internal acidification, and stretch) have been observed in atrial and ventricular myocytes of several species including rat, mouse, and pig. In terms of functional relevance, TREK-1 contributes to the “leak” potassium conductance in cardiomyocytes. As such, it aids normal repolarization and diastolic stability. However, increased TREK-1 current, for example during stretch, could shorten AP duration to a degree where this becomes proarrhythmic. In keeping with this, several well-established antiarrhythmic drugs, including lidocaine, mexiletine, propafenone, carvedilol, dronedarone, and vernakalant, inhibit TREK-1.

TREK-2 shares functional similarity with TREK-1, although little is known about its functional relevance in the heart. It appears active in chicken embryonic atrial myocytes and it is expressed in rat atria. TRAAK is a TREK-1 homologue with similar biophysical properties and regulation, expressed in the human heart and it is expressed in rat atria. TRAAK might form a human TREK-1 homologue, but its specific functional relevance in the heart is not yet known. The similarity of electrophysiological properties of TREK-1, TREK-2, and TRAAK, and the overwhelmingly high expression levels of TREK-1 in murine model systems, may explain the paucity of experimental data on TREK-2 and TRAAK function. It is possible to differentially study them, as volatile anesthetics activate TREK-1 and -2, but not TREK-2, whereas extracellular acidification inhibits TREK-1 but activates TREK-2. Detailed characterization of the functional relevance of these MGC in mammalian heart in general, and in human tissue in particular, is a prerequisite for consideration of their pathophysiological relevance and therapeutic target potential. This has, by and large, yet to occur.

**BK Channels**

BK channel activation is polymodal for a range of gating stimuli, which has given rise to different names for the same type of ion channel in a variety of studies (eg, SAKCa, BKCa, SLO1, MaxiK). They are present in a number of cell and tissue types, including vascular smooth muscle, atrium, and ventricles. BK channels are found not only in the sarcolemma but also in membranes of the endoplasmic reticulum, the Golgi apparatus, and mitochondria.

Mechanosensitivity of BK channels was established in membrane patches excised from cultured embryonic chick ventricular myocytes. However, as BK channels are activated by voltage changes and by alterations in intracellular Ca2+ concentration, it has been suggested that their mechanosensitivity is indirect, occurring secondary to stretch-induced changes in intracellular Ca2+ concentration. As implied by their name, BK channels have large conductances. They have been suggested to contribute to heart rate control and to offer cardioprotection during ischemia. Genetic variants of BK have been related with increased severity of systolic and general hypertension, as well as increased risk of myocardial infarction.

**Stretch-Activated Channels, Cation Nonselective**

Following on from the discovery by Guhary and Sachs of stretch-activated ion currents in avian skeletal muscle, Craelius et al identified SAC whole-cell currents in mammalian heart muscle. This current had the typical linear current–voltage relationship of weakly selective ion channels, which we now attribute to SACNS (SACns). In contrast to SACCa, SACns have smaller conductances and a reversal potential closer to 0 mV. With the reversal potential being positive to the resting potential of working cardiomyocytes, activation of SACns will depolarize resting heart muscle cells, potentially triggering premature or ectopic excitation.

In contrast to SAKκ, no SACns single-channel recordings have been obtained from freshly isolated adult ventricular cardiomyocytes. This has led to the suggestion that SACns may be hidden from patch pipette access, in membrane regions such as T-tub, caveolae, or at intercalated discs. A recent report suggests that α1A adrenergic agonists may cause translocation of a putative SACns (transient receptor potential channel, canonical [TRPC]6) from T-tub to the sarcolemma. Whether this occurs physiologically, and the extent to which it might serve as a useful experimental intervention to facilitate single-channel recordings of TRPC6 in adult ventricular myocytes, remains to be explored.

The main molecular candidates for SACns are Piezo and transient receptor potential (TRP) channels.

**Piezo1 and 2**

The discovery of Piezo1 and 2 by Coste et al represents a breakthrough in the field of mechanotransduction. Piezo proteins form true SAC that meet the 4 criteria listed above. Stretch activation was demonstrated by heterologous expression of Piezo1 in human embryonic kidney cells, which induced robust SACns. Purified Piezo1, reconstituted into asymmetric bilayers and liposomes, forms ruthenium-red (a well-established pore blocker) sensitive ion channels, demonstrating that Piezo1 proteins are a pore-forming subunit of the channel. Further investigation is required to clarify whether Piezo1 ion channel subunits are intrinsically mechanosensitive. Currently, no functional data have been published on Piezo1 or Piezo 2 in the heart. However, as Piezo channel properties are similar to those of endogenous cardiac SACns, including (weak) voltage dependency, single-channel conductance, inactivation, and sensitivity to Grammostola spatulata mechanotoxin #4 (GsMTx-4), it is tempting to think that Piezo contributes to cardiac MEF.

In terms of mRNA expression, Piezo1 has been observed in murine heart, albeit at low levels in comparison with expression in lung, bladder, or skin. However, mRNA or protein expression in tissue should be interpreted with caution. The two do not necessarily correlate to one another, and even if protein expression is confirmed, it does not necessarily prove the presence of functional ion channels. Vice versa, low expression levels do not rule out functional relevance of a
protein, in particular, if it is present in a minority cell population (such as Purkinje fibers, whose mechanosensitivity was subject of the paper that coined the term cardiac MEF)\textsuperscript{10}.

This is an exciting and area of dynamic development. Basic science questions concerning structure, protein partners, and regulation of Piezo channels need to be addressed, as does the question of whether they are present in, and relevant for, the human heart.

**TRP Channels**

Most mammalian TRP channels act as nonselective cation channels, passing Na\(^+\), K\(^+\), and in some cases Ca\(^{2+}\). They are widely expressed, involved in a variety of cell functions, and characterized by polymodal regulation.\textsuperscript{163} Although the presence and extent of direct stretch activation of these channels have remained controversial, results from heterologous expression systems suggest that some TRP are good SAC\(_{\text{NS}}\) candidates.

TRPC6 stretch activation was characterized in human embryonic kidney cells\textsuperscript{164} but was not confirmed in CHO and COS cells (CHO is a Chinese hamster ovary cell line; COS is a transformed monkey kidney fibroblast cell line).\textsuperscript{82} It has been suggested that TRPC6 requires the angiotensin II type 1 receptor to be mechanosensitive.\textsuperscript{163,165} TRPC6 is among a small number of SAC candidates that are highly expressed in human heart homogenates.\textsuperscript{166} In mouse heart, TRPC6 appears localized in T-tub and, in agreement with this observation, detubulation inhibits I\(_{\text{SAC,NS}}\) in murine ventricular cardiomyocytes.\textsuperscript{49} Whole-cell patch clamp experiments on mouse cardiomyocytes identified robust I\(_{\text{SAC,NS}}\) in response to shear stimuli, which was inhibited by pore-blocking TRPC6 antibodies.\textsuperscript{49}

Murine TRPC6 knockout models suggest that the channel contributes to the slow force response and may be involved in Duchenne muscular dystrophy,\textsuperscript{167} highlighting the potential clinical relevance of TRPC6 manipulation. In nonmyocytes, TRPC6 is proposed to promote myofibroblast transdifferentiation, and it could act as a positive regulator in wound healing.\textsuperscript{168} Putative roles, both in myocytes and nonmyocytes, make this channel an interesting target for therapeutic intervention.

TRPC1 mechanosensitivity is discussed controversially. Stretch activation of TRPC1 was first shown in Xenopus oocytes\textsuperscript{169} but has not been confirmed in other expression systems.\textsuperscript{82} Like TRPC6, this channel is likely to require the presence of a partner protein. Caveolin 1 has been reported to be a trafficking regulator of TRPC1.\textsuperscript{170,171} As caveolae are highly dynamic membrane regions, whose sarcolemmal integration is dynamically modulated by acute and sustained stretch,\textsuperscript{172,173} this observation points to the possibility that TRPC1 is mechano-modulated but not mechano-gated.

TRPC3 protein has been identified in rat ventricular myocytes, where it is located in T-tub.\textsuperscript{174} In mouse neonatal cardiomyocytes, this channel is involved in reactive oxygen species production in response to mechanical stimulation or to perfusion of 1-oleoyl-2-acyethyl-sn-glycerol (OAG), a nonspecific activator of MGC.\textsuperscript{174} Recently, TRPC3/4 and 6 channels have been shown to contribute to pathological structural and functional remodeling after myocardial infarction.\textsuperscript{175} Blocking these channels is suggested to reduce pathological remodeling and to improve contractility, making TRPC channels new therapeutic targets in the context of post-myocardial infarction.

TRPV2 is expressed in mouse heart\textsuperscript{157,176} and has been reported to be activated by both cell volume changes and patch pipette suction.\textsuperscript{177} Using the TRPV2 agonist probenecid in wild-type and TRPV2 constitutive knockout mice, it was proposed that this channel contributes to baseline function of the cardiac calcium-handling machinery.\textsuperscript{176} Like TRPC6, TRPV2 combines this putative physiological role with potential contributions to dystrophic cardiomyopathies in pathological settings. TRPV2 is overexpressed in dystrophic cardiomyocytes and contributes to Ca\(^{2+}\) influx. Interestingly, in cardiomyocytes challenged by osmotic shock, TRPV2 trafficking is impaired, leading to an accumulation of the protein on the sarcolemma and along T-tub, instead of their normal preferred location in the membranes of internal Ca\(^{2+}\) stores.\textsuperscript{178}

TRP melastatin (TRPM) 2 is primarily found on the endothoplasmic/SR and in primary cilia.\textsuperscript{180} However, a TRPP2-like protein seems to function as a channel in the plasma membrane of rat ventricular cardiomyocytes,\textsuperscript{181} acting as a modulator of the cardiac ryanodine receptor.\textsuperscript{182} Given its contribution to intracellular calcium cycling, TRPV2 dysregulation has been suggested to be involved in the development of heart failure.\textsuperscript{183}

**Pharmacological Modulators**

The best-known pharmacological tools to alter SAC activity are blockers of limited specificity, including gadolinium ions, amiloride, and cationic antibiotics (streptomycin, penicillin, and kanamycin). Caution is needed, in this context, when interpreting research on stretch effects in cultured cells, as standard media contain antibiotics that may alter (reduce) background availability of SAC.

Despite of their limited selectivity, the above SAC blockers have been highly productive for experimental cell research, as reviewed elsewhere.\textsuperscript{93,184} Their utility in whole animal or human studies is limited though. Thus, gadolinium is chelated almost completely in solutions that contain physiological pH buffer systems,\textsuperscript{185} while clinically used compounds (amiloride, antibiotics) exert their effect primarily and predominantly via their established pharmacological targets, rather than possible (side-)effects on SAC. Also, not always is in vitro behavior indicative of in situ response patterns. Thus, streptomycin’s ability to block stretch responses in isolated cells\textsuperscript{185} is not necessarily preserved over the same concentration ranges and response times in native tissue,\textsuperscript{186} highlighting the possibility of false-negative findings on SAC contributions probed using the antibiotic in more integrative model systems.

A number of other clinically applied compounds affect molecular SAC candidates with a relatively narrow spectrum.
of action. TREK-1 activity, for example, is modulated by the neuroprotective agent riluzole (10–100 μM), the antidepressant fluoxetine (Prozac; IC50–10 μM), and millimolar concentrations of volatile halogenated and gaseous general anesthetics, in the case of channel activation with the potential for unexpected false-positive effects in studies on anaesthetized mammals.

Among the few specific SAC inhibitors identified is the peptide GsMTx-4, isolated by the team of Fred Sachs from a Chilean tarantula venom. In contrast to SAC-K, SAC-Na are distinctly sensitive to this peptide, although the mechanism of this specificity is unknown. GsMTx-4 inhibits TRPC5, TRPC6, and Piezo1 channels, when applied to the external membrane. Both the δ and 1 enantiomers of GsMTx-4 block SAC-Na, showing that the mechanism of action is not stereospecific or chiral. Instead, the action of GsMTx-4 is thought to involve insertion into the outer membrane leaflet (GsMTx-4 is an amphipath) in the proximity of the channel, relieving lipid stress sensed by the channel and favoring the closed state of SAC. Counterintuitively, GsMTx-4 sensitizes bacterial MscS and MscL to increased tension, although it has no effect on TREK-1. The mode of action of GsMTx-4 on SAC requires further elucidation.

A SAC-K specific blocker is Spadin. It inhibits TREK-1 mechanoo-mechano-gating, without affecting TREK-2 and TRAAK. Additional TREK-1 modulators, including activators, have recently been identified by Bagrintsev et al., and the utility of these compounds for cardiac MEF research awaits exploration. Cardiac potassium-selective MMC that have been probed using specific inhibitors include KATP channels. The mitochondrial KATP is inhibited by the 5-hydroxydecanoate (5-HD), whereas HMR-1098 (Aventis Pharma) has been identified as an antagonist for the sarcolemmal KATP. Glibenclamide is also a popular pharmacological tool, inhibiting sarcolemmal KATP from either side of the membrane.

Concerning TRP channels, TRPC6 antibodies have been used as specific inhibitors of stretch responses in isolated cells. More generally, TRPC6 is modulated by hyperforin, TRPV1 by capsaicin, TRPV2 by probenecid, and TRPM4 by 9-phenanthrol.

If specific inhibitors are rare, even fewer specific activators have been identified. Recently, Yoda1 has been shown to be an agonist for both human and mouse Piezo1, affecting sensitivity and inactivation kinetics of the channel. This compound does not act via protein partners of the channel as it is still efficient in artificial bilayers. If confirmed in native heart tissue, it would represent a powerful tool to investigate Piezo1 functions in integrated systems, especially if the channel was closed in physiological conditions.

The potential for MGC and MMC modulators as pharmacological tools in heart rhythm management has been expertly reviewed by White. Considering the ubiquitous presence of these channels in all cell types of the human body, pharmacological interventions targeting a specific organ are challenging, requiring selective delivery and avoidance of side-effects on other body functions. A possibility here is genetic targeting, with the potential of not only aiming for a specific organ, but for a specific cell type in that organ (cardiomyocytes, endothelial, interstitial or immune cells, etc.) or a defined disease progression stage. Again, this is a domain of research requiring additional effort/focus.

**Mechanistic Projection Between Levels of Investigation**

The heart generates, experiences, and responds to a highly dynamic mechanical environment. Parameters relevant for cardiac mechanoreception change dynamically and on a range of different time scales, from years (eg, ontogenesis, disease development, or aging) to fluctuations that are circadian (eg, physical activity), spread over tens of seconds (eg, respiratory cycle induced alternations in venous return), or happen in milliseconds (eg, mechanical activation during a heartbeat). MGC are, jointly with phototransduction systems, among the most rapid sensors in biological systems, although they may make contributions over the whole range of time scales mentioned above.

Knowledge about SAC contributions to chronic cardiac conditions is limited, although they have been implicated in the development of cardiac hypertrophy and heart failure. Identification of causal chains of events is difficult in chronic disease, as a host of relevant parameters, from tissue and cell viscoelastic properties to ion channel expression, remodel.

Projection from SAC activity to cell and tissue levels during acute stretch-induced changes in cardiac electrophysiology is more straightforward. Activation of SAC-K with their reversal potential negative to the resting membrane potential of cardiac cells, will tend to hyperpolarize resting cells. SAC-Na, in contrast, with reversal potentials typically between 0 and −20 mV in cardiac cells, will depolarize resting cells, if sufficient current flow is generated. Interestingly, all stretch-induced changes in resting membrane potential of cardiac myocytes reported so far involve depolarization, up to and including mechanical induction of AP. Resting membrane depolarization can be explained by SAC-Na, but not by SAC-K, suggesting that the latter do not normally determine acute electrophysiological response patterns of the heart to diastolic mechanical stimulation.

Matters get more complicated during the AP, when timing of mechanical stimuli warrants closer examination. Although SAC-K activation would continue to have a re- or hyperpolarizing effect, SAC-Na will do so only while membrane potential levels are positive to the SAC-Na reversal potential. As a consequence, SAC-Na activation will shorten AP duration during early repolarization but may, if sustained or applied late in the AP, give rise to late AP prolongation, or even after-depolarization-like events (Figure 3). Indeed, both early and late stretch-induced after-depolarization-like behavior have been reported in cardiac research. If supra-threshold, these may mechanically trigger ectopic excitation.

Timing dependence of stretch effects on cellular electrophysiology matters also for the projection from cell to organ/organism levels, as both electrical activation and repolarization are characterized by a wave-like behavior across the heart. This means that the ECG offers an inherently limited temporal
reference for characterization of local events, as illustrated in more detail in Clinical Relevance section of this article.

Quantitative projection from molecule to organism, including elucidation of spatiotemporal modulators of stretch responses, has benefitted from computational modelling.\(^{209-211}\) Interestingly, the vast majority of observed acute electrophysiological responses of the heart to stretch can be successfully reproduced simply by invoking SAC\(^{212}\).

Of course, computational demonstration of quantitative plausibility is neither proof nor replacement of experimental validation. Occasionally, validation trails computational predictions by a decade or more. An example is models of impact-induced arrhythmogenesis (aka Commotio cordis [CC], agitation of the heart, without structural damage),\(^{3,4}\) which—using 2D\(^{213}\) and 3D\(^{214}\) simulations—concluded that the rare but devastating CC-induced ventricular fibrillation (VF) requires spatiotemporal overlap of mechanically affected tissue with the trailing edge of the repolarization wave. This has now been confirmed experimentally (see also Clinical Relevance section of this article).\(^{215}\)

Among the present challenges in projecting from protein to pathology is the difficulty of assessing and comparing actual effective mechanical parameters of ion channel stimulation. Few studies have quantified the stretch imparted upon patch-clamped membranes during SAC investigations.\(^{216}\) At the cell and tissue level, cell or sarcomere length are used as a readout of strain,\(^{217}\) and occasionally as an input parameter to gauge mechanical modulation of cardiomyocytes.\(^{218}\)

In native heart, implanted ultrasonic transducers,\(^{219}\) echo, and magnetic resonance imaging\(^{220}\) have all been applied to characterize transmural deformation patterns. But, and this is a crucial limitation, none of these techniques allows one to actually measure stress/force inside the preparation (whether a membrane patch, a cell, or a tissue/organ). An exciting development here is the advent of fluorescent force reporters,\(^{221}\) whose application to cardiac MEF research could revolutionize concepts and understanding of cardiac mechanosensitivity.

**Clinical Relevance**

In terms of physiological beat-by-beat effects, activation of SAC has been shown to underlie the non-neural component of the stretch-induced increase in spontaneous sinoatrial node cell pacemaking rate, aka the Bainbridge effect.\(^{20}\) Block of SAC using GsMTx-4 (but not using streptomycin, see Pharmacological Modulators section of this article) terminates this positive chronotropic response in native sinoatrial node tissue explants,\(^{216}\) which, in its guise of a respiratory sinus arrhythmia, persists at whole body level—even in heart transplant recipients and/or after additional pharmacological denervation.\(^{222}\)

Surprisingly, this is about all we know for sure regarding the physiological relevance of SAC in heart rate and rhythm regulation.

It is possible, perhaps probable, that the key role of cardiac SAC under normal conditions is related to autoregulation of contractile behavior. This could occur via stretch-dependent adjustment of trans-sarcolemmal Ca\(^{2+}\) influx,\(^{223}\) preservation of intracellular Ca\(^{2+}\) secondary to stretch-induced trans-sarcolemmal influx of Na\(^{+}\),\(^{152,224}\) and/or stretch-modulated grading of Ca\(^{2+}\) release from the SR.\(^{21}\) Any or all of these mechanisms could contribute to both acute (Frank–Starling response)\(^{225}\) and sustained (slow force response)\(^{226}\) adjustments of cardiac contractility to changes in the heart’s mechanical environment (highly relevant for contractile cells in the heart, given that they are not controlled via neuromuscular junctions, such as is the case in skeletal muscle), whereas their effects on cardiac electrophysiology would be secondary.

In contrast, SAC have been implicated in a host of clinically relevant scenarios, from acute arrhythmogenesis to sustenance and termination of arrhythmias, as explored next.

**Mechanical Induction of Arrhythmias (Acute)**

As mentioned before, stretch of resting myocardium, if strong enough to cause any change in membrane potential, gives rise to depolarization, potentially triggering ectopic excitation. In human volunteers, this has been shown to occur upon external mechanical energy delivery, such as by precordial tapping, at energy levels as low as 0.04 J (equivalent to dropping a golf ball [46 g] from a height of 9 cm).\(^{227,228}\) Mechanically induced ectopy is a common adjunct to intracardiac device-tissue interactions such as during cardiac catheterization,\(^{229}\) and it may even be triggered by the heart’s own mechanical activity, such as upon acute obstruction of ventricular outflow during balloon valvuloplasty.\(^{230}\)

Mechanically induced ectopy is usually benign, but in the context of pre-existing pathologies, it may give rise to sustained tachyarrhythmias. In a porcine model of pathologically prolonged QT-intervals, for example, β-adrenergic stimulation by bolus injection of isoproterenol can give rise to ventricular after-contractions, originating from near-endocardial locations. These after-contractions precede early afterdepolarization-like behavior in near-epicardial tissue. Upon
reaching threshold for induction of ectopic excitation, this can initiate torsades de pointes.\textsuperscript{231}

Much rarer than mechanically induced ectopy, but more widely known, is CC, in particular, in its most severe form of CC-induced VF. Here, a mechanical impact on the precordium, usually by a projectile such as an ice hockey puck or baseball, gives rise to acute MEF responses that, during a very narrow critical window of about 15–20 ms, can cause instantaneous induction of VF.\textsuperscript{232} The mechanisms underlying VF induction are discussed controversially, with focus either on abnormal repolarization\textsuperscript{233} or on abnormal excitation overlapping the trailing edge of normal repolarization.\textsuperscript{3} Also, the role of the impact-associated surge in intraventricular pressure is subject of debate, although it is accepted that local mechanically induced ectopy occurs in myocardium nearest to the site of mechanical stimulation, both in isolated heart\textsuperscript{215,234} and whole animal.\textsuperscript{234}

Recent optical mapping studies of isolated heart models of CC have confirmed that SAC\textsubscript{NS} are involved in the generation of ectopic excitation, and that deterrioration into VF is seen only if ectopy occurs right on the edge of the preceding normal repolarization wave.\textsuperscript{215} This confirms prior modeling-based predictions\textsuperscript{214} and sheds further light on the unusually narrow critical window for mechanical induction of VF in whole animal studies: the precondition for overlap of mechanical stimulus and trailing repolarization edge is met for any specific precordial impact location for a brief part of the ECG cycle only (if one could mechanically stimulate other parts of the heart by extracorporeal impact, the accompanying critical time window would be shifted). Thus, the critical window for VF induction during CC exists in time and in space, highlighting that systemic (ECG) and local (AP) timings are not interchangeable.

Mechanical Sustenance of Arrhythmias (Chronic)

Mechanical contributions to chronic arrhythmias have been implicated in the perpetuation of both atrial\textsuperscript{235–237} and ventricular tachycardias.\textsuperscript{238} Mechanisms here may range from activation of SAC that alter AP duration,\textsuperscript{239} slow conduction velocity,\textsuperscript{173,235,238} or increase dispersion of repolarization,\textsuperscript{238} over changes in expression of mechanically modulated ion channels\textsuperscript{206} and alterations in connexin phosphorylation\textsuperscript{240} to remodeling of tissue architecture, composition,\textsuperscript{241} and innervation.\textsuperscript{242}

Given the multiplicity of mechanisms and pathways involved and the variable time scales over which they manifest themselves, this area is understudied, in particular, with regard to ventricular arrhythmogenesis. A conceptually interesting approach to probing effects of sustained ventricular stretch in cardiac arrhythmogenesis has been employed by Waxman et al.,\textsuperscript{243} who used the Valsalva manoeuvre to temporarily reduce ventricular volume overload in tachycardic patients, achieving spontaneous return to normal sinus rhythm. Because the intervention also works in heart transplant recipients,\textsuperscript{13} the most probable explanation is that sustained stretch is proarrhythmogenic.

The demonstration of tachycardia termination by temporary reduction in ventricular load highlights that chronic mechanical effects on heart electrophysiology are a clinically relevant target for further research. A perhaps particularly important aspect is the border zone of local (post-)ischaemic foci,\textsuperscript{244} where mechanically promoted arrhythmogenesis\textsuperscript{245} has been prevented in whole animal studies by application of a mechanical constraint that curtails ischaemic segment lengthening during contraction of the surrounding healthy myocardium.\textsuperscript{246} The same intervention delayed extracellular potassium accumulation, suggesting mechanical modulation of transmembrane ion fluxes, perhaps involving sarcolemmal K\textsubscript{ATP} channels.

Mechanical Termination of Arrhythmias

Beyond removal of sustained overload, acute mechanical interventions, such as by precordial thump (“fist-aid”), have been known for at least a century to be of therapeutic potential.\textsuperscript{247} It is assumed that the short sharp impact on the chest is transmitted to the heart, where—presumably via activation of SAC\textsubscript{NS}—it triggers ectopic activation in excitable tissue.\textsuperscript{248} Such activation can be used to pace the quiescent heart or to obliterate excitable gaps in hearts containing re-entrant activation waves.

The latter scenario is less straightforward, given that excitable gaps, in particular, in the presence of multiple re-entry pathways, are unlikely to be accessible from the limited extent of precordial impact locations. In addition, in conditions of severe pre-existing ischaemia, the depolarizing effect of SAC\textsubscript{NS} activation may be off-set, in part, by ischaemic and mechanical coactivation of K\textsubscript{ATP} channels,\textsuperscript{249} potentially rendering mechanically induced depolarization less effective.\textsuperscript{229} Accordingly, early hopes, based predominantly on (publication-bias prone) case reports\textsuperscript{250} for thump version of tachyarrhythmias, have not been sustained in more recent prospective human study designs.\textsuperscript{250–253} Accordingly, precordial thump application has been discouraged in recent advice by the International Liaison Committee on Resuscitation (ILCOR).\textsuperscript{254}

Precordial thump-pacing in bradycardia or primary asystole, however, remains a potentially productive intervention,\textsuperscript{255,256} for example, to bridge between witnessed asystolic arrest and application of electrical device-based rhythm management.\textsuperscript{257} As evident form the 2010 ILCOR statement, precordial thump in asystole warrants further investigation, taking us full circle to the original 1920 paper by Schott,\textsuperscript{257} who kept patients with Stokes–Adams syndrome during episodes of intermittent atrioventricular conduction block conscious for extended periods of time by mechanical fist-pacing only.\textsuperscript{247}

Outlook

By and large, acute electrophysiological responses of the heart to mechanical stimulation can be explained, qualitatively and quantitatively, through activation of SAC. This in itself does not prove that they are sole or main drivers of responses. But, given the ability of increasingly selective pharmacological tools to probe responses in model systems from patch to patient, it seems reasonable to consider these ion channels as clinically relevant targets for management or correction of cardiac electrical activity.

So—What Are the Challenges?

At the basic science end, the perhaps most important question relates to the mechanism(s) of activation of MGC. Mechanical stimuli span all scales form nano to macro (an overview of the
sizes of main mechanosensitive structures and proteins can be found in Figure 4). Their quantification in living systems is particularly challenging at cellular and subcellular levels.

It is known from in vitro experiments that sarcolemmal in-plane tension, curvature and thickness, as well as lipid composition, affect MGC activity. In addition, the cytoskeleton can gate MGC. Nonsarcolemmal responses, such as the stretch-induced increase in calcium spark rate, require the integrity, here of microtubules. Sarcolemmal TREK channels are inhibited by actin, whereas Piezo1 channels need the actin cytoskeleton to fully activate. In addition, Piezo1 can be inhibited by the cytoskeleton-protein cross-linker filamin A in smooth muscle. Thus, the cytoskeleton may cause MGC activation or protect them from opening. An additional layer of complexity arises from the fact that cytoskeletal integrity affects membrane tension and shape of the cell and its organelles. This may provide a further, potentially indirect, path to affecting MGC gating. Thus, even though MGC were discovered 3 decades ago, the actual biophysics of channel activation in vivo has remained elusive.

That said, membrane stretch is usually taken to be the obvious driver of MGC activation, but what are the relevant properties and descriptors of such stretch? Existence and extent of in-plane tension in membranes of cardiac cells is not known. What is known is that lipid bilayers are not distensible (strain exceeding 2–3% of slack area causes membrane failure), so reservoirs (membrane invaginations, vesicles, caveolae) are known to buffer membrane tension variations via quick (<100 ms) membrane surface adaptation. This condition may be present in vitro more frequently than in vivo, as cells swell upon isolation, “losing” structurally identifiable caveolae, and

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**Figure 4. Size matters.** Strain is greatly influenced by size and shape of affected structures and spans all scales from nano to macro. Tools to study mechanosensors are listed on the left in parallel with milestones. Membrane (blue), cytoskeleton (red), measuring probes (green), and other structures (black) are annotated. Ø indicates diameter; AFM, atomic force microscopy; MRI, magnetic resonance imaging; MSC, mechanosensitive channel; MscL, mechanosensitive channel of large conductance; TREK-1, TWIK-related K+ channel; T-tub, transverse tubule.
hence, potentially biasing experimental observations. This could include either heightened mechanoresponsiveness or reduce differential stretch effects, as a result of preactivation of certain mechanosensitive pathways.

Related to tension (via the law of Laplace), but presumed to be an independent activator of MGC, membrane curvature is present both in the plasma- and in endomembranes. Effective strains are related to size and shape of mechanosensitive structures, and it is thought that curvatures only with radii in the range of ion channel dimensions (ie, few nanometers) will act as directly relevant signals. Although optical monitoring of membrane curvature in live samples (membrane patches and cells) is possible, this tracks larger radii only. A real understanding, therefore, of the biophysics involved in translating a macroscopic mechanical stimulus into a microscopic event, capable of increasing the open probability of an ion channel, is one of the key missing ingredients in developing a mechanistic understanding of mechano-electric coupling.

Thankfully, techniques are emerging that will allow quantification of mechanical properties experienced by individual cells in the tissue.

Förster resonance energy transfer-based tension probes have started to be used to assess dynamic changes in cytoskeletal tension, thus far in noncardiac cells. This measuring approach is based on the energy transfer between 2 chromophores whose distance changes as a function of the mechanical environment. As the efficacy of energy transfer (which is measured as a shift in emitted fluorescence) is inversely proportional to this distance with a power of 10^6, Förster resonance energy transfer tension probes are highly sensitive to minute changes at the ion channel level in living cells. Constructs like this could be used as tension reporters for various lipid bilayers, including nonsarcolemmal membranes.

At the applied end, one of the key challenges is to identify the molecular nature of individual ion channels involved in electrophysiological responses of the human heart to changes in the mechanical environment. Building on that, intervention tools that are organ-, or better cell- and/or disease-state specific, may hold a key to novel therapeutic opportunities.

A more long-term aim will be to decipher the complex pathways of mechanically induced changes in cell and tissue structure and function, which contribute to chronic responses to mechanical overload. Given that we are still unsure whether stress or strain is the key activators of MGC, it is difficult to decipher response patterns driven by volume- and pressure-challenges (or by pre- and afterload) in the whole organ.

The most complex setting—chronic overload combined with acute stretch (such as may occur in ischaemic border zone tissue)—is perhaps the clinically most relevant immediate target, as focal excitation occurs acutely and in relation to local stretch maxima. An MGC modulator that would be metabolic state specific (eg, perhaps pH-dependent) could potentially be applied systemically, yet act where needed. This is an area deserving targeted study.

Another challenge lies in the multiplicity of nonchannel structures that are mechanosensitive, making an understanding of the roles of mechanical forces more complex.

Caveolae are known to act as membrane reserves that can buffer variation of membrane tension. They were shown to limit VAC (I_{Ca,swell}) activation in rat ventricular myocytes and were implied as the substrate for stretch-induced conduction slowing (acting via mechanical unfolding, increasing effective surface membrane area).

Also, caveolin 3 can modify integrin function and mechanotransduction in cardiomyocytes and intact heart, whereas caveolin 1 in endothelial cells of murine coronary arteries is a critical requirement for shear stress-mediated vasodilatation. Caveolins are also tightly linked to some TRP channels, making caveolae another most interesting component of mechanical transduction pathways, including those relevant for MEF.

Last, but not least, T-tub—sarcolemmal membrane invaginations that dive deep into the centre of cardiac myocytes—contains MGC (eg, TRPC6) and MMC (eg, Kir2.3). They deform rhythmically on a beat-by-beat basis and may be one of the more underestimated physiologically relevant mechanotransducers in the heart. For a more extensive overview of nonchannel structures involved in mechanotransduction processes, we refer to Hirata et al.

Conclusion

MGC in the heart affect cardiac electrophysiology. Although mechanical activation of ion transport pathways may be an evolutionary inheritance of the need, even for the earliest cells to respond to osmotic challenges, SAC in (osmotically more stable) multicellular systems may have conferred other advantages. For muscle cells, this may include the adjustment of contractility to local mechanical demand. The fact that MGC carry ion currents that can affect heart rate and rhythm may, thus, be a side effect. But, even if so, it is one that matters for maintenance of normal heart rate and for the induction or termination of heart rhythm disturbances. Any therapeutic targeting will need to involve a 3-pronged approach that considers potential electrophysiological benefits in the context of the need to maintain volume regulatory capacity (say in ischaemia, and even more so, reperfusion) and the ability to tune cell contractility to the mechanical environment. In fact, the latter is particularly important for the heart as, in contrast to skeletal muscle, individual myocytes must match their mechanical performance to that of their neighbors in the absence of neuromuscular junctions or other cell external mechanisms to grade contractile activity. What is clear, therefore, is that any concept of cardiac electro-mechanical activity that ignores the information flow from mechanics to electrics is unnecessarily limited—and outdated.

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Disclosures

None.

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