TITLE
Cardiac Mechano-Electric Coupling: Acute Effects of Mechanical Stimulation on Heart Rate and Rhythm

RUNNING HEAD
Cardiac Mechano-Electric Coupling

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ABSTRACT

The heart is vital for biological function in almost all chordates, including human. It beats continually throughout our life, supplying the body with oxygen and nutrients while removing waste products. If it stops – so does life. The heartbeat involves precise coordination of the activity of billions of individual cells, as well as their swift and well-coordinated adaption to changes in physiological demand. Much of the vital control of cardiac function occurs at the level of individual cardiac muscle cells, including acute beat-by-beat feedback from the local mechanical environment to electrical activity (as opposed to longer-term changes in gene expression, and functional or structural remodelling). This process is known as Mechano-Electric Coupling (MEC). In the current review, we: present evidence for, and implications of, MEC in health and disease in human; summarise our understanding of MEC effects gained from whole animal, organ, tissue, and cell studies; identify potential molecular mediators of MEC responses; and demonstrate the power of computational modelling in developing a more comprehensive understanding of ‘what makes the heart tick’.

GRAPHICAL ABSTRACT
1. Normal cardiac rhythm requires the coordination of billions of individual heart cells’ activity, as well as their swift and well-coordinated adaption to changes in physiological demand. A critical factor for beat-by-beat control of cardiac function is an intracardiac, electro-mechanical auto-regulatory loop, involving feed-forward and feed-back interactions between the heart’s electrical and mechanical behaviour. This includes acute feedback from the local mechanical environment to cellular electrophysiology via mechano-sensitive sub-cellular components, a process known as Mechano-Electric Coupling (MEC).

2. In cardiac pacemaker cells, acute stretch (e.g., caused by an increase in venous return) results in increased spontaneous diastolic depolarisation (e.g., increasing rate of sinoatrial node excitation; the Bainbridge effect). This MEC response is intrinsic to the pacemaker cells themselves (i.e., independent of autonomic reflexes) and it is critical for the adaptation of heart rate to beat-by-beat changes in venous return. Age- and disease-related changes in myocardial mechanics can affect associated auto-regulatory mechanisms, and thus contribute to sinoatrial node dysfunction and disturbances of cardiac rhythm.

3. In the atria, acute stretch due to volume or pressure overload increases the vulnerability to, and sustainability of, atrial fibrillation – whether tissue remodelling is already present or not. This MEC effect has been attributed to heterogeneous, mechanically-induced changes in excitability, action potential duration, refractoriness, and/or conduction.

4. In the ventricles, acute mechanical stimulation, whether global (due to volume or pressure overload) local (due to contact of intra-cardiac devices with the endocardium, such as tips of catheters or pacing leads), or caused by external impacts (e.g., in the setting of Commotio cordis) can lead to premature excitation and induce tachyarrhythmias, including ventricular fibrillation. Outcomes depend on the interrelation of the mechanical stimulus with underlying substrate electrophysiology, creating an individually varying, spatio-temporally defined vulnerable window. In chronic diseases with ventricular overload, stretch contributes to the sustenance of ventricular arrhythmias, as evidenced by the anti-arrhythmic effect of a temporary reduction in ventricular loading (e.g., by the Valsalva manoeuvre).

5. Transcutaneous mechanically-induced excitation, whether by extracorporeal or epicardial impact, high-intensity focused ultrasound, or catheter-based device approaches, may be an effective means for transient pacing of the asystolic or severely bradycardic heart, potentially to the point of recovery of normal sinus rhythm. Current International Liaison Committee on Resuscitation (ILCOR) guidelines state that: “fist pacing may be considered in haemodynamically unstable bradyarrhythmias until an electric pacemaker (transcutaneous or transvenous) is available” and that: “there is insufficient evidence to recommend for or against the use of the precordial thump for witnessed onset of asystole caused by atrioventricular conduction disturbance”. This is an area requiring targeted research.
<table>
<thead>
<tr>
<th>Key Specialised Terminology</th>
<th>Definition</th>
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<tbody>
<tr>
<td><strong>ATP-sensitive potassium current</strong> (<em>I</em>&lt;sub&gt;K,ATP&lt;/sub&gt;)</td>
<td>A potassium channel that is activated both a reduction in intracellular adenosine triphosphate and modulated by stretch.</td>
</tr>
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<td><strong>Atrial fibrillation</strong> (AF)</td>
<td>Rapid, irregular excitation of some or all of the atria.</td>
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<td><strong>Bradycardia</strong></td>
<td>Slow heart rate.</td>
</tr>
<tr>
<td><strong>‘Calcium (Ca&lt;sup&gt;2+&lt;/sup&gt;) clock’</strong></td>
<td>Intracellular calcium cycling contribution to sinoatrial node automaticity.</td>
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<td><strong>Commotio cordis</strong></td>
<td>Mechanical “agitation of the heart” [Latin], usually by a precordial impact of sub-contusional energy, that may give rise to heart rhythm disturbances of varying severity and duration, including ventricular fibrillation.</td>
</tr>
<tr>
<td><strong>Reversal potential</strong> (<em>E</em>&lt;sub&gt;rev&lt;/sub&gt;)</td>
<td>Membrane potential at which there is no net flow through an ion channel.</td>
</tr>
<tr>
<td><strong>Gadolinium</strong> (*Gd&lt;sup&gt;3+&lt;/sup&gt;)</td>
<td>Chemical element that is a non-specific blocker of cation nonselective stretch-activated ion channels.</td>
</tr>
<tr>
<td><strong>Grammostola spatulata mechanotoxin-4</strong> (<em>GsMTx-4</em>)</td>
<td>Peptide isolated from the venom of the <em>Grammostola rosea</em> spider that is currently the most selective blocker of cation-nonselective stretch-activated channels.</td>
</tr>
<tr>
<td><strong>‘Funny’ current</strong> (<em>I</em>&lt;sub&gt;f&lt;/sub&gt;)</td>
<td>Hyperpolarisation-activated depolarising ‘inward’ current passed by cyclic nucleotide-gated channels, for example in sinoatrial node cells.</td>
</tr>
<tr>
<td><strong>Long QT syndrome</strong></td>
<td>Condition in which repolarisation of (part of) the ventricles is delayed, causing an increase in the QT interval of the electrocardiogram.</td>
</tr>
<tr>
<td><strong>Maximum diastolic potential</strong></td>
<td>Most negative membrane potential reached by pacemaker cells during their spontaneous cycle of de- and repolarisation.</td>
</tr>
<tr>
<td><strong>Maximum systolic potential</strong></td>
<td>Most positive membrane potential reached by pacemaker cells during their spontaneous cycle of de- and repolarisation.</td>
</tr>
<tr>
<td><strong>Mechano-electric coupling (MEC)</strong></td>
<td>Acute feedback from the mechanical status of the heart to its electrical activity.</td>
</tr>
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<td>-------------------------------------</td>
<td>---------------------------------------------------------------------</td>
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<tr>
<td><strong>‘Mechanics clock’</strong></td>
<td>System of mechano-sensitive mechanisms that contributes to sinoatrial node automaticity.</td>
</tr>
<tr>
<td><strong>Precordial thump</strong></td>
<td>A single fist impact, generally applied to the left of the lower half of the sternum, to re-set disturbed heart rhythms.</td>
</tr>
<tr>
<td><strong>Respiratory sinus arrhythmia</strong></td>
<td>Physiological fluctuation in heart rate that is synchronous with the respiratory cycle.</td>
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<tr>
<td><strong>Sinoatrial node (SAN)</strong></td>
<td>A region of tissue in the right atrium that contains the primary cardiac pacemaker.</td>
</tr>
<tr>
<td><strong>Spontaneous diastolic depolarisation</strong></td>
<td>Automaticity-providing shift in membrane potential in cardiac pacemaker cells.</td>
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<tr>
<td><strong>Spontaneous diastolic depolarisation</strong></td>
<td>Automaticity-providing shift in membrane potential in cardiac pacemaker cells.</td>
</tr>
<tr>
<td><strong>Stretch-activated channel (SAC)</strong></td>
<td>Ion channel which is gated by a mechanical stimulus (in the absence of cell volume changes).</td>
</tr>
<tr>
<td><strong>Stretch-modulated channel</strong></td>
<td>Ion channel whose activity is altered by a mechanical stimulus.</td>
</tr>
<tr>
<td><strong>Tachyarrhythmia</strong></td>
<td>Abnormally rapid heart rhythm.</td>
</tr>
<tr>
<td><strong>Ventricular fibrillation (VF)</strong></td>
<td>Rapid irregular excitation of the ventricles.</td>
</tr>
<tr>
<td><strong>‘Voltage (V_m) clock’</strong></td>
<td>System of sarcolemma-bound ion flux mechanisms that contributes to sinoatrial node automaticity.</td>
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<tr>
<td><strong>Vulnerable window</strong></td>
<td>A narrow period during which the heart is particularly susceptible to the induction of arrhythmias.</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional</td>
</tr>
<tr>
<td>AF</td>
<td>atrial fibrillation</td>
</tr>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>APD</td>
<td>action potential duration</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BK</td>
<td>big potassium channel</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>calcium ion</td>
</tr>
<tr>
<td>Ca&lt;sub&gt;V&lt;/sub&gt;</td>
<td>voltage-gated calcium</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>E&lt;sub&gt;rev&lt;/sub&gt;</td>
<td>reversal potential</td>
</tr>
<tr>
<td>Gd&lt;sup&gt;3+&lt;/sup&gt;</td>
<td>gadolinium ion</td>
</tr>
<tr>
<td>GsMTx-4</td>
<td><em>Grammestola spatulata</em> mechanotoxin-4</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>I&lt;sub&gt;b,Na&lt;/sub&gt;</td>
<td>background sodium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;Ca,L&lt;/sub&gt;</td>
<td>long-lasting calcium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;Ca,T&lt;/sub&gt;</td>
<td>transient calcium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;Cl,swell&lt;/sub&gt;</td>
<td>swelling-activated chloride current</td>
</tr>
<tr>
<td>I&lt;sub&gt;f&lt;/sub&gt;</td>
<td>‘funny’ current</td>
</tr>
<tr>
<td>I&lt;sub&gt;K,ATP&lt;/sub&gt;</td>
<td>adenosine triphosphate-sensitive potassium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>fast sodium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;NCX&lt;/sub&gt;</td>
<td>sodium-calcium exchanger current</td>
</tr>
<tr>
<td>I&lt;sub&gt;SAC,K&lt;/sub&gt;</td>
<td>potassium-selective stretch-activated current</td>
</tr>
<tr>
<td>I&lt;sub&gt;SAC,NS&lt;/sub&gt;</td>
<td>cation-nonselective stretch-activated current</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>potassium ion</td>
</tr>
<tr>
<td>K&lt;sub&gt;2P&lt;/sub&gt;</td>
<td>2 P-domain potassium channel</td>
</tr>
<tr>
<td>MEC</td>
<td>mechano-electric coupling</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>sodium ion</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
</tr>
<tr>
<td>SAC</td>
<td>stretch-activated channel</td>
</tr>
<tr>
<td>SAC&lt;sub&gt;K&lt;/sub&gt;</td>
<td>potassium-selective stretch-activated channel</td>
</tr>
<tr>
<td>SAC&lt;sub&gt;NS&lt;/sub&gt;</td>
<td>cation-nonselective stretch-activated channel</td>
</tr>
<tr>
<td>SAN</td>
<td>sinoatrial node</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>TnC</td>
<td>troponin C</td>
</tr>
<tr>
<td>TRAAK</td>
<td>TWIK-related arachidonic acid-activated potassium channel</td>
</tr>
<tr>
<td>TREK-1</td>
<td>TWIK-related potassium channel-1</td>
</tr>
<tr>
<td>TRP</td>
<td>transient receptor potential channel</td>
</tr>
<tr>
<td>TRPC</td>
<td>transient receptor potential channel, canonical protein</td>
</tr>
<tr>
<td>TRPM</td>
<td>transient receptor potential channel, melastatin protein</td>
</tr>
<tr>
<td>TRPP</td>
<td>transient receptor potential channel, polycystic protein</td>
</tr>
<tr>
<td>TRPV</td>
<td>transient receptor potential channel, vanilloid protein</td>
</tr>
<tr>
<td>TWIK</td>
<td>2-pore domain weak inwardly rectifying potassium channel</td>
</tr>
<tr>
<td>VF</td>
<td>ventricular fibrillation</td>
</tr>
<tr>
<td>V&lt;sub&gt;m&lt;/sub&gt;</td>
<td>transmembrane potential</td>
</tr>
</tbody>
</table>
I. CARDIAC MECHANO-ELECTRIC COUPLING (MEC)

A. MEC and the Mechano-electric Regulatory Loop

The heart is a remarkably dynamic, robust organ. It beats approximately once per second, and about 3-4 billion times in one’s lifetime. In doing so, it pumps the equivalent to the volume contained in an Olympic-sized swimming pool each year. The human heart is composed of billions of individual muscle cells (cardiomyocytes), as well as a host of other cell types (*e.g.*, fibroblasts, endothelial, fat, nerve, and immune cells). For effective pumping, this myriad of cells functions in a tightly controlled and well-orchestrated manner. Cardiomyocytes are electrically excited and mechanically contract in a well-coordinated pattern, while simultaneously adjusting their activity on a beat-by-beat basis to fluctuating haemodynamic conditions, so that local mechanical activity matches global circulatory demand. This demand is altered by exercise, when we change posture, and even with every breath we take, affecting the passive mechanical stretch of cells before contraction (referred to as ‘strain’ when normalised to resting length) and the load against which cells actively contract (referred to as ‘stress’ when expressed as force per cross-sectional area). In the ventricles, pre-contraction stretch can be approximated by end-diastolic volume (called ‘preload’), and the force opposing ventricular ejection is determined by the pressure in the downstream aortic or pulmonary vessels (called ‘afterload’). One result of the inherent cardiac mechano-sensitivity is the fact that cardiac output (ejection) matches venous return (filling), maintaining balanced cardiovascular system performance, while also matching the throughput of left and right sides of the heart over any period of time.

Incredibly, the heart’s coordinated activity and its adaption to haemodynamic changes occur in the absence of the kind of neuro-muscular junctions that organise skeletal muscle activity (although neuro-muscular interaction sites with the autonomic nervous system may be much more wide-spread and regular in the heart than previously thought (478)). And while heart function is clearly influenced by extra-cardiac factors such as sympathetic and parasympathetic innervation and circulating hormones, beat-by-beat adaptation of cardiac function to changes in the mechanical environment continues even when the heart is removed from the body, or when it lacks nervous system inputs such as in freshly transplanted hearts. This is possible because the heart possesses highly efficient intrinsic (intra-cardiac) auto-regulatory mechanisms that are based on feed-forward and feed-back interactions between the heart’s electrical and mechanical activity.

In the direction classically regarded as feed-forward, electrical excitation of the myocardium, physiologically initiated by the leading pacemaker in the sinoatrial node (SAN, a region of the
right atrium), results in a spreading wave of cellular action potentials (AP) that, through a process known as excitation contraction coupling, give rise to mechanical activation (47, 168). In the opposite direction, the heart’s mechanical status, including internal and external mechanical perturbations, affects cardiac electrical activity. This acute feedback (as opposed to medium-term gene expression changes or longer-term electrophysiological, mechanical, and structural remodelling that occur with chronic mechanical alterations and during heart disease (397)) has been termed ‘Mechano-Electric Feedback’ (which, strictly, considers only cardiac mechanical activity as an input signal), or more broadly, ‘Mechano-Electric Coupling’ (MEC, which encompasses mechanical perturbations of the heart irrespective of their origin) (318, 499) (illustrated in Figure 1).

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**Figure 1.** The feed-forward and feed-back links between cardiac electrophysiology and mechanics, forming the intra-cardiac mechano-electric regulatory loop. The feed-forward between electrical excitation and mechanical contraction involving intra-cellular calcium (Ca^{2+}) handling and actin-myosin cross-bridge cycling, is a process known as 'Excitation-Contraction Coupling'. Feedback from myocardial deformation to cell electrophysiology and intra-cellular Ca^{2+} dynamics occurs via multiple interdependent mechano-sensitive mechanisms, which in turn affect the origin and spread of excitation, a phenomenon known as 'Mechano-Electric Feedback' (which, strictly, would consider only cardiac mechanical activity as an input signal) or more broadly 'Mechano-Electric Coupling' (which encompasses mechanical perturbations of the heart irrespective of their origin). [Adapted from (487).]
MEC is an expression of the heart’s exquisite mechano-sensitivity. It is evident at all levels of structural and functional integration (from sub-cellular to whole organ), in numerous cell types (ventricular and atrial myocytes, SAN and Purkinje pacemaker cells, as well as cardiac non-myocytes), and it is present both in invertebrates and vertebrates from fish to human (318). Mechanical stimuli, acting through stretch-activated ion channels (SAC, which are directly gated by a mechanical stimulus), stretch-modulated ion channels (whose primary mechanism of activation is non-mechanical, but whose activity is modulated, usually increased, by mechanical stimulation), changes in calcium handling, and second messenger systems have far-ranging physiological effects on the heart, from altered electrophysiological properties including excitability, refractoriness, and electrical load, to changes in heart rate (HR) and rhythm, AP shape, and electrical conduction. These effects have important clinical consequences, including the induction or termination of arrhythmias.

In this review, following a brief look at the history of MEC research, we will present the evidence for and implications of MEC in human, summarise insight into MEC effects that have been gleaned from whole animal, organ, tissue, and cell studies, explore potential molecular mechanisms of MEC, and reflect upon the utility of integration of mechano-electric interaction data by computational modelling.

B. Brief History of MEC Research

Early case reports on mechanically-induced changes in heart rhythm date back, in the European medical literature, at least to the late 19th century (e.g., on mechanically-induced sudden death by Felice Meola (394), Auguste Nelaton (426), and Ferdinand Riedinger (519)). At the turn of the 20th century, systematic experimental studies in whole animals explored phenomena ranging from Commotio cordis (e.g., Georg Schlomka (543)) to stretch-induced increase in HR (23). Mechanistic insight from AP recordings in isolated cardiac tissue started to emerge in the 1960s, when Klaus Deck characterised the stretch-induced positive chronotropic response in isolated SAN from rabbit and cat (147). These intra-cardiac, mechanically-induced electrophysiological effects (discussed in ‘II.A. Modulation of Heart Rate’) were recognised as an expression of Mechano-Electric Feedback (“Mechano-Elektrische Rückkoppelung”) in a paper by Raimund Kaufmann and Ursula Ravens (née Theophile) that reported a stretch-induced increase in automaticity of Purkinje fibres from rhesus monkeys (292).

Stretch-induced electrophysiological effects in working myocardium (e.g., acceleration of early repolarisation, depolarisation in later phases of the AP, and the potential of triggering ectopic beats) were demonstrated in frog by Max Lab in the 1970s (329). Ten years later, Michael Franz
and colleagues showed that an acute increase in intra-ventricular volume in hearts from dogs caused diastolic depolarization that could be used to pace the asystolic heart (180). Around the same time, direct evidence for the presence of MEC in human ventricles came from Peter Taggart and colleagues, who reported an acute decrease in AP duration upon increased left ventricular pressure in patients being weaned from cardiopulmonary bypass (593), and Joseph Levine and colleagues, who showed similar results during acute right ventricular outflow tract occlusion in patients undergoing balloon valvuloplasty (347); these observations are discussed in ‘II.B. Transient Effects on Whole Heart Electrical Activity’). Another ten years on, similar MEC responses were shown to exist in the atria by Flavia Ravelli and Maurits Allessie (507; described in the section on ‘II.C.6. Atrial Fibrillation’).

Molecular MEC mechanisms started to emerge in 1984, with single channel recordings of currents through SAC in cultured embryonic chick skeletal muscle by Falguni Guharay and Frederick Sachs (209). This was followed soon after by recordings of SAC currents in rat isolated ventricular myocytes (133; discussed in ‘II.A. Modulation of Heart Rate’), and later by cloning of SAC ion channels (accomplished in Escherichia coli (580)) and structural analysis using x-ray crystallography (99). At the tissue and whole heart level, investigations of the role of SAC currents in observed MEC responses have involved the use of pharmacological blockers (described in ‘III. Molecular Mechanisms of MEC’), while the structural homologue to the bacterial SAC in the mammalian heart remains unknown (a particular focus of recent research has been on determining its molecular identity). At the same time, significant attention has been paid to non-sarcolemmal mediators of MEC, particularly stretch-effects on intracellular calcium (Ca^{2+}) handling (described in ‘III.B.3. Mechano-Sensitivity of Intra-Cellular Ca^{2+} Handling’).

Importantly, the experimental innovations mentioned above have been complemented by rapid advancement of computational modelling, which has enabled the integration and interpretation of data, and the generation of novel, experimentally-testable hypotheses (312, 493, 616) (described in ‘IV. Integrative Computational Models of MEC’). Thus, building from a strong history, the present and future of MEC presents exciting possibilities, as is elucidated below.
II. PHYSIOLOGICAL AND CLINICAL RELEVANCE OF MEC

A. Modulation of Heart Rate

1. Mechanisms of SAN Automaticity

The perhaps clearest example of a physiological role for MEC is the response of the heart’s primary intrinsic pacemaker to stretch (23, 147). Normal excitation of the heart originates from the SAN, a tissue region located in the wall of the right atrium that displays spontaneous rhythmic excitation (295). At the whole cell level, SAN electrophysiology has been well described (269): rhythmic SAN firing requires spontaneous diastolic depolarisation of the transmembrane potential (V_m) from its most negative value (maximum diastolic potential) towards threshold for AP firing (illustrated in FIGURE 2, A).

Early spontaneous diastolic depolarisation is driven by a depolarising inward current through hyperpolarisation-activated cyclic nucleotide-gated channels (‘funny’ current, h) and background conductances (e.g., for sodium [Na⁺], h_Na (438)), facilitated by a continual reduction in repolarising outward potassium (K⁺) currents (152). As diastole progresses, spontaneous diastolic depolarisation rate increases by activation of inward Ca²⁺ flux through voltage-gated ‘transient’ Ca²⁺ channels (Cav3.1 carrying I_Ca,T) and, upon further depolarisation, long-lasting Ca²⁺ channels (Cav1.2/1.3 carrying I_Ca,L), whose activation ultimately drives the AP upstroke in SAN cells of large mammals (395) (in mice, fast Na⁺ channels carrying h_Na also contribute to SAN AP upstroke (342), affecting the utility of mice for translational studies into SAN electrical function). This system of membrane-bound ion channels can independently give rise to cyclic spontaneous AP generation, as illustrated by quantitative computational models (680). This cardiac pacemaker mechanism has been referred to as a ‘voltage clock’ (V_m clock (380); summarised, along with potential stretch effects, in FIGURE 2, C).

Spontaneous diastolic depolarisation of SAN cells is also facilitated by Ca²⁺ release from the sarcoplasmic reticulum (SR), occurring either spontaneously or triggered by Ca²⁺-induced Ca²⁺-release upon activation of I_Ca,L (612). Cytosolic Ca²⁺, extruded from the cell by the Na⁺-Ca²⁺ exchanger (h_NCX), gives rise to membrane depolarisation, as h_NCX is ‘electrogenic’ in that it moves three Na⁺ ions into the cell for each Ca²⁺ ion removed. As SR Ca²⁺ release remains rhythmic for a period of time, even in the absence of cyclic changes in V_m, this electrogenic effect is also sufficient to drive SAN pacemaking (335), and it has been referred to as the ‘Ca²⁺ clock’ of cardiac pacemaking (380); summarised, along with potential stretch effects, in FIGURE 2, B AND C).
When considering mechanisms that drive pacemaker activity, it is important to remember that pacemaker function adapts to changes in haemodynamic load on a beat-by-beat basis. The $V_m$ and Ca$^{2+}$ ‘clocks’ do not inherently account for this rapid response to circulatory demand (cellular Ca$^{2+}$ balance changes over multiple beats, while mechanically-driven variation of sarcolemmal ion channel expression takes even longer). Thus, another set of mechanisms,
sensitive to the SAN’s cyclically changing mechanical environment, must contribute to spontaneous diastolic depolarisation. In analogy to the above terminology, this may be considered a ‘mechanics-clock’ (496, 622). Of course, the concept of multiple ‘clocks’ providing one ‘time’ (initiation of each heartbeat) is somewhat counterintuitive: if one considers SAN activation as the uniform ‘time’ output, then the various underlying mechanisms may better be conceptualised as a system of three coupled oscillators.

Pacemaker electrophysiology has been studied largely in isolated, unloaded cells. In vivo, the SAN is subjected to cyclic yet variable changes in its mechanical environment. During atrial diastole the SAN is stretched by the downward shift of the atrioventricular valve-plane during ventricular contraction (214) and the associated filling by venous return. Peak stretch levels coincide with spontaneous diastolic depolarisation, which is affected by stretch-induced inward currents (120) (discussed further in ‘III. Molecular Mechanisms of MEC’), thus ‘priming’ SAN cells during the very period when their $V_m$ moves towards threshold for excitation.

The contribution of mechanical load to spontaneous diastolic depolarisation and SAN excitation timing was established in 1964, when Klaus Deck reported microelectrode recordings of $V_m$ during equi-biaxial stretch of cat and rabbit isolated SAN tissue, demonstrating an increase in spontaneous diastolic depolarisation and spontaneous beating rate (147). The critical nature of the mechanical environment for spontaneous, rhythmic SAN excitation was confirmed soon after, as it was shown that slack isolated SAN tissue often shows no, or irregular, excitation, while moderate stretch restores rhythmic pacemaker activity in previously quiescent or arrhythmic SAN tissue (73, 337) (FIGURE 3). Preload may in fact be critical to SAN

**Figure 3. Effects of stretch on isolated sinoatrial node beating rate.** Floating microelectrode recordings of transmembrane potential in cat isolated sinoatrial node, showing a stretch-induced shift of the maximum diastolic potential towards less negative values, resulting in restoration of regular rhythm in a preparation with irregular activity at slack length (A), or initiation of spontaneous excitation in a previously quiescent preparation (B). In both examples, tissue length was increased by ~40% from slack, with periods of stretch indicated by the lower horizontal lines. [Adapted from (337).]
pacemaker activity from the very first heartbeat during embryonic development (e.g., day 22 in the human embryo), as physiological loading (fluid pressure build-up in the quiescent cardiac tube) may be a pre-requirement for initiation and pre-neuronal control of cardiac excitation during ontogenesis (104, 504, 505).

Ultimately, through the combined actions of the various pacemaking oscillators, spontaneous diastolic depolarisation causes $V_m$ to cross the activation threshold for AP generation, resulting in the initiation and spread of a new wave of cardiac excitation (395). While the roles and interrelation of the mechanisms of SAN automaticity are still debated ($V_m$, $Ca^{2+}$, and mechanics oscillators can, in the experimental setting, each independently induce SAN excitation (153, 379, 525)), they represent overlapping and redundant systems that do not operate in isolation. Their interplay supports a robust and flexible system that integrates multiple functionally relevant inputs to provide a reliable basis for cardiac rhythmicity (259).

2. SAN Mechano-Sensitivity

SAN automaticity is influenced by extrinsic cues, such as biochemical signals from the autonomic nervous system and circulating hormones (374), as well as biophysical factors including preload. Mechano-sensitivity of SAN pacemaking was first established in the laboratory of Albert von Bezold, who reported sinus tachycardia induced by an increase in venous return in rabbits in whom the heart’s sympathetic and parasympathetic connections with the nervous system had been cut (‘denervated’) (570). More generally known is the work by Francis Bainbridge, who demonstrated that right-atrial distension by intravenous fluid injection in anaesthetised dogs causes an increase in HR (23) – a response now known as the ‘Bainbridge effect’.

Demonstrating that the Bainbridge effect also occurs in humans was difficult, as most non-invasive interventions that raise central venous pressure (such as tilt-table studies) tend to also increase arterial pressure and trigger the (dominant) baroreceptor-mediated depressor reflex. It was not until 1978 that David Donald and John Shepherd overcame this challenge by passively elevating the legs of volunteers in the supine position (155), which raised central venous pressure (by favouring venous return) without a simultaneous rise in arterial pressure. This resulted in an increase in HR that unequivocally established the presence of a positive chronotropic response to stretch in humans.

Under most conditions, the degree of filling of the right atrium, and thus the extent of stretch of the SAN, is primarily determined by venous return. Venous return is modulated, for example, by breathing, posture, physical activity, and vascular tone. Through the Bainbridge effect, HR in
large mammals is raised in response to an increase in right atrial filling. Along with cell length-dependent changes in stroke volume (a consequence of the ‘Frank-Starling Law’ – for further discussion of 'Mechano-Mechanical Coupling' see (81, 428, 497)), this stretch-induced response helps match cardiac output (HR × stroke volume) to changes in venous return. The Bainbridge effect also opposes the baroreceptor response (the ‘Bezold-Jarisch’ or ‘depressor’ reflex, which reduces HR when arterial blood pressure is increased (271, 636)), thus preventing excessive slowing of beating rate or diastolic (over-)distension of the right atrium, while maintaining cardiac output and adequate circulation during haemodynamic changes that increase both venous return and arterial pressure. Interestingly, a response similar to the Bainbridge effect may also occur in cells of lower order pacemaker and conduction system, where Purkinje fibres – stretched during ventricular diastole (91) – show a mechanically-induced increase in automaticity (292, 536) and conduction velocity (146, 154, 524).

The fundamental importance of SAN mechano-sensitivity is indicated by its presence across the invertebrate (546) and vertebrate phyla (464). Originally assumed to be a neurally-mediated reflex (the near-instantaneous response suggests that circulating humoral factors are not responsible), it can be observed not only in intact animals, but also in isolated hearts, tissue, and single pacemaker cells, indicating that intra-cardiac mechanisms are indeed key contributors (489, 496).

Ex vivo evidence has added further support to the notion of a nervous system-independent mechanism for the stretch-induced increase in HR, as the chronotropic response to stretch is insensitive to ablation of intra-cardiac neurons (663) and pharmacological block of Na⁺ channels (103, 663) or adrenergic and cholinergic receptors (30, 52, 61, 72, 73, 103, 337, 465, 663).

There is evidence, however, for an interaction between mechanical and autonomic HR modulation. Stretch causes both an increase in HR and a decrease in the response to vagus nerve stimulation in whole animals (62) and isolated tissue (664). Conversely, when HR is reduced by vagus nerve stimulation, the stretch-induced increase in HR is enhanced (61, 72, 664), possibly in part through stretch-inactivation of the stretch-modulated acetylcholine-activated K⁺ current (219). The stretch response is similarly enhanced when HR is first reduced by pharmacological parasympathetic or cholinergic stimulation, and diminished when HR is increased by adrenergic stimulation (30, 60, 72, 147, 219, 527, 664). In the case of excessive adrenergic stimulation, the direction of stretch-induced changes in HR may reverse (i.e., give rise to slowing (30)), similar to the response seen in mouse (a species with an inherently high HR (119), limiting the utility of mice for translational studies of cardiac MEC responses).
whether these changes in chronotropic stretch-responses are driven by an interaction of intrinsic (stretch) and extrinsic (autonomic nervous system) effects, or simply result from HR-dependent differences in the electrophysiological response to stretch is difficult to tell (several studies have reported that the positive chronotropic response to stretch is enhanced at lower HR, regardless of the nature of the HR reduction (113, 119)).

Combined actions of stretch- and neuronally-mediated effects on HR are evident also from variations in HR that are synchronous with the respiratory cycle: HR rises during inspiration and declines during expiration. This phenomenon, noted in humans more than 170 years ago by Carl Ludwig (372) and referred to as ‘respiratory sinus arrhythmia’ (even though it is a physiological fluctuation in heart rhythm, not an arrhythmia per se), has long been considered to be a consequence – and, hence, useful clinical indicator – of ‘vagal tone’. Yet, respiratory sinus arrhythmia continues to exist, albeit at a reduced magnitude, in the transplanted (i.e., denervated) human heart (44, 45, 502), during autonomic block (558, 569), and in acutely vagotomised animals (472), indicating a contribution of intrinsic, mechanically-mediated mechanisms.

The MEC contribution to respiratory sinus arrhythmia is driven by fluctuations in right atrial volume during respiration, as venous return is favoured – by reduced intra-thoracic and increased abdominal pressure – during inspiration and impeded during expiration. During physical activity, non-neuronal responses appear to dominate respiratory sinus arrhythmia-mediated fluctuations in HR even in healthy volunteers, as cyclic fluctuations in venous return are increased with increased respiratory effort, while ‘vagal tone’ is reduced during physical activity (45, 95). In keeping with this, during positive pressure ventilation, which reverses the thoraco-abdominal pressure gradients relative to the respiratory cycle, respiratory sinus arrhythmia can switch phase, so that HR decreases during inspiration, as intrathoracic positive pressure application impedes venous return to the heart below levels present during passive expiration (370).

3. SAN Dysfunction

Mechanical modulation of HR appears to be functional only within a certain range of mechanical loads, as excessive stretch can result in irregular rhythms (337) and multifocal activity (234). This may be relevant in cardiac pathologies associated with atrial volume overload (417, 535, 565), where natural occurring HR variability is reduced by SAN stretch (240, 391) – an adverse prognostic marker. Decreased SAN distension upon increased myocardial stiffness, resulting from cardiac fibrosis or structural remodelling in advanced age (410, 530), atrial
fibrillation (AF) (170, 325) or other cardiac pathologies (2, 59, 322, 421), may also contribute to SAN dysfunction, and may be further exacerbated by mechano-sensitive non-myocytes (315, 317). The potential importance of age-related SAN remodelling for stretch-induced responses is supported by the greater increase in HR that occurs with similar stretch of the SAN from younger versus older animals (147).

Another important consideration in the context of SAN mechanics is the structural heterogeneity of the SAN, which results in regional differences in tissue stiffness and stretch (433). Changes in HR have been shown to correlate best with maximum SAN stretch, which occurs at its periphery, a region more distensible than the central node (279). This regional difference may be important for transmission of electrical activity from the SAN to atrium (195), as the SAN periphery is where (the possibly stretch-modulated) If is thought to play the largest role in SAN pacemaking. This is due, in part at least, to the more negative maximum diastolic potential in that region (caused by electrotonic influences from coupled working cardiomyocytes), which activates more If and increases the driving force for cation-nonselective stretch-activated channels (SACNS) (323, 431). These regional differences in SAN mechano-sensitivity may be exasperated by heterogeneous changes in SAN mechanical properties, or by variable expression and activity of SAC and stretch-modulated ion channels during disease or ageing (65, 575, 600).

It is important to note, however, that it is not entirely clear what constitutes a normal or a pathophysiologically-altered SAN mechanical environment. In this context, questions that should be explored in further research include: whether the key mechanical parameter for changes in SAN function is stretch (279, 536), stress (14, 73, 103), or a combination of both (337); whether the rate of change in mechanical load affects SAN electrophysiology (73, 337) or not (103); and whether certain spatial loading patterns (e.g., linear, equi-biaxial, multi-axial) are more appropriate than others (147). Additionally, as mechanical-responses, at least in ventricular myocytes, are AP shape- and phase-dependent (86, 181, 222, 437), the timing of mechanical stimulation is an important variable that may be affected by disease. This could help to explain species differences in the chronotropic response to stretch (i.e., mouse versus larger mammals (119), as discussed further in ‘III.A.1. Mechano-Sensitivity of ‘Mechanical Oscillator’ Components’).

4. Summary

The SAN, the heart’s intrinsic pacemaker, initiates the heartbeat. Its rate of firing is determined by the interaction of multiple oscillators (Vm, Ca2+, mechanics) whose integrated
activity sets the clock for robust automaticity and regular heart rhythm. The chronotropic response to SAN stretch (Bainbridge effect) allows HR to be tuned to haemodynamic demand on a beat-by-beat basis, governed by effects intrinsic to SAN pacemaker cells. Abnormal stretch can result in a disturbance of rhythm, representing a potential contributor to SAN dysfunction with age and in disease. While much is understood about SAN function and its control by the mechanical environment, most ex vivo studies are performed in unloaded preparations, leaving questions regarding the (patho-)physiological importance of MEC in the SAN unanswered, thus warranting further investigation.

B. Transient Effects on Whole Heart Electrical Activity

1. Diastolic Stretch

Electrophysiological effects of acute mechanical stimulation are cardiac electrical cycle-dependent. In ‘electrical diastole’ (here used to describe the period when cells of the working myocardium are at their resting \(V_m\)), a sufficiently large mechanical stimulus will cause depolarisation, in a stretch-amplitude dependent manner. If supra-threshold, this can trigger excitation in whole heart (FIGURE 4, A), tissue, and cells (495). In the whole heart, this is true both for transient increases in intra-ventricular volume (54, 150, 151, 161, 180, 181, 221-223, 245, 301, 424, 459, 460, 513, 547, 568, 650, 688) and for local tissue deformation, such as upon contact of intra-cardiac devices (e.g., the tips of catheters or pacing leads) with the endocardium (35) or by epicardial and precordial impact (492, 494) (FIGURE 4, B). Interestingly, both for global and local mechanical stimuli it appears that depolarisation depends on tissue stretch, rather than stress. With an increase in intra-ventricular volume, the amount of volume required for excitation is remarkably consistent between hearts of the same species, while the associated change in intra-ventricular pressure shows high variability (222), depending on the speed of volume changes applied (in contrast, stretch-induced changes in refractoriness have been shown to correlate best with ventricular wall stress (215)). With local impact-induced tissue deformation, the extent of tissue indentation needed for excitation is similar between subjects and for various regions in a given heart, while the pressure under the probe can be highly variable (117, 492), depending on probe contact surface area and impact location. Depolarisation also appears to depend on the rate of stretch application (320), which increases the magnitude of stretch needed to cause excitation at lower deformation rates (172, 181). This application rate-dependence may be a consequence of myocardial visco-elasticity, where faster application of an external mechanical stimulus will be associated with a larger transient
overshoot in local peak stretch levels, while slow application of a mechanical stimulus may cause mild depolarisation and partial inactivation of $h_{Na}$ (in keeping with experimental observations showing that the threshold for electrical stimulation is also affected by stretch dynamics, being reduced due to stretch-induced $V_m$ depolarisation (620)).

It should be noted that in the setting of a ‘global’ stimulus, such as an increase in intraventricular volume, there will be spatially heterogeneous mechanical effects. Myocardial stiffness varies regionally throughout the heart, resulting in non-uniform stretch and depolarisation (101, 547). Upon global mechanical stimulation, excitation originates from areas where the largest stretch is observed, typically in the left ventricular free-wall or the right ventricular outflow tract, depending on the cardiac chamber affected (101, 181, 547) (FIGURE 4, C). This again highlights the notion that stretch, not stress, is a main input signal for MEC.

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**Figure 4. Mechanically-induced excitation upon diastolic stimulation of rabbit isolated whole heart.** A, top row: Monophasic action potential recording from the left ventricular (LV) epicardium (EPI; top trace) during intraventricular volume pulses ($\Delta$VOL; bottom trace) by inflation of an intraventricular balloon (schematic on left) during complete heart block, showing transient membrane depolarisations upon each balloon inflation whose amplitude increases with pulse volume; above a certain amplitude, each LV balloon-inflation causes LV excitation (note: first two action potentials are spontaneous ‘escape beats’ of the preparation). [Adapted from (181).] A, bottom row: Optical mapping of right ventricle near-epicardial membrane potential showing focal excitation at the site of maximum stretch during an intraventricular volume pulse (scale bar = 4 mm). [Adapted from (547).] B, top row: Optical mapping of LV near-epicardial membrane potential showing focal excitation resulting from a sub-contusional local impact of the epicardium. B, bottom row: LV excitation pattern with an electrical stimulus applied to the same site as the local impact, showing a similar activation pattern (scale bar = 5 mm). [Adapted from (492).]
2. Systolic Stretch

When an increase in intra-ventricular volume is applied during ‘electrical systole’ (i.e., during the AP), or maintained over the entire cardiac cycle, electrophysiological effects are generally characterised by a heterogeneous decrease in AP duration (APD) and refractoriness (54, 76, 86, 87, 101, 129, 130, 144, 161, 208, 215, 238, 331, 346, 511, 513, 514, 583, 642, 644, 688, 689), although some studies have reported an increase in both (40, 42, 131, 143, 655). These effects are also HR dependent (238, 511, 644) and generally thought to be accompanied by a decrease in tissue conduction velocity (150, 403, 554, 583, 655, 689), though an increase in conduction velocity has been seen in isolated ventricular tissue and engineered myocyte strands (252, 392). The stretch-induced decrease in conduction velocity has been attributed to effects on passive cable properties of interconnected cardiomyocytes through an increase in axial tissue resistance (75), caused by an increase in sarcoplasmin resistance (116, 652), as well as to an increase in cardiomyocyte membrane capacitance caused by incorporation of sub-sarcolemmal caveolae into the cell surface membrane (311, 477). The reported discrepancies in electrophysiological responses to systolic or sustained stretch mentioned above (some of which come from the same groups) may relate to differences in species (e.g., small versus large animal), preparation (e.g., intact animal versus isolated heart or tissue or cell), physiological factors (e.g., baseline heart rate, AP morphology), experimental considerations (e.g., mechanical stimulus magnitude, measurement technique, temperature), or data handling (e.g., measurement algorithms).

Similar effects of acute changes in ventricular preload have been seen in humans (448). For instance, an increase in intra-ventricular volume upon discontinuation of cardiopulmonary bypass results in a heterogeneous decrease in APD (593). In contrast, however, acutely reducing intra-ventricular volume (over 15 s) by the Valsalva manoeuvre (which involves forced expiration against a closed glottis, causing an increase in intrathoracic pressure that impedes venous return) in patients undergoing routine cardiac catheterisation procedures has been shown to also decrease APD, even in transplant recipients with denervated hearts, in whom a concomitant autonomic response is eliminated (590). This decrease in APD with reduced intra-ventricular volume has been suggested to relate to a reduction in myocardial shortening (rather than the change in intra-ventricular volume), as APD changes correlated with ventricular wall-motion changes (589). A similar decrease in APD occurs in experimental studies when myocardial shortening is restricted during isometric contraction (293, 329, 571). Naturally occurring oscillations in ventricular preload, much like for respiratory sinus arrhythmia in the
SAN, may also cause respiratory-related (225, 625) and lower-frequency (‘Mayer wave’) fluctuations (224, 484) in ventricular repolarisation.

An acute increase in ventricular afterload, on the other hand, for instance due to aortic constriction (449, 591) or pulmonary balloon valvuloplasty (347), also results in a heterogeneous decrease in APD, and can lead to after-depolarisation-induced ectopy (347). Ectopic excitation also occurs in experiments involving rapid increases in aortic blood pressure (550, 552, 553), potentially due to post-systolic myocardial deformation (212), which is reduced by anti-hypertensive treatment (551, 553). Conversely, an acute reduction in ventricular pressure overload, as occurs following balloon valvuloplasty or angioplasty, increases APD (347) (although this may partly reflect mechanically-induced after-depolarisation) and decreases dispersion of repolarisation (538).

MEC may play a role in coordinating whole heart electrical activity by helping to generate homogeneity out of the complex, physiologically-necessary electrophysiological and mechanical heterogeneity that exists across the heart (291). The interplay of regional mechanical effects of a contraction-induced intra-ventricular pressure wave and the phase of the AP in early and late activated regions may act to regionally synchronise ventricular repolarisation (446, 486). A similar effect has been shown using duplexes of individually controlled, mechanically interacting (in-parallel or in-series) cardiac muscle segments that allow for the simulation of mechano-electric interactions in heterogeneous myocardium (382, 561). This experimental model has demonstrated that mechanical heterogeneity contributes differently to APD changes when muscle segments are coupled in in-parallel or in-series, which may play a role in mechanical tuning of electrical activity in distant tissue regions. Also, the electro-mechanical activity of interacting contractile elements is affected by their activation sequence, which may optimise myocardial performance by reducing intrinsic APD differences. Pathophysiological, non-uniform ventricular contractions, on the other hand, can lead to electrocardiogram (ECG) T-wave vector displacement (563) and, along with increased intra-ventricular volume, may be partly responsible generation of the ECG U-wave (105, 124, 185, 542, 584-586).

3. Summary

Acute electrophysiological effects of MEC depend on the timing of mechanical stimulation relative to the AP cycle of affected cells. Mechanical stimulation, whether local or global, during electrical diastole will – if large enough to give rise to any change in electrophysiology – cause depolarisation of \( V_m \). This may trigger premature and/or ectopic excitation. If instead timed during the AP, or sustained over the entire cardiac cycle, mechanical stimulation affects APD,
refractoriness, conduction, and the dispersion of those parameters across the heart, potentially causing a pathological increase in electrophysiological heterogeneity, while otherwise playing a physiological coordinating role. While these effects are well established, critical parameters determining MEC outcomes, such as the relative importance of stretch versus stress, the actual levels of stretch or stress experienced by individual cells within the tissue, the rate of mechanical stimulus application, and underlying mechanical and electrical heterogeneities are unclear, necessitating future research.

C. Induction of Sustained Arrhythmias

1. Tissue-Level Mechanisms of Stretch-Induced Arrhythmias

As mentioned above (in ‘II.B.1. Transient Effects on Whole Heart Electrical Activity’), diastolic mechanical stimulation may cause depolarisation and trigger excitation (FIGURE 5, A). While extra beats in healthy heart will mostly have benign consequences, ectopic excitation accompanied by mechanically-induced effects on electrophysiological tissue properties during an AP can interact with underlying electrical activity to cause severe tachyarrhythmias (487). Ventricular tachyarrhythmias are thought to arise as a result of untoward interactions of an arrhythmogenic trigger and a substrate for re-entry (699). Both of these may be favoured by, or result from, MEC effects (268, 330, 510). Across the heart, electrical systole involves dispersion of $V_m$, as cells in atria and ventricles de- (P- and QRS-waves of the ECG) and repolarise sequentially (atrial repolarisation is not normally discernible on the ECG, ventricular repolarisation is reflected by the T-wave). This gives rise to electrical tissue gradients that are relatively short upon activation, and more drawn-out upon repolarisation, as witnessed for ventricles by the smaller-amplitude broad ECG T-wave, compared to the QRS complex. As a result, mechanical stimuli during electrical systole tend to encounter locally differing stages of the underlying cellular AP, which – during repolarisation – can furnish a substrate for re-entry, creating a vulnerable window for mechanically-induced ventricular tachyarrhythmia (FIGURE 5, B).

Early studies of MEC, where intra-ventricular volume was acutely increased in ex vivo whole hearts, reported a decrease in the threshold for electrically-induced excitation and tachyarrhythmias (including ventricular fibrillation, VF) (265, 513, 526); the same holds for AF inducibility during acute atrial dilatation (179, 506) (discussed in more detail in ‘II.C.6. Atrial Fibrillation’). Also in isolated hearts, mechanically-induced excitation resulting from intra-ventricular volume pulses can trigger ventricular ectopy and tachyarrhythmias (54, 222, 547,
In vivo, an acute increase in intra-ventricular volume in experimental preparations (101, 180, 208), in patients during balloon valvuloplasty (347), or as a consequence of mitral valve prolapse, stenosis, or insufficiency (31, 122, 348) is associated with a high incidence of arrhythmias. Outcomes are typically mechanical stimulus magnitude- and ECG timing-dependent and may in part result from heterogeneous stress-stretch patterns due to the spatio-temporal dissociation between a globally uniform stimulus and its regional effect. In the volume overloaded ventricle, there may also be a contribution of excessive Purkinje fibre stretch to arrhythmias, as it has been suggested to contribute to reduced conduction velocity (524) or loss of AP conduction (158, 292), sub-threshold after-depolarisations (175) and ectopic excitation (158, 234, 557), or rapid firing-induced tachycardia (158, 515, 608).

Similarly, local mechanical stimulation can result not only in ectopic excitation, but also in ventricular tachyarrhythmias, as reported upon tissue contact of central venous and pulmonary artery catheter tips (140, 169, 176, 251, 326, 340, 567, 577) or intra-cardiac catheters and electrodes (58, 339, 359, 398). The same is true for extra-corporeal mechanical stimuli, such as chest compressions during cardio-pulmonary resuscitation (43), or impacts to the precordium (97) for instance in the setting of non-contusional mechanical stimuli causing Commotio cordis (FIGURE 6, A) (316, 385, 427).

2. Commotio Cordis

Commotio cordis may be the most dramatic example of the consequences of mechanically-induced ventricular tachyarrhythmias, having been reported to result in VF-related sudden death at least as far back as the 1870s (394, 426). Even though VF by Commotio cordis is a rare event, it is one of the most common causes of sudden death in youth athletes in the US (383). Electrophysiological outcomes of Commotio cordis are determined by mechanical stimulus characteristics such as anatomical location and impact area, duration, and energy (363, 364, 543). Studies in pigs have characterised mechanical inducibility of VF as inversely-related to impact area and duration, rising with projectile stiffness and occurring only when impact-induced ectopy occurs during a vulnerable window that exists during a 10-20 ms period immediately prior to the peak of the ECG T-wave (360, 361, 365, 383) (FIGURE 6, B). Results indicate the susceptibility to VF by Commotio cordis is subject-specific (8) and, as for the chronotropic effects of stretch on SAN rate, is not affected by autonomic block (576) or denervation (543).

Computational modelling has helped to explain how precordial impact in the vulnerable window may lead to VF. Two- (2D) and three-dimensional (3D) simulations have demonstrated that Commotio cordis-induced VF should arise only when a supra-threshold mechanical stimulus
occurs at the trailing edge of the preceding wave of repolarisation, such that the mechanically-induced premature excitation forms directly adjacent to tissue that is functionally refractory (still repolarising). This results in the generation of both a trigger (ectopy, via SAC_{NS} in the model)

Figure 6. Mechanically-induced ventricular fibrillation in the setting of Commotio cordis. A: Summary of location of lethal precordial impacts in victims of Commotio cordis. [Adapted from (384).] B: Global endocardial activation map (right anterior oblique, RAO, orientation) of impact-induced electrical excitation preceding ventricular fibrillation, highlighting the focal nature of the initial trigger event. [Adapted from (7).] C: Electrocardiogram recording of instantaneous, impact-induced ventricular fibrillation in an anesthetised pig model of Commotio cordis. [Adapted from (360).] D: Surface electrocardiogram from rabbit isolated heart showing the effect of local epicardial mechanical stimulation applied to the left ventricle (LV) during the early T-wave, resulting in instantaneous ventricular fibrillation. E: Spatial interrelation of mechanical stimulation site and 50% repolarisation isochrone of the preceding sinus beat, obtained from epicardial voltage mapping (green) in those cases where sub-contusional mechanical stimulation did trigger ventricular fibrillation: only when mechanically-induced excitation (red) occurs directly adjacent to still inexcitable tissue (yellow) is a region of functional block (black rectangle) formed, around which re-entry can occur (as predicted from computational modelling shown in FIGURE 5, C). [Adapted from (492).]
and a substrate (conduction block) around which sustained re-entry can occur (194, 355) (FIGURE 5, C; discussed further in ‘IV.B.2. Triggering and Sustenance of Arrhythmias’). Conceptually, this is similar to the vulnerable window for extracorporeal electrical stimulation, which was systematically studied since the 1930s (660), but whose duration is significantly larger (≥100 ms in large animals (549)) than that described for precordial impacts (365). The difference in the length of the two vulnerable windows is a consequence of the fact that repolarisation is spatially heterogeneous across the ventricles. Therefore, the condition for overlap of mechanically-induced excitation with the trailing wave of repolarisation will be met in different cardiac locations at different time-points of the cardiac cycle, and at each of these locations for brief periods only. This means that the vulnerable window for mechanical VF induction is determined by space and time (as is also the case for point electrical stimulation).

This theoretical concept has been tested in ex vivo rabbit hearts (492), demonstrating that local epicardial stimulation causes focal excitation underneath the contact site (as seen with intra-cardiac mapping during extracorporeal impacts in the pig model of Commotio cordis (7)). As predicted by modelling, this ectopic excitation results in VF if, and only if, the stimulus overlaps with the trailing edge of repolarisation from the preceding sinus beat (492) (FIGURE 6, C).

3. Acute Regional Ischaemia

Mechanical heterogeneity is thought to contribute to the induction of arrhythmias in regional ischaemia (267). In patients with myocardial ischaemia, there is a strong correlation between the presence of regional wall motion abnormalities and arrhythmogenesis (84, 557). In the acute phase, a large proportion of ectopic beats originates from the ischaemic border zone (123, 328) (FIGURE 7, A), an area of particularly high stretch due to ‘paradoxical segment lengthening’ of the ischaemically-weakened myocardium during mechanical systole (189, 479, 533, 609, 626). Also, in ischaemic hearts that develop VF, stretch magnitude is related to the timing of VF onset (232). Similarly, the end-diastolic length of the ischaemic region is a strong predictor of VF (26, 27, 29). In acute regional ischaemia, a contribution of MEC to arrhythmogenesis is further supported by an increase in the incidence of ectopic activity in isolated hearts with elevated left ventricular pre- and afterload (established by an intraventricular balloon, connected to a fluid filled column with a clamp to control ejection resistance), compared to an unloaded ventricle (FIGURE 7, B). In addition, it has been shown that following a potentiated contraction due to an increased diastolic interval (which is presumed to increase stretch at the ischaemic border) there is an increase in the likelihood of ectopic excitation (123), which is also seen after an increase in
intra-ventricular volume (123, 459). These findings are supported by computational modelling that suggests mechanically-induced depolarisation originating from the ischaemic border zone (through SACNS) contributes to the formation of ectopic foci (if supra-threshold), or to the slowing and block of conduction (if sub-threshold) (274) (FIGURE 7, C; discussed further in 'IV.B.2. Triggering and Sustenance of Arrhythmias').

![Figure 7. Mechanically-induced arrhythmias during acute regional ischaemia. A: Activation map of ventricular premature excitation (VPE) originating at the ischaemic border in a pig isolated heart model. B: Plots summarising greater frequency of arrhythmias in loaded versus unloaded pig hearts. [Adapted from (123).] C: Computational simulation of mechanically-induced ventricular ectopy (black circle) and re-entry during acute regional ischaemia (top) and simulated activation patterns without re-entry when omitting from the model either stretch-activated channels (SAC) or ischaemic electrophysiological changes (bottom). [Adapted from (274).]}

4. Mitral Valve Prolapse

Another pathological setting, in which regional changes in ventricular mechanics are thought to contribute to arrhythmogenesis, is mitral valve prolapse (31, 442). In mitral valve prolapse, one or both leaflets of the mitral valve bulge into the left atrium during ventricular systole, resulting in stretch of the leaflets, chordae tendinae, papillary muscles, and infero-basal ventricular wall (471). The resulting arrhythmias that occur in some patients involve complex premature ventricular excitation, arising from sites close to the anchor points of prolapsing leaflets and supporting structures, such as the papillary muscles, fascicular tissue, LV outflow tract, or mitral annulus, which can lead to sudden cardiac death (32). This excitation appears to occur due to mechanically-induced after-depolarisations of tissue associated with the mitral valve (662) (particularly the papillary muscles (186, 204, 248)), by contact of the prolapsing leaflets snapping back against the ventricular myocardium during diastole (136), or by stretch of the valve itself (muscle fibres in the mitral valve leaflet have been shown to develop diastolic depolarisation when stretched, potentially leading to automatic activity that may propagate into
the surrounding myocardium \((668)\)). The key role of abnormal mechanical forces is further supported by a series of cases in which surgical correction of bi-leaflet mitral valve prolapse resulted in a reduction of ventricular arrhythmias by relieving myocardial stretch \((623)\).

5. Chronic Pathophysiological States

In a host of chronic cardiovascular diseases, alterations in myocardial mechanical properties may contribute to electrophysiological changes that promote arrhythmogenesis. This had initially proposed based on the observation that ventricular tachyarrhythmias are frequently encountered in pathologies associated with volume or pressure overload \((327, 592)\). It is difficult, however, to identify causal relationships between tissue mechanics, MEC, and cardiac rhythm disturbances in chronic disease settings, as structural and functional remodelling, as well as fluctuations in metabolic and autonomic state, may be arrhythmogenic in their own right. Considering effects of acute changes in mechanical load, and in particular the temporary removal of chronic overload, on ventricular electrophysiology in chronic pathophysiological states has been an alternative, yet effective way to elucidate the potential relevance of MEC in the induction and sustenance of ventricular arrhythmias.

One of the most striking examples is the anti-arrhythmic effect of an acute temporary decrease in intra-ventricular volume in patients suffering from chronic ventricular volume overload and tachyarrhythmias. In these patients, acute haemodynamic unloading \((249)\), or a temporary reduction in cardiac chamber volume by the Valsalva manoeuvre \((341, 648)\), rapid pacing \((473)\), or repeated forceful coughs \((651)\) can result in temporary termination of ventricular tachyarrhythmias (for as long as the reduced load is maintained) (FIGURE 8), even in transplant recipients \((10)\). In a similar vein, in patients with a dilated left atrium due to mitral stenosis, the associated arrhythmogenic dispersion and delays of conduction can be immediately reversed upon normalisation of pressure gradients by percutaneous transvenous mitral balloon valvotomy \((122)\).

In the opposite direction, an increased mechanical load on top of a chronic disease background can be pro-arrhythmic \((587)\). For instance, in heart failure patients average daily median pulmonary artery pressure has been shown to correlate with the risk of ventricular tachyarrhythmias \((512)\). In the setting of heart failure, acute increases in afterload occur on the background of pro-arrhythmic metabolic (mitochondrial oxidative capacity, fatty acid and glucose oxidation, rate of glycolysis), humoral (circulating catecholamines), electrophysiological (APD, conduction velocity, repolarisation) and mechanical (structural remodelling, volume overload) changes, and it has been shown that an acute increase in intra-ventricular pressure alone may
be as arrhythmogenic as the acidified catecholamine-rich milieu, or the electrical remodelling associated with heart failure (500).

Figure 8. Temporary termination of ventricular tachyarrhythmia with acute haemodynamic unloading. A: X-ray images of the thoracic cavity of a patient in ventricular tachycardia after deep inspiration (INSP., top left) and after an identical inspiration followed by a strong Valsalva manoeuvre (top right, timing corresponding to label in B), with a tracing of the cardiac silhouettes below (solid line = INSP., dashed line = INSP.+VALSALVA). B: Surface electrocardiogram leads 1, 2, 3 (L1-3), bipolar right atrial electrogram (BAE), and aortic blood pressure (BP) from the same patient showing as background activity atrioventricular dissociation and ventricular tachycardia. During the Valsalva manoeuvre, there is an initial increase in BP (corresponding to a period of blood redistribution away from the chest cavity, reducing heart size), followed by a decrease in BP, which is associated with a return to normal sinus rhythm. After the Valsalva manoeuvre is stopped, BP and cardiac volume return to control levels, and arrhythmia resumes. [From (648).]

Similarly, increased intraventricular preload may help in sustaining established ventricular tachyarrhythmias, as stretch accelerates activation and increases the complexity of ventricular tachyarrhythmias, potentially by producing more areas of transmural excitation breakthrough and/or conduction block (68, 106, 108-111, 413, 614). These effects can be eliminated by stabilising ryanodine receptors (RyR) in their closed state (148), highlighting the crucial contributions of intra-cellular Ca\(^{2+}\) handling to MEC (discussed further in ‘III.B.3. Mechano-Sensitivity of Intra-Cellular Ca\(^{2+}\) Handling’). Interestingly, an increase in intra-ventricular volume has also been shown to acutely reduce the effectiveness of antiarrhythmic drugs (514), while Na\(^+\) channel block by flecainide may be potentiated by atrial distension (166).

In the case of ischaemia, if infarction occurs, viable myocardium is replaced by scar tissue (518). The consequence is considerable mechanical heterogeneity (and stretch) at the infarct border zone (16), such that acute increases in intra-ventricular volume result in ventricular tachyarrhythmias, arising from the site of the largest stretch-induced change in repolarisation (87). This effect of post-infarction mechanical heterogeneity on electrophysiology may be enhanced by mechano-sensitive non-myocytes in cardiac lesions, if electrically-coupled to surviving cardiomyocytes (317), and may explain why, in patients with myocardial infarction,
acute afterload reduction can abolish arrhythmias (159, 419). Yet, little direct evidence exists regarding the role of MEC in post-infarction arrhythmias, and while mechanical heterogeneity does overlap with sites of arrhythmogenesis suggesting MEC could be involved, computational models have demonstrated that arrhythmias can arise from such regions without evoking MEC (15, 115, 393). Moreover, in the case of wall motion abnormalities, while non-uniform ventricular contraction is associated with increased dispersion of repolarisation, dispersion appears to increase primarily in normally contracting regions of hearts, independent of the presence of infarction (447).

Potential contributions of MEC to arrhythmogenesis in chronic cardiac diseases may also be related to an increase in tissue mechano-sensitivity, as demonstrated in various animal models (272, 273, 281, 304, 485, 646). Chronic disease-related hyper-sensitivity of MEC may result from: increased expression or sensitivity of SACNS current (ISACNS; (281)); increased microtubule density (460), altered viscoelastic properties (646), a reduced compensatory response to increased load (345); altered intra-cellular Ca²⁺ handling, including changes in mechano-sensitive RyR function (273) due to impaired regulation (287) or to mitochondria function due to microtubule rearrangement (405).

Some chronic diseases, attributed primarily to cardiac electrical dysfunction, may also include underappreciated beat-by-beat mechanical contributions. An example is long QT syndrome, in which spatially heterogeneous prolongation of repolarisation results in increased dispersion of APD, QT prolongation, and a propensity for developing polymorphic ventricular tachyarrhythmias that may give rise to sudden cardiac death (522). Both in transgenic and pharmacological models of long QT syndrome, there is a spatial correlation between regional APD changes and diastolic dysfunction (443), also seen in patients (66), whose extent correlates with individual arrhythmic risk (336). In fact, diagnosis of long QT syndrome may be more straightforward and accurate using spatially-resolved deformation imaging (e.g., by MRI) than the more global read-outs provided by ECG. This regional mechanical heterogeneity may contribute to disturbed electrical activity (309). One possible scenario, observed in whole animal models of pharmacologically-induced long QT syndrome, are after-contractions in the ventricular sub-endocardium which stretch (17) and depolarise sub-epicardial tissue regions, causing after-depolarisations that may give rise to torsades de pointes (188, 601).

6. Atrial Fibrillation

While severe ventricular arrhythmias are lethal, an increasing proportion of our ‘aging population’ lives with AF. Many factors contribute to the initiation and progression of AF,
including atrial dilatation, with left atrial enlargement being an independent risk factor for the development of the disease (483, 629, 630). Atrial overload can be acute (e.g., acute pulmonary embolus, myocardial ischaemia), transient (e.g., pregnancy), and chronic (e.g., mitral valve disease, hypertension, or changes secondary to HF) (630). Experimental studies of AF have confirmed that acute atrial dilatation increases AF inducibility and sustenance (FIGURE 9, A AND B) (55, 56, 107, 166, 167, 179, 184, 351, 399, 434-436, 506, 621, 672, 673, 691), while an acute reduction of atrial dilatation reduces the vulnerability to AF (263). The increase in AF vulnerability upon acute atrial dilatation is thought to occur due to stretch-induced AP shortening and altered refractoriness (285, 507, 555). Other pro-arrhythmic effects of atrial tissue distension

Figure 9. Acute stretch increases AF inducibility. A: Photographs of the right atrium of a rabbit isolated heart during acute atrial stretch caused by raising intra-atrial pressures from 0 (left) to 10 cm H2O (right, scale bar = 1 cm; expanding atria highlighted by dashed circle). [Adapted from (506).] B: Bipolar atrial electrograms showing an increase in atrial fibrillation (AF) inducibility (triggered by bursts of high-frequency pacing, end of burst-pacing indicated by arrow) with increasing intra-atrial pressure. C: Effect of application of the cation-nonselective stretch-activated channel blocker Grammostola spatulata mechanotoxin-4 (GsMtx-4, 170 nM) on AF inducibility (top panel: open circles = control, filled circles = GsMtx-4, dashed line = washout) and AF duration (bottom panel) as a function of intra-atrial pressure (* = p < 0.05 versus baseline). [Adapted from (56).]
include decreased conduction velocity (107, 562) and altered APD, refractoriness, and conduction – effects that vary locally, in part as a result of highly heterogeneous atrial wall thickness (20, 167, 246, 540). Consequently, regions of altered re-entrant cycle length (679) and conduction block (167) can be observed. AF inducibility is also increased upon removal of the pericardium, adding weight to the suggestion that electrophysiological effects of acute atrial dilatation depend on stretch, rather than tissue stress (435).

Some of the above experimental findings have been confirmed in human. Increased atrial pressure promotes the induction of AF (12), while atrial stretch modulates re-entrant cycle length (508, 649). In patients undergoing cardiac surgery, rapid atrial dilatation decreases conduction velocity and causes signal fractionation (640), while atrial loading modified by atrioventricular pacing decreases the refractory period, conduction velocity, and increases the vulnerability to AF (85, 509, 618). In keeping with these reports, relief of chronic atrial stretch after percutaneous mitral balloon commissurotomy results in an increase in refractoriness and a decrease in its heterogeneity (564).

5. Summary

Depending on magnitude and timing, as well as on the underlying electrical and mechanical background, global and local mechanical stimulation can generate excitation and a substrate for re-entry (resulting in sustained arrhythmias during a narrow and regionally varying vulnerable window for mechanically-induced tachyarrhythmias), or lower the threshold for electrically-induced arrhythmias, both in experimental models and humans. This occurs, for example, in the settings of Commotio cordis, acute regional ischaemia, mitral valve prolapse, and AF. In chronic diseases associated with mechanical changes, such as an increase in preload (end-diastolic volume overload) of afterload (increased arterial blood pressure or outflow resistance), there may also be a contribution of MEC to arrhythmogenesis that is additional to the existing pro-arrhythmic substrate, as evidenced by temporary changes in arrhythmia incidence with acute changes in load. Determining direct causal effects of MEC on cardiac rhythm is challenging and requires further experimental and computational consideration.

D. Arrhythmia Termination

1. Tachyarrhythmias

The anti-arrhythmic potential of cardiac mechanical stimulation had been first noted anecdotally as far back as the 1930s. Mechanical interaction of needles with the myocardium in the context of the then so-called ‘intra-cardiac therapy’ (for adrenalin injections to ‘revive the
dead’) were shown to have the potential of re-starting the asystolic heart even in the absence of drug injections (250). Later on, targeted myocardial contact of intra-cardiac catheters has been used to terminate atrial, junctional, and ventricular tachycardia (35, 57, 94, 381, 444, 466, 669), as well as AF (338). Several reports have also found a link between an abrupt increase in intra-thoracic pressure, due to coughing (135) or during the Valsalva manoeuvre (648) and termination of ventricular tachyarrhythmias.

The potential for extracorporeal mechanical stimulation for termination of tachyarrhythmias, on the other hand, received little attention until the 1970s, when a paper detailing the use of precordial thump (a single forceful blow to the lower half of the sternum using the lateral aspect of a closed fist) to defibrillate the tachycardic heart was published (470). It has been shown since that precordial thump may be used in some settings to terminate a host of ventricular tachyarrhythmias, as reported in case reports and uncontrolled studies (summarised in (469), with additional reports since (266, 560, 624, 656)).

Few prospective studies of precordial thump have been published, all of which demonstrated extremely low success for termination of ventricular tachyarrhythmia (below 2%; (11, 82, 216, 467)). In contrast, precordial thump applied to the heart in primary asystole may make relevant contributions to restoration of spontaneous circulation in patients (467) (as discussed in ‘II.D.2. Bradycardia and Asystole’). It is important to note that the clinical utility of precordial thump in emergency settings is a function of time-since-collapse, as all reported successful cases of precordial thump-induced cardioversion occurred early during the development of ventricular tachycardia or in early VF (21, 35). Animal models of precordial thump have shown a matching disparity of results, with success rates ranging from 0% in an asphyxiated dog model of VF (equivalent to very late application; (675)) to 95% in a post-infarction pig model (198), suggesting that the utility of precordial thump may be inversely related to myocardial tissue energy availability.

Computational modelling has helped in understanding probable mechanisms of successful precordial thump. In these models, successful precordial thump interrupts tachyarrhythmias by stretch-induced excitation of cells in the excitable gap(s), which obliterates re-entrant activity and results in return to sinus rhythm if no re-entrant waves survive or are created (310) (discussed further in ‘IV.B.3. Modifying and Terminating Arrhythmias’). However, when the heart is severely ischaemic, as will often be the case in out-of-hospital VF, the mechanical augmentation of metabolically pre-activated adenosine triphosphate (ATP)-sensitive K\(\text{+}\) current (\(I_{\text{K,ATP}}\) can
account for the reduced efficacy of precordial thump (310) (discussed further in ‘IV.B.2. Triggering and Sustenance of Arrhythmias’).

2. Bradycardia and Asystole

One of the first reports in the Western medical literature of the anti-arrhythmic effects of precordial mechanical stimulation was published in 1920, when Eduard Schott demonstrated that rhythmic fist thumps, applied to the precordium (now commonly referred to as ‘precordial percussion’, ‘percussion pacing’, or ‘fist pacing’), each triggered competent ventricular contractions, which maintained patient consciousness during acute Stokes-Adams attacks (disturbances in atrio-ventricular conduction that decrease cardiac output and can give rise to loss of consciousness and death; (545)). Unlike the utility of precordial thump for termination of ventricular tachyarrhythmias, which has been generally disappointing, triggering contractions in the bradycardic or asystolic heart seems to work more reliably, so that the use of precordial percussion pacing to treat asystole in the emergency setting has been a well-received concept (396, 541, 661).

In a number of case reports (3, 41, 134, 156, 157, 164, 165, 199, 200, 396, 411, 445, 537, 619) percussion pacing has been shown to be relatively effective in triggering electrical activation and competent contractions in the bradycardic or asystolic heart. In the few case series of precordial percussion pacing reported in the literature, a total of 139 patients have been mechanically paced, with a 93% success rate (306, 692, 701). Similarly, finger-tapping of the heart is generally an effective means for cardiac surgeons to restore rhythmic contractile activity while weaning the heart from cardio-pulmonary bypass, especially when electrical defibrillation attempts have put the heart into asystole.

Experimental studies of the asystolic post-electrical defibrillation shock period (377) or of cardiac standstill due to complete atrioventricular block (637) have also demonstrated that percussion pacing is a relatively effective means of mechanically stimulating heart beats (FIGURE 10). It has also been shown that passive chest compressions, applied for cardio-pulmonary resuscitation, can lead to ventricular excitation, resulting in active cardiac contractions (451, 452). However, since its inception, interest in percussion pacing has always been contrasted by questions about its utility (675, 702), fuelled by a lack of prospective clinical studies (469) and mechanistic explanations.

Overall, it appears that single or serial precordial thump (often called precordial percussion) may have some utility in the asystolic or severely bradycardic heart. Importantly, ventricular
contractions resulting from mechanically-induced excitation (which triggers active contraction) are haemodynamically more productive than external chest compressions (which generate circulation by passive ventricular ejection): cardiac output is 77% of baseline for mechanically-induced excitation, compared to 38% of baseline even with optimally performed chest compressions (98, 262). Thus, percussion pacing could have some utility in maintaining adequate circulation in the asystolic heart in emergency setting.

![Figure 10. Precordial fist thumps for mechanical pacing. A: Technique of percussion pacing, using short sharp blows with the ulnar side of the clenched fist from a height of about 30 cm to the lower left sternal edge. [From (200).] B: Termination of ventricular fibrillation by external defibrillator shock in an anesthetised pig, followed by a single premature ventricular contraction and two seconds of asystole. A series of chest thumps then results in active ventricular depolarisation (electrocardiogram Lead 2; top row) and left ventricular (LV) contractions (LV pressure; bottom row). [From (377).]](image)

Based on the potential of precordial mechanical stimulation as a rapid and non-invasive means of cardiac pacing, several techniques for applying mechanical stimuli to the heart have been developed (488). In 1976, pacemaker, defibrillator, and resuscitation pioneer Paul Zoll developed a device for temporary mechanical pacing (the “Cardiac Thumper”) (700), which was effective in evoking repetitive heartbeats in patients with asystole after VF, with AF, or with atrioventricular block, as well as in dogs with normal sinus rhythm or atrioventricular block (701) (FIGURE 11). Other mechanical stimulation devices have been devised, including patents for an extracorporeal mechanical pacer that stimulates the heart via pressure waves applied to the precordium (228), and an implantable mechanical defibrillator that applies a mechanical shock to the heart by a piezo-transducer generated pressure wave transmitted through a hydraulic line to a balloon-head in contact with the myocardium (233).
While precordial percussion is an immediately accessible form of extracorporeal pacing that is potentially well-suited for out-of-hospital emergency settings, more recent device-based efforts have focused on the use of extracorporeal high-intensity focused ultrasound (319) as a potentially more controlled means of externally applying mechanical stimuli to specific regions of the myocardium, over longer periods. The bio-effects of ultrasound have been extensively studied (motivated by the assessment of its safety for use in echocardiography), and are dependent on tissue properties (e.g., density, attenuation, absorption), exposure (e.g., frequency, intensity, pulse duration / duty cycle), and beam configuration (137). Ultrasound-induced tissue deformation can occur as a result of acoustic radiation force (a consequence of momentum transfer from the ultrasound wave to the tissue) which can lead to excitation of the heart - one of the known side-effects of high-intensity focused ultrasound lithotripsy (121, 138, 149, 162, 207, 286, 665, 667). The first report of the excitatory effects of ultrasound on the heart came from E. Newton Harvey in 1929, who noted that in frog and turtle hearts high frequency ultrasound caused an increase in HR or the resumption of regular beating of an otherwise quiescent ventricle (226). Subsequent studies have shown similar ultrasound-induced excitation in frog (139), mouse (376), and rat (229) hearts, as well as cultured neonatal ventricular cardiomyocytes (177), which, as for direct mechanical stimulation, appears to be driven by activation of SAC (324). Short periods of mechanical pacing by repetitive high-intensity focused ultrasound-induced have also been used for excitation in pigs with hypoxia-induced bradycardia (613), in anesthetised rats (368), and in ex vivo and in vivo pig hearts, occasionally using intra-ventricular contrast agents to enhance energy transfer (387) (FIGURE 12, A).
Beyond precordial percussion and high-intensity focused ultrasound, there have been reports of the use of time-varying magnetic fields to excite the heart, which by-and-large has proven to be impractical due to high energy requirements and unreliable pacing capture \((63, 260, 418, 653, 676-678)\). These have inspired an alternative approach for the delivery of cardiac mechanical stimulation using electromagnet-manipulated intravenously-injected magnetic microparticles \((528)\). Using an electromagnet to localise intravenously-injected magnetic microparticles in the ventricles, and then periodically forcing them against the myocardium using an alternating magnetic field, allowed mechanical pacing in \textit{ex vivo} and \textit{in vivo} rat hearts, as well as \textit{in vivo} in pigs \((\text{FIGURE 12, B})\).

![Figure 12. Mechanical pacing. A: Electrocardiogram from right atrium (RA) and left ventricle (LV) and haemodynamic recordings (LV pressure) of ultrasonic LV pacing in a pig isolated heart, showing that upon ultrasound application LV excitation and pressure development precede atrial excitation. [Adapted from (387).] B: Arterial pressure (blue line) and the current through an electromagnet coil (red line) during mechanical pacing using electromagnet-manipulated intravenously-injected magnetic microparticles in an anesthetised pig (+ signs indicate pacing capture). [From (528).]}

3. Advantages and Limitations of MEC-based Anti-Arrhythmic Interventions

The above discussion highlights the potential utility of mechanical pacing for the asystolic or severely bradycardic heart. Perhaps most importantly for its use, by triggering active contractions, mechanical pacing generates a greater stroke volume than external chest compressions that passively squeeze blood from the cardiac chambers \((98, 262)\). On top of this, extracorporeal mechanical pacing is more targeted, has low energy requirements \((0.04-1.5 \text{ J} (701))\), compared to 150 J or more that are used for electrical defibrillation \((572)\), and it is less painful than transthoracic (transcutaneous) electric stimulation (the alternative available method for temporary extracorporeal pacing), so it has some advantages that ideally one would wish to garner in the clinical setting. For instance, due to nerve and skeletal muscle activation causing

(39)
painful spasms, transthoracic electric stimulation typically necessitates the use of a sedative or aesthetic agent, which may further impair the critical haemodynamic condition of a patient (416).

However, mechanical pacing is not without its own limitations. High-intensity focused ultrasound has been shown to cause cell and tissue damage (352, 353, 400-402, 690), and direct mechanical stimulation may give rise to contusional effects (117, 492, 494), so stimulation energy levels must be carefully considered and well controlled (not usually possible with manual application – though the maximum energy most physicians will be able to apply by fist thumps from the recommended 30 cm height is below 10J (468)). Another major concern for cardiac mechanical stimulation is the potential for the induction of sustained arrhythmias (discussed in ‘II.C. Induction of Sustained Arrhythmias’). Precordial thump, for instance, has been shown to carry a risk of causing rhythm deterioration (425, 559) and, while rare, ventricular tachyarrhythmias have been reported to occur with precordial percussion (306), high-intensity focused ultrasound (when used with an intra-ventricular contrast agent) (387), and even with chest compressions (451, 452). Thus, in applications of mechanical pacing, timing relative to any underlying rhythm should be considered – as is common for cardiac electrical stimulation. For mechanical pacing with magnetic microparticles, there are additional concerns relating to particle biocompatibility and their excretion, as well potential coronary blockage and vascular embolism (528).

Another important consideration for the utility of mechanical pacing is the loss of capture that has been observed in many studies upon repeat application of mechanical stimuli. High-intensity focused ultrasound-based mechanical pacing in anesthetised rats, for example, was effective for a maximum of 7 consecutive stimuli, despite low pacing rates (once per breathing cycle (368)). Mechanical pacing in the presence of magnetic microparticles in the heart was marginally more successful, with ~30 stimulated beats in anesthetised rats and pigs before loss of 1:1 capture occurred (528) (FIGURE 12, B). Even though fist-pacing has been reported to be possible over longer periods in severely bradycardic patients (described in ‘IV.D.2. Bradycardia and Asystole’), the published case reports generally did not monitor whether 1:1 capture was indeed sustained, and in many cases, treatment was interspersed with periods of spontaneous circulation, so the sustainability of mechanical pacing in human is currently unknown.

The apparent lack of sustainability of mechanical pacing in the above studies was attributed to a loss of magnetic microparticles (528) or contrast agent (when used to enhance the effects of high-intensity focused ultrasound-based stimulation) (387) at the pacing site, or to disruption of myocyte homeostasis (such as a mechanically-induced increase in intra-cellular Ca^{2+} levels)
Studies of mechanical pacing by gentle mechanical contact with the epicardium of ex vivo hearts have corroborated a loss of capture (494, 696) and demonstrated that this effect is pacing rate dependent, suggesting that loss of mechanical stimulation efficacy may be a fundamental biological limitation of mechanical stimulation itself (494) (FIGURE 13). While the mechanisms for the loss of capture with repetitive mechanical stimulation have not yet been identified, loss of capture appears to relate to an MEC adaptation period during which mechanical, but not electrical, excitability is reduced. As capture with mechanical stimulation is restored after a period of normal sinus rhythm, it appears mechanical and electrical stimulation are limited by different types of ‘refractoriness’. This concept is supported by: i) ex vivo studies of intra-ventricular balloon inflation (151) and stimulation of myocyte monolayers by fluid jets (320), in which repeat stimulations were effective only after periods of rest up to 1 min for full recovery of mechanically-induced excitability; and ii) in vivo studies of repetitive local ventricular stimulation that demonstrated a decrease in the effective refractory period with electrical but not mechanical stimulation (18), in which mechanical stimulation during the (electrically-established) relative refractory period resulted in excitation only with every second or third mechanical stimulus (71).

![Figure 13. Loss of mechanical pacing capture.](image)

Figure 13. Loss of mechanical pacing capture. A: Electrocardiogram (ECG, top curve) and left ventricular pressure (LVP, bottom curve) recorded from an isolated Langendorff-perfused rabbit heart during sinus rhythm, followed by a train of focal left-ventricular mechanical stimulations (MS; see short pressure spikes preceding mechanically-induced contractions, bottom curve), with a transient period of 1:1 capture, followed by return to sinus rhythm with intermittent MS beats. B: Effect of varying rate of MS on the number of stimulations to loss of 1:1 capture. [Adapted from (494).]

There are several potential mechanisms that could account for mechanical refractoriness that is distinct from electrical refractoriness, including effects of mechanical stimulation on tissue mechanical properties, ion distributions in cardiac cells, or SAC or stretch-modulated ion channel activity.
Mechanical stimulation is known to affect myocardial mechanics. Repeated axial stretch of ventricular myocardium (by 5 - 15%) has been reported to cause a small decrease in muscle stiffness, which recovers after ~30 s of rest (302). This apparent viscoelastic effect could contribute to formation of an MEC adaptation period, especially considering that loss of capture with mechanical pacing is accelerated as stimulation frequency is increased. On the other hand, a possible role for changes in cellular ion balance(s) in the loss of mechanical pacing capture, specifically via mechanical modulation of Ca^{2+} handling (81), could be based on an acute stretch-induced increase in localised SR Ca^{2+}-release events (‘Ca^{2+} sparks’), which may reduce SR Ca^{2+} levels (190, 257, 258, 474, 481, 482), or on Ca^{2+}-release from mitochondria, whose intra-organelle Ca^{2+} concentrations may also be affected (36, 37, 405, 412). If Ca^{2+} is involved in mechanically-induced excitation, then a depletion of mechanically releasable sub-pools of Ca^{2+} could affect the efficacy of mechanical stimulation. Stretch-induced Ca^{2+}-release from the SR may result either from direct mechanical stimulation of RyR (258) or arise via effects mediated by mechanically-stimulated reactive oxygen species (ROS) production (481). Both mechanisms could be affected by the frequency of cyclic mechanical stimulation, which could help to explain the decrease in the number of mechanical stimulations before a loss of pacing capture when stimulation rate is increased (494).

Perhaps the most convincing potential mechanism contributing to pacing loss, however, is the fact that SAC_{NS} themselves show ‘mechanical refractoriness’ in the heart (i.e., the first response to stretch is larger than subsequent ones). This is supported by the observation in cardiac cells that repeated mechanical stimulation causes a cumulative reduction in I_{SAC,NS} due to channel inactivation, unless stimulations are spaced minutes apart (48, 49, 242). At the whole heart level, this reduction in I_{SAC,NS} results in a continuously increasing delay between mechanical stimulation and excitation with successive stimuli (696). Although it has not been studied in cardiac myocytes, mechanically-induced current through Piezo channels has been shown to decrease with repetitive stimulation (e.g., in HEK293t cells expressing the ion channel). In sensory dorsal root ganglion neurons this leads to a stimulation frequency dependent loss of mechanically-induced excitation (349), as seen with mechanical pacing in the heart. SAC ‘desensitisation’ or ‘rundown’ is in fact a broadly reported phenomenon and a common observation in patch clamp studies. It has been observed, for instance, in the 2-pore K^{+} domains in a weak inwardly rectifying K^{+} (TWIK)-related K^{+} channel-1 (TREK-1) (237). A similar use-dependent decrease in SAC could be responsible for the inverse dependence of the
number of mechanical stimulations to a loss of pacing capture on the mechanical stimulation rate.

Even though it appears that 1:1 capture may not be maintainable for extended periods of repetitive mechanical stimulation at physiological rates, it is possible that mechanical pacing is effective at rates below normal sinus rhythm, accounting for clinical case reports on its utility in patients with bradycardic or asystolic hearts, who have been kept conscious during prolonged ventricular asystole for periods of close to 3 hours (3).

4. Summary

Precordial mechanical stimulation, whether by precordial impact, high-intensity focused ultrasound, or other device-based means, can cause excitation of the heart and may hold important therapeutic potential for temporary pacing or, less compellingly, tachyarrhythmia termination in emergency settings. Mechanical rhythm management is not without limitations, however, including lack of sustainability, safety, and ethical (hitting a patient) concerns. Based on the 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations (440), current International Liaison Committee on Resuscitation (ILCOR) (321, 582) and American Heart Association (74, 362) guidelines state that: "precordial thump may be considered for patients with witnessed, monitored, unstable ventricular tachyarrhythmias, including pulseless ventricular tachycardia if a defibrillator is not immediately ready for use" (74), but "should not be used for unwitnessed out-of-hospital cardiac arrest" (74). The need for further research into the utility of mechanical heart rhythm management in severe bradycardia is highlighted as follows: “there is insufficient evidence to recommend for or against the use of the precordial thump for witnessed onset of asystole caused by atrioventricular conduction disturbance” (321). Regarding percussion pacing, the guidelines state that: “fist pacing may be considered in haemodynamically unstable bradyarrhythmias until an electric pacemaker (transcutaneous or transvenous) is available” (582), but that “there is insufficient evidence to recommend percussion pacing during typical attempted resuscitation from cardiac arrest” (96). These recommendations reflect a paucity of prospective data and a general lack of understanding of the efficacy, limitations, and mechanisms of mechanical rhythm management, justifying future study of its optimisation and potential clinical utility in particular in asystole and severe bradycardia – the rhythm disturbance for which precordial thump was originally reported.
C. Knowledge Gaps and Future Directions

- The critical characteristics of mechanical stimulation, such as timing, force versus deformation, rate, and site of application are largely unexplored, deserving further exploration.
- The mechanisms and importance of SAN mechano-sensitivity for maintaining normal sinus rhythm, and mechanical contributions to SAN dysfunction with age and disease, are unknown; this, and the interplay of $V_m$, $\text{Ca}^{2+}$, and mechanics oscillators in sustaining SAN activity and autoregulation warrant further investigation in experimental models and human studies.
- For ventricular mechanical stimulation, observed responses include a wide variety of electrophysiological changes, but the source of this variability is unclear; aspects relating to differences in species, mechanical stimulation characteristics, or underlying physiology will need to be explored.
- The potential role of non-myocytes (such as fibroblasts, macrophages, or intracardiac neurons) in MEC responses is just emerging, and much remains to be explored; the use of innovative targeted technologies, such as optogenetics, holds promise for addressing this new frontier.
- While hallmark examples of the arrhythmogenic and anti-arrhythmic potential of MEC in the acute settings have driven conceptual and computational model development to link molecular and clinical observations, mechanistic insight into MEC-mediated behaviour in chronic diseases, their mechanisms of action, and potential for therapeutic exploitation, have not been established; this is a critical area for targeted investigation.
- MEC contributions to anti-arrhythmic interventions seem most relevant in the asystolic or severely bradycardic heart. The source of the observed loss of mechanical trigger efficacy with repetitive stimulation is unknown; further investigations are essential for the clinical translation of mechanically-based pacing.

III. MOLECULAR MECHANISMS OF MEC

A. Pacemaker Cells

1. Mechano-Sensitivity of ‘Mechanical Oscillator’ Components

   In the microelectrode recording experiments of Klaus Deck (FIGURE 14, A), the increase of spontaneous diastolic depolarisation and beating rate during SAN stretch were accompanied by a decrease in the absolute values of both maximum diastolic and maximum systolic potentials
In other experiments, the need for a minimum mechanical preload to establish rhythmic SAN excitation also involved a progressive reduction in the absolute value of the maximum diastolic potential with stretch, which resulted initially in the appearance of sub-threshold oscillations of $V_m$, followed by spontaneous beating (337) (FIGURE 3, A). Isolated Purkinje fibres also responded to stretch with diastolic depolarisation, which, once it exceeds a certain level, gives rise to arrhythmic AP generation, followed by loss of excitation (292)).

These findings helped in narrowing the range of plausible molecular mechanisms involved in the chronotropic response to stretch, as any components affecting $V_m$ would be expected to have their reversal potential ($E_{rev}$) somewhere between maximum diastolic and systolic potentials. Initial targeted electrophysiology studies used positive pressure inflation (via the

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**Figure 14. Stretch-induced increase in SAN beating rate.** A: Intra-cellular sharp electrode recordings of transmembrane potential (top) and applied and generated force (bottom; passive stretch and active contraction pointing upwards) in cat isolated sinoatrial node tissue, showing an increase in beating rate during stretch, combined with a reduction in absolute values of maximum diastolic and maximum systolic potential. [From (147).] B: Axial stretch, applied to a spontaneously beating rabbit sinoatrial node cell using a pair of carbon fibres (scale bar $= 10$ mm). C: Patch-clamp recordings of transmembrane potential showing a stretch-induced increase in spontaneous beating rate of the pacemaker cell, accompanied by a reduction in the absolute values of maximum diastolic and maximum systolic potential (light curve $= $ before stretch, dark curve $= $ during stretch). [From (120).] D: Whole-cell stretch-induced current (I) / voltage (V) relation (I is the difference current in absence versus presence of streptomycin to block cation-nonselective stretch-activated channels, normalised to cell capacitance) from rabbit isolated sinoatrial node cells, showing a reversal potential of $\sim 11$ mV (dotted lines $= 95\%$ confidence limits). [From (120).]
patch pipette in whole-cell mode) of rabbit isolated SAN cells, which activated the swelling-activated chloride current ($I_{\text{Cl,swell}}$) \(^{(213)}\), as well as $I_{\text{Ca,L}}$ \(^{(389)}\). With an $E_{\text{rev}}$ near 0 mV in cardiac myocytes, $I_{\text{Cl,swell}}$ could theoretically account for the observed stretch-induced changes in SAN electrophysiology. However, there is a time-lag between the onset of cell swelling and activation of $I_{\text{Cl,swell}}$ (usually exceeding 1 min), rendering it too slow for the near-instantaneous changes upon acute stretch. Additional studies using hypo-osmotic swelling of spontaneously beating rabbit SAN cells in perforated patch mode demonstrated a reduction, rather than the expected increase, in beating rate \(^{(343)}\). These experiments were accompanied by computational simulations suggesting that the decrease in beating rate is caused by cytosol dilution \(^{(343)}\). It was shown that this effect, via a decrease in intra-cellular K\(^+\) concentration, could have reduced the rapid delayed rectifier K\(^+\) current, shifting the maximum diastolic potential towards more depolarised levels and reducing the hyperpolarisation-activated depolarising ‘funny’ current $I_f$, as confirmed experimentally in voltage-clamped SAN cells \(^{(343)}\).

It should be noted that cell inflation, whether by positive pressure inflation or swelling, is mechanically different from axial stretch, as swelling is associated with an increase in cell diameter and negligible changes in length. In contrast, axial stretch causes cell lengthening, a reduction in diameter (as cell volume is understood to remain constant during acute length changes), and an increase in beating rate of spontaneously beating rabbit SAN cells \(^{(120)}\). Even in isolated cells, this is accompanied by a reduction in the absolute values of maximum diastolic and maximum systolic potential, as seen in SAN tissue (FIGURE 14, B AND C).

Subsequent $V_m$-clamp studies revealed that stretch of single SAN cells indeed gives rise to a stretch-activated current with an $E_{\text{rev}}$ near -11 mV \(^{(120)}\) (FIGURE 14, D). This current is compatible with SAC\textsubscript{NS} \(^{(133, 209)}\) (FIGURE 15), whose block indeed causes a reduction of the chronotropic response to SAN stretch \(^{(119)}\).

Interestingly, while in larger mammals with inherently slow background HR the chronotropic response to SAN stretch is ‘positive’ (increase in beating rate), in smaller mammals such as mouse and rat with resting HR of 600 bpm or more, SAN stretch can decrease beating rate \(^{(119)}\). Perhaps counterintuitively, both responses may be accounted for by activation of SAC\textsubscript{NS}. In mammals with slower background HR, the SAN AP is characterised by a relatively slow AP upstroke (carried mainly by $I_{\text{Ca,L}}$) and a relatively prominent plateau-like early repolarisation phase, while mammals with faster HR exhibit faster upstrokes (often carried by a mix of Na\(^+\) and Ca\(^{2+}\) currents \(^{(344)}\)) and swift initial repolarisation, giving rise to a more spike-like AP shape.
Consequently, ‘slower’ SAN AP spend the majority of each cycle moving their $V_m$ towards the $E_{rev}$ of SAC$_{NS}$, while ‘faster’ SAN AP spend a larger proportion of time moving their $V_m$ away from it (FIGURE 16). Thus, activation of $I_{SAC,NS}$, which ‘pulls’ $V_m$ in the direction of its $E_{rev}$, would be expected to increase slower and reduce faster beating rates (118), a concept that has been supported quantitatively by computational modelling (120) (discussed in ‘IV.A.1. Consequences of SAC Activation’). This concept is also supported by recent experimental data from zebrafish, whose HR (~120 bpm) is closer to that of mammals larger than mice, and whose SAN AP show a relatively slow upstroke and a prominent plateau: stretch of the zebrafish SAN causes a stretch-amplitude dependent increase in beating rate (375). The difference in the chronotropic stretch response between mouse and other mammals may also relate in part to species-specific structural and mechanical SAN properties, in particular distinct collagen architecture and acute changes that occur with stretch.
2. Mechano-Sensitivity of $V_m$ Oscillator Components

Quantitative plausibility is no substitute for experimental validation (498), and SAN stretch responses might also result from direct effects on stretch-modulated components of the $V_m$ and/or Ca$^{2+}$-oscillators (summarised in FIGURE 2, C). In cell expression systems, mechanical stimulation increases the amplitude (88) and both activation and deactivation rates (358) of $I_f$. Interestingly, these effects are dependent on background beating rate: when membrane patches are AP-clamped to cyclic SAN or Purkinje cell AP waveforms, membrane deformation causes an increase in $I_f$ at higher, and a decrease at lower beating rates, which would be expected to have opposite effects to the observed direction of stretch-induced chronotropic effects. Similarly, $I_{Ca,L}$ but not $I_{Ca,T}$ (77, 373, 594), several K$^+$ currents (via Kv1, Kv3, Kv7, KvCa) (415), and the Na$^+$ window current (50) have been shown to be mechanically modulated. This means that their opening probability is sensitive to the mechanical environment, even though stretch per se is not normally sufficient to trigger channel openings on its own. In the beating heart, $I_{Ca,L}$ and the Na$^+$ window current could be involved in accelerating SAN (390, 409) and Purkinje fibre pacemaking rates (173, 270, 422, 450). In pathological settings associated with myocardial ischaemia, $I_{K,ATP}$ may also become important in the mechanical modulation of pacemaker function (51). This current, whose activity – if pre-activated by a reduction in ATP-levels – is increased by stretch in atrial and ventricular myocytes (627, 628), has been shown to be present in rabbit isolated SAN

(48)
cells (220). Activation of $I_{\text{K,ATP}}$ in the ischaemic SAN could be expected to hyperpolarise $V_m$, opposing spontaneous diastolic depolarisation and, potentially at least, the positive chronotropic effect of stretch, yet the ultimate effect on beating rate is unclear, as SAN beating rate is set by competing changes in several current systems whose partially reciprocal dependence on the maximum diastolic potential can stabilise beating rate (such as $I_f$ and $I_{b,Na}$ (438)).

3. Mechano-Sensitivity of Ca$^{2+}$ Oscillator Components

Stretch-effects on intra-cellular Ca$^{2+}$ handling may also modulate SAN beating rate. Stretch has been shown to directly affect intra-cellular Ca$^{2+}$ handling in cardiac cells (5, 81, 480, 602). In keeping with a contribution of these mechanisms to beating rate regulation, it has been reported that the response to stretch is reduced by interventions that decrease SR Ca$^{2+}$ content by lowering extracellular Ca$^{2+}$ concentration or blocking sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (14), inhibit SR Ca$^{2+}$ release by RyR block (14), or decrease trans-sarcolemmal Ca$^{2+}$ fluxes, such as upon $I_{\text{Ca,L}}$ block (230). As mentioned above (in ‘II.D.3. Advantages and Limitations of MEC-based Anti-arrhythmic Interventions’), axial stretch of ventricular myocytes causes an acute increase in Ca$^{2+}$ spark rate (258) (FIGURE 17). In ventricular cells, this may further involve mechanically-induced mitochondrial Ca$^{2+}$ release through mitochondrial $I_{\text{NCX}}$ (36, 37, 405, 412). If similar stretch-effects on Ca$^{2+}$ handling are present in SAN cells, they could be relevant for mechanical modulation of SAN activity, as changes in SAN intra-cellular Ca$^{2+}$ concentration (682) or mitochondrial $I_{\text{NCX}}$-mediated changes in Ca$^{2+}$ spark rate (683) may affect beating rate.

![Figure 17](image-url)
In addition, there may be secondary effects of altered Ca\(^{2+}\) handling through store-operated Ca\(^{2+}\) channel current (an inward Ca\(^{2+}\) current whose magnitude depends on the level of depletion of SR Ca\(^{2+}\) stores (548)). While not generally included as a component of the Ca\(^{2+}\)-oscillator, this current has been implicated in modulation of SAN beating rate, both directly via a background inward current that is modulated by beat-by-beat changes in SR Ca\(^{2+}\) content (277), and through secondary effects via electrogenic I\(_{NCX}\) activity (276). Moreover, it has been suggested that the store-operated Ca\(^{2+}\) channel may be accounted for by transient receptor potential (TRP) canonical protein (TRPC) expression (277). Since type 1 of TRPC has been suggested to underlie SAC\(_{NS}\) (386), it could play a much more important role in direct mechanical effects on cardiac pacemaker activity than hitherto appreciated.

4. Summary

Mechanical activation of SAC\(_{NS}\) can explain most aspects of the chronotropic response to SAN stretch. Stretch causes an increase of spontaneous diastolic depolarisation and a decrease in the absolute values of maximum diastolic and maximum systolic potentials, due to the \(E_{\text{rev}}\) of SAC\(_{NS}\) between -20 and 0 mV. As a result, beating rate is increased with stretch in most animals. In species with high HR and fast early repolarisation such as mouse, however, beating rate can be decreased with SAN stretch, even though this response may be explained by the same molecular mechanism – SAC\(_{NS}\) activity. While SAC\(_{NS}\) can explain most aspects of the chronotropic response to SAN stretch, there may be important contributions of mechanical effects on components of the \(V_m\) and Ca\(^{2+}\) oscillators. Many factors involved have been shown to be subject to mechanical modulation, including various ion currents (\(I_i\), \(I_{Ca,L}\), the \(Na^+\) window current, and several \(K^+\) currents) and intra-cellular Ca\(^{2+}\) handling, although most of the related evidence comes from other cardiac cell types or expression systems, so direct confirmation of their involvement in SAN mechano-sensitivity is missing.

B. Working Cardiomyocytes

1. SAC\(_{NS}\)

    Stretch-induced changes in \(V_m\) of working cardiomyocytes can be explained by \(I_{SAC,NS}\) (FIGURE 5A). Due to an \(E_{\text{rev}}\) at levels that are somewhere half-way between peak AP and resting \(V_m\), activation of \(I_{SAC,NS}\) depolarises \(V_m\) in resting cells (132), causing after depolarisation-like events in isolated cardiomyocytes (308) and, if supra-threshold, premature excitation (521, 658). During the AP plateau, \(I_{SAC,NS}\) accelerates \(V_m\) repolarisation, causing a shortening of early APD (659, 688). As a result, AP shortening has frequently been observed with sustained stretch (50).
However, as the cell membrane repolarises and becomes more negative than the $E_{\text{rev}}$ of SAC_{NS}, this can give rise to late AP prolongation (693), potentially resulting in a cross-over of the repolarisation curve (687).

The role of SAC_{NS} in causing the typical stretch-induced changes in cardiac electrophysiology is further supported by studies using pharmacological blockers (657) (although results must be interpreted with caution, as discussed in ‘III.D.3. Considerations for the Use of Pharmacological Probes’). Gadolinium (Gd^{3+}) and amiloride, two non-specific blockers of SAC, have been shown to reduce the incidence of stretch-induced ectopy in ex vivo hearts (221, 245, 547), and to prevent stretch-induced changes in electrophysiology in myocyte monolayers (320), single myocytes (281, 556, 693), cardiovascular smooth muscle cells (670), and expression systems (217). More specific compounds have shown similar inhibition of stretch effects, such as streptomycin (20, 40, 150, 161, 192, 245, 320, 371, 423, 614, 644, 650), which at low-concentrations serves as a reasonably selective SAC blocker (657), and the selective SAC_{NS} inhibitor Grammostola spatulata mechanotoxin-4 (GsMTx-4(578)) (28, 430, 492, 578, 641).

SAC_{NS} have also been implicated in mechanically-induced ventricular arrhythmias. In the rabbit isolated heart model of Commotio cordis, it was shown that impact-induced excitation was dependent on SAC_{NS} (492), as it was blocked by GsMTx-4. This is in apparent contrast to results from an anaesthetised pig model of Commotio cordis, where streptomycin did not alter the probability of VF induction after precordial impact (193). The rabbit heart study, however, also showed no effect of streptomycin on mechanically-induced excitation (492), which may reflect the limited efficacy of streptomycin for acute SAC_{NS} block in native myocardium (119) (for a discussion of utility and limitations of SAC blockers, see ‘III.D.3. Considerations for the Use of Pharmacological Probes’).

During acute ischaemia, a role of SAC_{NS} in stretch-induced excitation has been suggested by computational modelling (274). This is in contrast to recent experimental reports, where application of Gd^{3+} (25) or GsMTx-4 (28) did not alter the incidence of arrhythmias in whole animal (pig) experimental models. The interpretation of data from pharmacological intervention in whole animals is challenging, of course, as the lack of an observation of an effect is not the same as observation of a lack of an effect, given that pharmacological agents used to target SAC have a number of restrictions that affect their utility in vivo (discussed further in ‘III.D.3. Considerations for the Use of Pharmacological Probes’). In particular, Gd^{3+} has been shown to precipitate almost completely upon interaction with anions in physiological buffers, causing a drastic reduction in its free concentration (83), so that it cannot be recommended for in vivo
MEC studies. In the pig experiments using GsMTx-4, the effective concentration at the level of cardiac cells would have been extremely low, given that the peptide is diluted not only in plasma (which would have yielded 170 nM, a concentration that has been found to be effective in some, but not all, in vitro studies), but also in interstitial fluid (which has a 3 times greater volume than plasma), yielding an effective concentration of GsMTx-4 of less than 50 nM; this may not have blocked SAC NS effectively.

Along with SAC NS, additional stretch-modulated mechanisms may contribute to arrhythmogenesis in acute ischaemia (239), such as the stretch-modulated $k_{\text{ATP}}$ (acting as a K$^+$-selective stretch-activated channel, SAC$_K$) (627, 628), sympathetic stimulation (241), or altered Ca$^{2+}$ handling (33). In particular, a role for mechanical augmentation of $k_{\text{ATP}}$ in ischaemia is supported by the fact that preventing paradoxical segment lengthening of affected tissue in a pig model of myocardial ischaemia using a mechanical splinting device delays K$^+$ accumulation in the ischaemic zone, and prevents electrophysiological changes such as APD shortening and occurrence of alternans (59).

In the atria, the importance of SAC NS for stretch-induced excitation is supported by experiments showing that Gd$^{3+}$, (which blocks $k_{\text{SAC,NS}}$ in isolated atrial cells; (698)) suppresses ectopy (599). Indeed, in stretch-augmented rapid pacing-induced AF models, Gd$^{3+}$, streptomycin, and GsMTx-4 all reduce AF inducibility (FIGURE 9, C), without affecting refractoriness (55, 56, 179, 436). Interestingly, the stretch-mediated increase in AF inducibility can be reduced by altering the fatty acid composition of cardiac cell membranes with dietary fish oil, possibly by changing physical membrane properties and altering mechanical stimulus transmission from macroscopic input (pressure/volume overload) to SAC and stretch-modulated currents as molecular sensors (434).

There may also be a contribution of stretch-induced excitation, mediated via SAC NS, in pulmonary vein automaticity, a key clinical contributor to AF. Here, stretch results in an increased incidence and rate of firing (278), which is blocked by Gd$^{3+}$ and streptomycin (100) (although with Gd$^{3+}$, simultaneous block of $k_{\text{Na}}$ may also contribute to suppression of excitation (350)). Streptomycin, on the other hand, can also block L-type Ca$^{2+}$ channels (39) and Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels may be one of the contributors to the decrease in refractoriness with atrial dilatation, as changes in refractoriness and AF inducibility are also prevented by block of $k_{\text{Ca,L}}$ (618, 691). Interestingly, a recent report has implicated caveolae-mediated activation of stretch-activated $I_{\text{Cl,swell}}$ as a critical cause of pulmonary vein automaticity with stretch, however
based on the considerations of a role of $k_\text{Cl,swell}$ in a stretch-induced increase in automaticity discussed above, this finding requires further investigation (163).

2. SAC$K$

While all of the known acute stretch-induced effects on cardiac electrophysiology in healthy myocardium can be reproduced in quantitative computational models by simply invoking $I_\text{SAC,NS}$, there is strong evidence supporting a contribution by SAC$K$, specifically in ATP-deprived tissue. During systole, activation of SAC$K$ (whose $E_{\text{rev}}$ is close to the reversal potential of $K^+$) would be expected to enhance APD shortening (297) and, if activated in resting cells, to hyperpolarise $V_m$. The latter has not been reported, which suggests that SAC$K$ do not dominate MEC responses in healthy cardiomyocytes. In ischaemia, there may be an additional contribution to APD shortening by the stretch-modulated $k_{\text{ATP}}$ (627, 628). $k_{\text{ATP}}$ has additionally been implicated in VF induction in the setting of Commotio cordis, as administration of the non-specific $I_{\text{K,ATP}}$-blocker glibenclamide reduced the incidence of VF-induction in pigs upon precordial impact (366). That said, under conditions of normal oxygen supply $k_{\text{ATP}}$ is inactivated (441) and not responsive to mechanical stimulation (627, 628) (in fact, the combined sensitivity to stretch and ATP-reduction may explain why to cause channel opening in vitro ATP concentrations must be reduced far more than might be expected to occur in vivo). In keeping with non-specific effects of the blocker used (glibenclamide) (19, 501), the decrease in the incidence of mechanically-induced VF may instead represent $k_{\text{ATP}}$-induced changes in repolarisation timing and refractoriness, causing a shift in the exceedingly narrow vulnerable window for mechanically-induced re-entry (492) and, hence, a potentially false-positive conclusion regarding glibenclamide effects on cardiac mechano-sensitivity. In the case of increased AF incidence with atrial dilatation, $K^+$ influx via SAC$K$ may contribute to decreased refractoriness and it has been shown that acidotic conditions, which amplify stretch activation of $K^+$ channels such TREK-1 (378), cause an additional reduction in refractory period and increase in AF susceptibility with atrial dilatation (436).

3. Mechano-Sensitivity of Intra-Cellular Ca$^{2+}$ Handling

Stretch directly affects intra-cellular Ca$^{2+}$ handling in cardiac cells. Stretch has been shown to acutely increase SR Ca$^{2+}$ release in guinea pig (257), rat (190, 258, 482), and mouse (481) ventricular myocytes, via either direct mechanical (258), ROS-mediated (481), or mitochondrial Ca$^{2+}$-related (36, 37, 405, 412) influences on RyR open probability, which may contribute to Ca$^{2+}$-induced after-depolarisations (182). This stretch-induced increase in SR Ca$^{2+}$ release may
be especially important in acute ischemia as it is enhanced, along with the stretch-induced increase in ROS production, in that setting (90). Intra-cellular free Ca$^{2+}$ is also acutely affected by a length-dependent change in the affinity of troponin C (TnC) for Ca$^{2+}$ (4), such that with increased stretch (4, 6) or tension (685) more Ca$^{2+}$ is in the bound state. Upon rapid shortening during relaxation, the dissociation of Ca$^{2+}$ from TnC causes a surge in intra-cellular Ca$^{2+}$ (638). In this period RyR can have sufficiently recovered to allow additional Ca$^{2+}$-induced Ca$^{2+}$-release from the SR (24), which can cause propagating Ca$^{2+}$ waves (FIGURE 18, A). Cellular excitation may then occur (141, 605, 639) by depolarisation of V$_{m}$ via electrogenic Ca$^{2+}$ removal through $I_{\text{NCX}}$ (408). It is important to note that in the given example, it is the relaxation of stretched muscle, rather than the stretch per se, that causes this Ca$^{2+}$-mediated arrhythmic trigger. Such response would be in keeping with the observation that Ca$^{2+}$ release is enhanced by increasing the rate of relaxation (639), independently of the involvement of SAC$_{\text{NS}}$ (407). Importantly, in the case of non-uniformly contracting myocardium, for instance with regional changes in contraction as occurs in ischaemia or long QT syndrome, acute mechanically-induced changes in Ca$^{2+}$ dynamics may give rise to arrhythmogenic Ca$^{2+}$ waves (406, 603, 604, 606). These waves themselves can then result in after-contractions that cause after-depolarisations in other cardiac tissue segments that may trigger tachyarrhythmias (188, 601) (FIGURE 18, B).

4. Summary

SAC$_{\text{NS}}$ can explain most electrophysiological MEC responses in working myocytes of the heart. With an $E_{\text{rev}}$ approximately half-way between peak AP and resting V$_{m}$, $I_{\text{SAC,NS}}$ can cause depolarisation and excitation in resting cells, while during the AP plateau it can accelerate early repolarisation and shorten APD. As a result, SAC$_{\text{NS}}$ are implicated in atrial and ventricular arrhythmogenesis, including settings such as AF, Commotio cordis, or acute ischaemia. These effects may be reduced or prevented by SAC$_{\text{NS}}$ blockers, but they must be used with careful consideration to avoid false conclusions. Other sub-cellular stretch-modulated components may also contribute to pro-arrhythmic stretch effects, including after depolarisations, ectopic excitation, and tachyarrhythmias, including SAC$_{\text{K}}$ (such as the stretch-modulated $k_{\text{ATP}}$) and intra-cellular Ca$^{2+}$ handling (via changes in SR Ca$^{2+}$ release and the affinity of TnC for Ca$^{2+}$).
C. Cardiac Non-Myocytes

1. SAC<sub>NS</sub>

While electrophysiological responses to stretch in native cardiac tissue can be explained generally by direct MEC effects on myocytes, they may also be mediated through mechano-sensitivity of electrically-coupled non-myocytes, such as macrophages (which have been shown to both express SAC<sub>NS</sub> (455) and electrically-couple to myocytes (247)) or fibroblasts (313). Fibroblasts are relatively depolarised cells that possess SAC<sub>NS</sub> (573). They have been shown to form structural connexin-based links with SAN cells (89) that support active electrototoxic coupling with cardiomyocytes (490, 529) in native tissue. In cell cultures, fibroblasts can act as current...
sinks that affect cardiomyocyte excitability, repolarisation, and conduction (305, 404), and they may serve as passive conductors between structurally separate myocyte groups (196, 205).

Stretch has been shown to cause fibroblast depolarisation (282, 315, 317), a response that is sensitive to pharmacological depletion or buffering of fibroblast intra-cellular Ca^{2+} (303), and that has been implicated in mechanically-induced electrophysiological changes in fibrotic cardiac tissue (610). A role for non-myocytes in stretch-induced electrophysiological responses may underlie the observation that the magnitude of mechanical effects on HR increases with structural complexity of the biological model: isolated SAN cells ~5%, whole-heart/SAN tissue ~15%, intact dog up to 30% (even though the inherent reduction in beating rate that occurs with more reduced preparations should have the opposite effect, i.e., the largest chronotropic response in isolated cells) (119). This phenomenon suggests a loss of contributory factors involved in transmission or sensing of mechanical stimuli as one moves towards more reduced biological model systems (which, in denervated preparations, will also include loss of autonomic nervous system contributions).

2. Summary
Mechano-sensitivity of non-myocytes, electrically coupled to cardiomyocytes, may contribute to MEC responses in the heart. Fibroblasts in particular have been shown to express SAC_NS and to electrically connect with cardiomyocytes, which may give rise to mechanically-induced changes in cardiac electrophysiology.

D. Molecular Candidates for SAC

1. SAC_NS
The first single channel recordings of depolarising \( I_{\text{SAC,NS}} \) were reported in 1984 by Falguni Guharay and Frederick Sachs, in cultured embryonic chick skeletal muscle (209). Since then, \( I_{\text{SAC,NS}} \) has been recorded in isolated ventricular myocytes from various mammalian species, including human (133, 281, 283, 539). This current has a near-linear current-voltage relationship (as is typical for weakly selective ion channels), a single channel conductance between 10 and 30 pS, and an \( E_{\text{rev}} \) somewhere between -20 and 0 mV. In contrast to atrial cells, where single-channel recordings of SAC_NS have been reported (300), SAC_NS channels in adult ventricular cardiomyocytes appear to be hidden from direct patch pipette access, for example in membrane regions of the transverse tubular system (244), caveolae, or at intercalated discs (264). Two principal families of candidates for SAC_NS have been identified: TRP and Piezo channels, both of
which have been shown to be expressed in the plasma membrane of numerous human and animal cell types (476) (FIGURE 19).

Most TRP channels found in mammals are cation non-selective, and depending on the specific channel, pass some combination of Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), K\(^{+}\), and Cs\(^{+}\). They are generally widely expressed, play a critical role in sensory physiology by enabling cells to sense changes in their local environment (including chemicals, temperature, osmolarity, vibration, and pressure). They can be activated by multiple factors, including mechanical stimuli (such as osmotic stress, shear force, and membrane stretch (255), although direct stretch activation of TRP channels is controversial (432)). Particularly interesting candidates for cardiac SAC\(_{NS}\) include members of the TRPC, TRP vanilloid (TRPV), TRP melastatin (TRPM), and TRP polycystic (TRPP) protein sub-families.

**Figure 19. A selection of the more well-known mechano-sensitive ion channels and receptors in different organisms.** Channels in red are expressed in the heart, underlined channels have been clearly identified as mechano-gated, while channels with an asterisk have no known mammalian homologues. Examples of mammalian channels with homologues in other organisms include: NOMP, OSM9, TRP4, TRPY1, and LOV-1, which are transient receptor potential homologues; MEC channels, which are members of the degenerin / epithelial sodium channel superfamily that in mammals are Asic; TPK which is a homologue of K\(_{ATP}\) channels; and Mid1 which is homologous to voltage-gated calcium channels. AchR, acetylcholine receptor; Asic, acid-sensing ion channels; BK, big potassium (K\(^{+}\)) channels; CFTR, cystic fibrosis transmembrane conductance regulator; CLC, chloride channels; K\(_{ATP}\), ATP-inactivated K\(^{+}\) channel; KCNQ, KQT-like voltage-gated K\(^{+}\) channel; LOV, location of vulva; Mid1, mating induced death; MCA, mechanosensitive Ca\(^{2+}\) channel; MSC, mechanosensitive channel of small conductance homolog in *Chlamydomonas reinhardtii*; MscA, K, L, M, MJ, S, mechano-sensitive channel of archaeon, K\(^{+}\), large, medium, *Methanococcus jannashii*, small conductance, mito, mitochondria; MSL, mechanosensitive channel of small conductance like; Msy, MscS homologues in fission yeast; NOMP, no mechanoreceptor potential C; NMDA, N-methyl-D-aspartate; SAC\(_{NS}\), cation-nonselective stretch-activated channel; SAC\(_{K}\), stretch-activated K\(^{+}\)-selective channel; SR, sarcoplasmic reticulum; TRAAK, TWIK-related arachidonic acid-activated K\(^{+}\) channel; TREP, TWIK-related K\(^{+}\) channel; TRPA, C, M, N, P, V, and Y, transient receptor potential ankyrin, canonical, melastatin, NOMP, polycystin, vanilloid, and yeast channels; TPK, 2-pore domain K\(^{+}\) channels; OSM, OSMotic avoidance abnormal family; TWIK, 2-pore K\(^{+}\) domains in a weak inwardly rectifying K\(^{+}\) channel. [From (476).]
In terms of candidates in the TRPC sub-family of channels, TRPC6 is highly expressed in the human heart (516), being localised in transverse tubules of mouse ventricular myocytes (160). Stretch activation of TRPC6 was first characterised in human embryonic kidney cells (566). Lack of mechano-sensitivity of TRPC6 expressed in Chinese hamster ovary (CHO) or monkey kidney fibroblast (COS) cell lines (206) may be attributed to the requirement for co-expression of angiotensin II receptor type 1 to yield SAC activity (255, 631). $I_{SAC,NS}$ in mouse cardiomyocytes during shear stress is reduced by pore-blocking TRPC6 antibodies or detubulation (160). TRPC3 expression has been shown in rat ventricular myocytes, where – like TRPC6 – it is found in the transverse tubular system (183). In mouse neonatal cardiomyocytes, the channel is involved in ROS production upon mechanical stimulation or application of 1-oleoyl-2-acetyl-sn-glycerol (OAG), a non-specific activator of SAC (183). Stretch activation of TRPC1 is controversial. It was initially demonstrated in Xenopus oocytes (386), however like for TRPC6, it was not confirmed in other expression systems (206), suggesting that this channel may also require the presence of a partner protein. Caveolin 1 may be a trafficking regulator of TRPC1 (254, 458). As caveolae can be integrated into the sarcolemmal surface membrane of ventricular cardiomyocytes in response to stretch (311, 477), TRPC1 mechano-sensitivity may be linked to stretch-induced changes in membrane topology, including knock-on effects on conduction velocity (due to a stretch-induced increase in membrane capacitance) rather than ion channel function of the protein.

Regarding candidates in other TRP sub-families, TRPV2, expressed in the mouse heart (264, 531), is activated by patch pipette suction and cell volume changes (420), and it has been proposed to contribute to Ca$^{2+}$-handling in cardiac cells (531). TRPM4 is expressed in cardiomyocytes of several species, including mouse, rat, and human (631), and it has been implicated in stretch-activated responses of vascular smooth muscle (414), though its physiological role in the heart is currently unknown. TRPP2 is primarily found on the endoplasmic/SR and in primary cilia (647), but a TRPP2-like protein seems to function as an ion channel in the sarcolema of rat ventricular myocytes (634), potentially acting as a modulator of RyR activity (13).

The discovery of Piezo channels (Piezo 1 and 2) constituted a breakthrough in the field of mechano-transduction (127). Stretch activation of Piezo 1 has been demonstrated with heterologous expression in human embryonic kidney cells, resulting in $I_{SAC,NS}$ (128). While Piezo has not been detected at the protein level in cardiomyocytes, and no functional data have been published for the Piezo in the heart at the time of writing (34), low level Piezo1 mRNA
expression (compared to lung, bladder, or skin) has been observed in mouse heart tissue homogenates (127). As Piezo current properties are similar to those of cardiac $I_{\text{SAC,NS}}$, including weak voltage dependency, single-channel conductance, inactivation, and sensitivity to GsMTx-4 (635), it is tempting to think that Piezo may indeed contribute to cardiac MEC. Whether this is through a direct involvement in cardiomyocyte function, or via non-myocyte mediated effects (which would be in keeping with the low-level tissue homogenate mRNA data) remains to be elucidated.

2. SAC$_K$

A whole cell SAC$_K$ current ($I_{\text{SAC,K}}$) in cardiac cells was first reported by Kim in 1992 (299). SAC$_K$ is outwardly rectifying, allowing K$^+$ to move out of the cell more easily than in, has a relatively large single-channel conductance (~100 pS), and inactivates in a time-dependent manner. Single-channel $I_{\text{SAC,K}}$ recordings have been made in adult mammalian atrial (299) and ventricular myocytes (597, 643). $I_{\text{SAC,K}}$ is thought to be carried primarily by 2 P-domain K$^+$ channels (K$_{2P}$) in the mammalian heart (367) (FIGURE 19).

One of the most studied K$_{2P}$ channel is K$_{2P2.1}$ (TREK-1). TREK-1 is active over a range of physiological $V_m$ and activated by a number of stimuli, including intra- and extra-cellular pH, temperature, fatty acids, anesthetics, and membrane deformation or stretch (46, 70, 236). TREK-1 in the rat heart is arranged in longitudinal stripes on the surface of cardiomyocytes, a pattern that could support directional stretch sensing (671). TREK-1 shows heterogeneous expression in rat heart, increasing transmurally from sub-epi to sub-endocardium (574, 596). This heterogeneity seems to correlate with transmural differences in mechanical sensitivity of myocardium, where stretch causes the most pronounced AP shortening in the sub-endocardium (203, 297). While TREK-1 mRNA expression has been reported in rat atria and ventricles (1, 367, 596, 607), it has not yet been identified in human heart (1, 211). Whole-cell currents exhibiting the characteristics of TREK-1, including sensitivity to internal acidification, anesthetics, and stretch, have been observed in atrial and ventricular myocytes of several mammalian species including rat, mouse, and pig (203, 544). TREK-1 contributes to the “leak” K$^+$ conductance in cardiomyocytes, aiding in repolarisation and diastolic stability (203, 463). During stretch, increased K$^+$ current could cause excessive, pro-arrhythmic AP shortening (297). TREK-1 can be particularly pro-arrhythmic in patients with channel mutations. In a patient with right ventricular outflow tract tachycardia, a heterozygous point mutation in the selectivity filter of TREK-1 has been identified, which increased Na$^+$ permeability and mechano-sensitivity of the channel (145). TREK-2 shares functional similarity with TREK-1, is expressed in rat atria (367) (59).
and appears active in chick embryonic atrial myocytes, yet little is known about its relevance in the heart. The TWIK-related arachidonic acid-activated K+ channel (TRAAM) is a TREK-1 homologue with similar biophysical properties and regulation. TRAAM is expressed in human heart and might form the human TREK-1 homologue, although its functional relevance has not yet been demonstrated.

There may also be a contribution to ISAC,K from big K+ (BK) channels, which are activated by various stimuli and which therefore have been given multiple names (e.g., SAKCA, BKCA, SLO1, MaxiK). BK channels have a large conductance (100 - 300 pS), are present in many cardiovascular cell types, including vascular smooth muscle and atrial and ventricular cardiomyocytes. They are found in the sarcolemma, as well as in membranes of the endoplasmic reticulum, the Golgi apparatus, and mitochondria. Stretch-activation of BK channels was observed in membrane patches excised from cultured embryonic chick ventricular myocytes. However, as BK channels are activated also by Vm changes and by intracellular Ca2+, it has been suggested that their mechano-sensitivity may be indirect, occurring secondary to stretch-induced changes in intracellular Ca2+ concentration caused by ISAC,NS. In terms of their physiological role, BK channels have been suggested to contribute to HR control and to offer cardio-protection during ischaemia, along with Ic,ATP.

3. Considerations for the Use of Pharmacological Probes

Pharmacological modulators are among the most effective tools available for investigating molecular mechanisms of MEC. The principal agents for this are blockers and activators of stretch-modulated sarcolemmal ion channels. The most widely used probes in the heart to date have been two types of non-specific inhibitors of ISAC,NS: lanthanides and aminoglycosidic antibiotics, which have been highly productive for experimental cell research in vitro. Caution is needed, however, with their use, as limitations can lead to false-positive or -negative conclusions.

Lanthanides, most commonly Gd3+, non-specifically reduce ISAC,NS at concentrations of 1 - 100 μM. Their mechanism of action is thought to be multi-site, involving open channel block of SACNS and screening of surface negative charges, which alters the properties of the lipid bilayer. In isolated cardiac cells they have been shown to block whole cell ISAC,NS in atrial and ventricular myocytes, and to block the increase in sub-sarcolemmal Ca2+ during cell prodding. However, in preparations where ISAC,NS cannot be isolated from other currents, interpretations of results is difficult. Within the concentration range used to inhibit ISAC,NS, Gd3+ is known to also inhibit Ic,LL (IC50 = 1.4 μM), INa (IC50 = 48 μM)
Another important consideration is that fact that Gd$^{3+}$ interacts with anions present in physiological buffers (HCO$_3^-$, CO$_3^{2-}$, HPO$_4^{2-}$, and PO$_4^{3-}$), forming precipitates that drastically reduce its free concentration (addition of 1 µM to solutions containing standard amount so phosphate and bicarbonate, results in a free concentration of less than 1 pM) (83). This can be a problem even with solutions employing synthetic buffers, if continually exposed to room air (as CO$_2$ dissolves in water to form carbonic acid), and it will certainly alter available free Gd$^{3+}$ in vivo.

Aminoglycosidic antibiotics are composed of two or more amino sugars joined to hexose by glycosidic links; they are thought to act on SAC by partial occlusion of the channel pore (666). The most commonly used compound is streptomycin. As for Gd$^{3+}$, streptomycin is not specific for SAC$_{NS}$ and it has been shown to also block Ca$^{2+}$ and K$^+$ channels (39), as well as to reduce Ca$^{2+}$ transients and contraction (38). In cardiac cells, it inhibits $I_{Ca,L}$ at relatively high concentrations (IC$_{50}$ = 1-2 mM), but not at concentrations that inhibit $I_{SAC,NS}$ (40 µM) (40), demonstrating a superior demarcation between inhibition of SAC and other currents compared to Gd$^{3+}$, at least in vitro. ‘Pure’ streptomycin has a molecular weight of 581.6 g/mol, but the most commonly available commercial form is streptomycin sulphate with a molecular weight of 1457.4 g/mol. This requires an extra level of attention, as streptomycin concentration may differ by a factor of three if the wrong molecular weight is used for calculation (sadly, clear information needed to replicate experiments is not always provided; (491)). A further consideration for the use of streptomycin is its limited utility for use in tissue (if streptomycin was an effective SAC blocker in vivo, it might not be suitable for prescription as an antibiotic). While it is an efficient blocker of SAC$_{NS}$ in isolated or cultured cardiac cells (40), it appears to have a limited efficacy in native myocardium (119). This limitation results in the need for higher concentrations for effects on stretch-induced responses (>200 µM) (161, 534) and a disparity of positive and negative results, some of which may have been caused by lack of effect or off-target actions (492). It is also important to note that streptomycin (and other aminoglycosidic antibiotics) are common components of standard cell-culture media, so caution is needed when interpreting studies on stretch effects in cultured cells, as background SAC$_{NS}$ availability may be reduced in these preparations. Thankfully, streptomycin seems to wash off reasonably well in vitro.

Among the more specific pharmacological agents for modulating SAC$_{NS}$ activity, the best-known is the 35 amino acid peptide toxin GsMTx-4 (64). In particular in its native (toxin-isolated) form, it has been shown to be a highly-potent and specific inhibitor of $I_{SAC,NS}$ (578). GsMTx-4 has been shown to effectively block the stretch-induced increase in AF inducibility at a concentration
of 170 nM (56) (2-3 orders of magnitude less than what is needed for similar effects by Gd³⁺ or streptomycin (55)), while showing no effects on AP shape at concentrations up to 4 μM (532). GsMTx-4 is an amphipath, having both hydrophobic and hydrophilic groups, and is thought to act by insertion into the outer leaflet of the sarcolemma in the proximity of SACNS, causing deformation of the bilayer and preventing mechano-sensing by the channel (579). This mechanism of action is not stereospecific or chiral, as both the D- and L-enantiomers of GsMTx-4 inhibit ISAC,NS (579). GsMTx-4 cDNA has been sequenced and a cloned 34 amino acid version of the wild-type toxin synthesised. In the hands of serval investigators, the synthesised version has been found to require much higher concentrations to inhibit ISAC,NS, potentially indicative of the difficulty in properly folding peptides ex vivo (453). It has been shown, though, that GsMTx-4 blocks the SACNS candidate proteins Piezo1 (22, 475) and TRPC6 (456, 566).

Specific activators (Yoda1 (334, 588) and Jedi (645)) and inhibitors (Dooku1 (171)) of Piezo1 are also now available, offering a potentially powerful new tool for determining whether Piezo1 is indeed a key player in cardiac MEC. Similarly, a number of activators and inhibitors of various TRP channels have been reported (174), which will be useful in probing the role of those ion channels in tissue- and organ-level MEC.

Another interesting class of pharmacological probes for investigating sub-cellular mediators of MEC responses are those that target the cytoskeleton (657), although results have been inconsistent. Cytochalasin D, which prevents actin polymerization, has been shown to cause an increase in SACNS in atrial myocytes (300), but similar results were not seen by others (698). Conversely, application of cytochalasin (280) and colchicine (which prevents microtubule polymerisation) (261) causes a reduction in SACNS in ventricular cells. On the other hand, the microtubule stabiliser paclitaxel has been shown to cause both an increase in the susceptibility to stretch-induced arrhythmias in rabbit isolated hearts (460) and a decrease during acute ischaemia in rat isolated hearts (93), while inhibiting the stretch-induced increase in AF inducibility (673). Colchicine, however, had no effect (151, 460). Thus, overall there is no general agreement regarding the role of the cytoskeleton in MEC effects. This lack of consensus may in part be due to the fact that, as with other pharmacological agents discussed above, actin and tubulin modulators are not specific to the cytoskeleton, and the degree to which they actually disrupt the cytoskeleton is rarely monitored (79). Also, the individual molecular components of the cytoskeleton may have their own, independent effects on intracellular Ca²⁺ handling and ion channel function (201, 202, 298), although this is debated (78, 80, 461).

4. Summary
While the electrophysiological consequences of stretching the heart have been well characterised, the molecular mechanisms involved are still unclear. In particular, the specific identities of cardiac SACNS and SACK in different cardiac cell types are largely unknown. Based on the biophysical characteristics of $I_{\text{SAC,NS}}$ and $I_{\text{SAC,K}}$ in cardiac cells, there are numerous candidates, including Piezo and various TRP channels (for SACNS), and $K_{\text{2P}}$ channels such as TREK-1 and BK channels (for SACK). While pharmacological tools have been helpful in narrowing down ion channel contributions, agents have been limited in their specificity and applicability to whole heart and organisms, hindering progress; more reliable probes are emerging, and they will drive further insight into sub-cellular mechanisms of MEC.

C. Knowledge Gaps and Future Directions

- The molecular mechanism(s) responsible for the chronotropic response to SAN stretch are still unconfirmed, including the role of mechano-sensitivity of ion fluxes in SAN automaticity; targeted genetic and pharmacological studies are needed to address this fundamental aspect of cardiac function.
- It remains unclear how exactly the chronotropic response of the SAN to stretch differs between animal species; this requires comparative studies utilising innovative approaches to determine underlying causes.
- Novel, more specific pharmacological agonists and antagonists are becoming available, which may lead to transformative insight into sarcolemmal and non-sarcolemmal SAC effects in normal homeostasis, pathogenesis, and therapy.

IV. INTEGRATIVE COMPUTATIONAL MODELS OF MEC

A. Single Cell

1. Consequences of SAC Activation

In early computational studies of MEC, SAC currents were approximated either by simulation of currents with an appropriate $E_{\text{rev}}$ through ohmic conductances – i.e., as structures conducting charge not matter (ions) (315) – or by an increase in background Na$^+$ and K$^+$ conductances (191). Since then, more detailed models of SAC have been developed, which include activation through biophysically-relevant parameters, allowing for simulation of stretch effects in cells, tissue, and whole hearts. In these formulations, SAC-activation is scaled by either: i) stretch (or strain) (227, 312, 693); ii) stretch (or strain) rate (274); or iii) tension (312), reflecting the lack of agreement on which biophysical input determines SAC activity. While these
models have largely been successful in replicating known MEC responses, it is worth noting that even when they consider ion movements, they account for effects on bulk cytosolic ion concentrations only. If a subset of effectors interacted with physiologically-relevant sub-cellular compartments, such as sub-membrane spaces like the dyadic cleft, or with SR and mitochondrial Ca\(^{2+}\) stores, their influence on cardiac electrophysiology may not be captured by current models. This is true, also, for close proximity effects of mechano-sensors, such as the putative secondary activation of BK channels in response to Ca\(^{2+}\) fluxes through co-localised SAC (256).

SAC incorporated into cardiac electrophysiology models have been used extensively in single cell simulation studies that recapitulate experimental data (307), occasionally in direct iteration with experimental studies. For instance, computational simulations correctly suggested that block of SAC with Gd\(^{3+}\) does not reduce the stretch-induced increase in resting Ca\(^{2+}\) in single ventricular myocytes, due to additional effects of Gd\(^{3+}\) on the delayed rectifier K\(^{+}\) current and Ca\(^{2+}\) extrusion via \(I_{\text{NCX}}\) (235). Similarly, stretch effects on the delayed rectifier K\(^{+}\) current, along with a transmurally varying \(I_{\text{SAC,K}}\) were shown to plausibly explain transmural differences in AP changes with stretch (227). Differences in experimentally reported stretch effects (including triggering of premature excitation and changes in AP shape and duration) have also been explained by computational modelling, which suggested that the various responses relate to different sub-cellular mechanisms (for instance SAC activation versus changes in the Ca\(^{2+}\) affinity of TnC versus modulation of SR Ca\(^{2+}\) handling) (312). These effects have been shown in cellular models to be further exasperated in conditions of Ca\(^{2+}\) overload, such as occurs with decreased activity of the Na\(^{+}\)-K\(^{+}\) pump (581).

The source of the experimentally established importance of stretch timing for cell-level responses has also been elucidated with computational simulations. That transient stretch, applied at the end of an AP or during diastole, causes depolarisation and premature excitation, while stretch during the AP plateau causes early repolarisation (and during later repolarisation has little effect), can be explained by the interrelation of SAC\(_{\text{NS}}\) \(E_{\text{rev}}\) and cell \(V_m\) during those phases of the AP (312, 520, 688). Simulations have also demonstrated that this relation can explain the increase in beating rate of sinoatrial node cells with sustained stretch in rabbit (120), and that this stretch response may be enhanced by effects of SAC\(_{\text{NS}}\) activation in electrotonically-coupled fibroblasts (315, 317).

Not only does MEC result in premature excitation and enhanced automaticity, it may also modulate pro-arrhythmic electrical alternans. Computational simulations have shown that
increasing $I_{\text{SAC,NS}}$ suppresses electro-mechanically concordant alternans (positive $V_m$-Ca$^{2+}$ coupling or voltage-driven alternans), while it promotes electro-mechanically discordant alternans (negative $V_m$-Ca$^{2+}$ coupling or Ca$^{2+}$-driven alternans) (187). In addition, the interaction of MEC with Mayer waves, involving oscillations in sympathetic nervous system activity and changes in ventricular preload, enhance this alternans promoting effect, which can ultimately result in premature excitation (484).

2. Consequences of Altered Intra-Cellular Ca$^{2+}$ Handling

The increase in diastolic Ca$^{2+}$ concentration, SR Ca$^{2+}$ release, and TnC-Ca$^{2+}$ affinity seen with stretch have all been reproduced in computational models. A stretch-induced increase in diastolic Ca$^{2+}$ levels has been modelled on the basis of secondary effects of an increase in Na$^+$ and K$^+$ background conductances on $I_{\text{NCX}}$ (191), highlighting the close interrelation of stretch effects on SAC and Ca$^{2+}$ handling, the importance of identifying secondary effects of mechanical perturbations, and the relevance of further enhancing the 3D structural detail on sub-cellular compartmentalisation of cardiac cells. The effect of stretch on SR Ca$^{2+}$ release has been simulated by including, in a formulation of RyR Ca$^{2+}$ flux, an exponential dependence on sarcomere length or tension (although the effect this has on cardiac electrophysiology was not investigated) (312). This has been extended to specifically simulate the stretch-induced increase in Ca$^{2+}$ spark rate, linked to mechanically-stimulated ROS production, by developing an RyR model that includes a ROS-dependent mode switch (357). This model has also not yet been used to investigate the potential role this increase in Ca$^{2+}$ spark rate may have on cardiac electrophysiology, but it has been used to make relevant predictions. Simulations using this model have demonstrated that stretch during diastole is expected to cause a local increase in ROS near the RyR complex, which increases the magnitude of the subsequent Ca$^{2+}$ transient, potentially helping to match contractile force to haemodynamic load (290) and coordinating contraction across the heart (92). Further, it has been suggested that when the chemical reducing capacity of cells is decreased, such as in ischaemia, mechanically-stimulated ROS production contributes to global oxidative stress and enhances SR Ca$^{2+}$ leak, which may increase the possibility of stretch-induced arrhythmias. Computational modelling has also suggested that the above effects may be microtubule dependent (275). These computational predictions, by and large, remain to be experimentally confirmed.

Stretch-induced changes in the Ca$^{2+}$ affinity of TnC have been studied extensively, using computational simulations to understand effects on cardiac electrical activity (617). In the context of MEC, simulations have shown that with constant stretch, changes in TnC-Ca$^{2+}$ affinity
would be expected to delay repolarisation, due to elevated Ca$^{2+}$-buffering by TnC causing an increase in total SR Ca$^{2+}$ release and a delay in re-uptake that leads to an increase and prolongation of $i_{\text{NCX}}$ (312, 429, 598). These stretch-induced AP changes differ from those predicted to arise from SAC activation, which, as described above, tends to cause AP shortening or cross-over of the repolarisation curve. Differences in the extent to which stretch effects are mediated by SAC or Ca$^{2+}$ handling may therefore partly account for discrepancies in experimental findings of stretch effects on APD. This could be the case due to differences in timing or magnitude of applied stretch, differing (patho-)physiological states, or unintended experimental influences (for example the ‘clamping’ of intra-cellular ion concentrations in whole-cell patch, as opposed to sharp electrode recordings). In fact, with stretch applied during the late plateau phase, TnC–Ca$^{2+}$ binding (in contrast to SAC$_{\text{NS}}$-activation) shortens the AP (312, 611), as the increase in Ca$^{2+}$ buffering occurs at a time when SR Ca$^{2+}$ release has terminated, thus reducing cytosolic Ca$^{2+}$ concentration and $i_{\text{NCX}}$ (312, 429, 598). The effects of mechanically-induced changes in TnC-Ca$^{2+}$ affinity are also influenced by the mechanical state of the cell. Computational simulations suggest, for example, that increased cellular viscosity results in the occurrence of premature excitation due to an increase in intra-cellular Ca$^{2+}$ levels, to the point of triggering spontaneous Ca$^{2+}$ releases from the SR (289), and that Ca$^{2+}$ overload and the ensuring incidence of arrhythmias can be enhanced by a decrease in the afterload imposed upon auxotonically contracting cells, or a decrease in preload for isometrically contracting cardiomyocytes (288). Again, many of the modelling-based hypotheses in this context still await experimental validation.

3. Summary

Single cell computational simulations have been successfully used to reproduce observed MEC effects. This has helped in integrating and interpreting experimental data to elucidate underlying mechanisms, including the role of SAC$_{\text{NS}}$ and SAC$_{\text{K}}$ and of changes in intracellular Ca$^{2+}$ dynamics or ROS, the importance stretch timing, non-specific effects of pharmacological agents, and the potential relevance of non-myocytes. That said, modelling of the effects of MEC at the cell level remains limited by a lack of fundamental information about characteristics of SAC function, such as mechanistic links between mechanical stimuli and channel opening, including their dependence on cytoskeletal elements and membrane properties such tension and curvature, as well as three-dimensional distribution in highly structured cardiac cells, necessitating assumptions for model parameterisation. This is, of course, is a reflection of limits in experimental insight. Technological developments, such as membrane (114) and protein
tension \((125, 333, 681)\) sensors, their application to cardiac research, and improved 3D nano-scale reconstructions of cells \((67, 523, 686)\), are needed to obtain and integrate the required information to further develop our conceptual and computational models.

**B. Multi-cellular**

1. **Physiological Mechanical Activity and Electrical Instability**

   While single cell simulations are useful for recapitulating and explaining known cell-level MEC effects, tissue and whole organ simulations are necessary for exploring mechanically-induced changes in heart rhythm that involve more integrative mechanisms. This aids in the understanding of causally-linked effects involving complex interactions between factors such as tissue composition and structure, electrophysiological and mechanical heterogeneity, and haemodynamics and organ geometry, while ideally allowing for the generation of novel experimentally-testable hypothesis and predictions \((493, 616)\).

   2D computational simulations of MEC have been used to investigate the effects of myocardial deformation caused by cardiac contraction. Theoretical studies have shown that contraction-induced deformation affects AP conduction, due to effects of tissue inertia \((126)\) and stress-assisted diffusion \((102, 369)\), which under pathological conditions can promote vulnerability to re-entry (especially for curved wave-fronts) \((314, 654)\). This effect can lead to spiral wave breakup \((457)\) and reduces the dispersion of repolarisation between adjacent regions of interacting muscle \((633)\). In addition, as in single cell simulations, stretch may influence electrical alternans, as computational simulations have demonstrated that SAC\(_{NS}\) can cause a transition from concordant (in-phase) to discordant (out-of-phase) alternations due to a change in the slope of the conduction velocity restitution curve \((503)\), while spatially distributed stretch can instead suppress alternans \((684)\).

   3D computational simulations have also been useful for investigating experimentally intractable questions. For instance, 3D simulations have allowed examination of the importance of myocardial mechanics in MEC effects, suggesting that during ventricular loading AP changes are mediated by stretch in both the longitudinal and transverse myocyte axes \((632)\), and that changes in conduction velocity are driven by reduced intercellular resistance and a concurrent increase of effective membrane capacitance \((142, 403)\), for example due to caveolar membrane integration. The stability of sustained transmural scroll waves – whose 3D organisational centres are only just becoming experimentally tractable \((112)\) – has been explored during stretch using biophysically-detailed 3D electro-mechanical models of the ventricles. These demonstrated that

(67)
MEC can cause deterioration of otherwise stable waves into turbulent patterns (296). In particular, wave stability depends on the $E_{\text{rev}}$ and conductance of SAC: an $E_{\text{rev}}$ of -60 mV reduces scroll wave breakup for all values of conductance by heterogeneously flattening the APD restitution curve, while an $E_{\text{rev}}$ of -20 mV partially prevents scroll wave breakup at low conductance values by heterogeneously flattening the conduction velocity restitution curve (but not when conductance is increased, as $I_{\text{Na}}$ inactivation in regions of large stretch leads to conduction block, counteracting the increase in scroll wave stability (243)). The putative impact of pathological states has also been investigated in a 3D electro-mechanical model of the ventricles, suggesting an increased importance of MEC effects in failing hearts (9).

2. Triggering and Sustenance of Arrhythmias

By their very nature, re-entrant cardiac arrhythmias require multiple electrically connected cells. A prime example of the utility of 2D and 3D computational modelling for the prediction of mechanisms underlying mechanically-induced re-entry is Commotio cordis. Simulating the application of a mechanical stimulus to a 2D sheet of ventricular myocytes by activating SAC$_{\text{NS}}$ in a sub-population of cells at different timings after the preceding 'normal' wave of excitation (and therefore with differing overlap on the trailing wave of repolarisation) demonstrated that SAC$_{\text{NS}}$ causes depolarisation in cells that have regained excitability, while it accelerates repolarisation in cells at a $V_m$ more positive than the $E_{\text{rev}}$ of SAC$_{\text{NS}}$. Mechanical stimuli applied too early (i.e., when most of the tissue is still refractory) do not cause an excitatory response, while stimuli timed to occur well after a previous excitation, when most of the tissue is repolarised, trigger a single premature excitation. If, and only if, a mechanical stimulus overlaps the trailing wave of repolarisation, it may provide both a trigger (premature excitation) and sustaining mechanism (increased electrophysiological heterogeneity, including a functional block line at the intersection of the new and preceding excitation) for the development of sustained ‘figure-of-eight’ re-entry (194) (FIGURE 5, C). This computationally-generated ‘overlap hypothesis’ was confirmed in a 3D whole ventricle model by simulation of an epicardial mechanical stimulation, which added confidence to the original prediction and highlighted the fact that mechanical stimulus location (relative to the ventricular surface) is a variable that may affect the absolute timing (though not the duration) of the vulnerable window for VF induction (355). The modelling-based prediction that the vulnerable window for precordial impact-induced VF exists in time and space gave rise to a subsequent targeted investigation, which experimentally confirmed this hypothesis ten years later (492) (FIGURE 6, B).
A similar computational approach, using a combination of electrical trigger and subsequent mechanical stimulus in a 2D sheet of ventricular myocytes, has been utilised to model the stretch of mechanically weakened ventricular regions that occur in acute regional ischaemia. In this case, when a mechanically-induced wave of excitation is initiated perpendicularly to an initial excitation after it has propagated through almost the entire sheet of tissue, the mechanically-induced excitation interferes with the trailing end of the original excitation, causing formation of a ‘spiral’ re-entrant wave (similar to the arrhythmic effect of a critically-timed S1-S2 stimulus) (517). Alternatively, when a centrally located area of ischaemic tissue is simulated, the wave of sinus excitation is slowed in the ischaemic area, causing the formation of two wave fronts that circumvent the ischaemic region. In this case, subsequent contraction of the surrounding myocardium that distends the ischaemic tissue (paradoxical segment lengthening) may lead to SACNS activation and a mechanically-induced ectopic beat (314). These effects of acute ischaemia may in fact be increased in diseased hearts where fibrosis is present, through mechano-sensitive fibroblasts, as computational simulations suggest that relatively small fibroblast-rich tissue and low levels of fibroblast-myocyte coupling are sufficient to give rise to stretch-induced excitation in ventricular muscle (317, 694). As for studies of Commotio cordis, the computationally-derived concept that stretch of the ischaemic border zone during acute regional ischaemia may be responsible for mechanically-induced excitation and re-entry has been supported by 3D computational modelling, which further showed that a combination of MEC and ischaemic effects is necessary for the induction of ventricular tachyarrhythmias: when SACNS are omitted from the model, ectopic excitation and block of conduction do not occur, while when ischaemia-induced electrophysiological changes are absent, the mechanically-induced ectopy and conduction effects do not result in re-entry (274) (FIGURE 7, C). Computational modelling further suggests that an increase in intra-ventricular volume during acute ischaemia can result in ectopic excitation from the more compliant ischaemic region (314), which has since been shown experimentally (459).

3. Modifying and Terminating Arrhythmias

In the case of sustained arrhythmias, MEC can affect re-entry dynamics. 2D computational simulations involving a fully-coupled electro-mechanical model have been used to investigate mechanisms underlying the increased prevalence of VF in mechanically compromised heart. These studies suggest that sustained stretch shortens APD and flattens the APD restitution curve. Stretch applied specifically near an excitation wave-front, however, creates a distribution of stretch during spiral re-entry that is characterised by a large gradient at the core of the spiral
wave, which then prolongs APD and creates an extended refractory region at the wave-end. This localised effect facilitates wave breakup and the occurrence of VF (231). In terms of atrial arrhythmias, computational simulations have suggested that MEC affects spiral wave frequencies, trajectories, and stability in AF (69), acting as a major source of cycle length variability during atrial flutter through effects of both respiration (as with respiratory sinus arrhythmia) and ventricular contraction, as atrial flutter interval (time between consecutive atrial activations) becomes phase-locked to ventricular contraction during periodic ventricular pacing (388).

The mechanisms by which mechanical stimulation may instead terminate established re-entry have also been investigated with computational modelling of precordial thump. When a mechanical stimulus is applied to a simulated 2D sheet of tissue in which sustained re-entry is occurring, activation of SACNS causes depolarisation of excitable tissue and, if stretch is large enough, initiation of excitation in these regions. If the proportion of excitable gaps in the simulated tissue in which stretch eradicates the excitable gap is large enough, this will terminate the re-entrant wave (310). In cases of established VF however, the myocardium will often be in an ischaemic state, due to a lack of coronary blood flow, resulting in co-activation of stretch-modulated $k_{ATP}$. Computational modelling suggests that the combined activation of SAC$_K$ and SAC$_{NS}$ during mechanical stimulation will result in a more negative $E_{rev}$ of the overall stretch-induced current, compared to control conditions (-35 mV versus -10 mV). This reduces excitable gap depolarisation, prevents formation of ectopic foci, and shortens APD (310), thus explaining one possible mechanism underlying the reduced efficacy of precordial thump in severely hypoxic conditions (675). MEC may also play a role in reducing the efficacy of electrical defibrillation in the arrested, volume-overloaded heart. 2D computational simulations have demonstrated that SAC$_{NS}$ activation in this setting results in a more positive $V_m$ at the end of the effective refractory period, a reduction in conduction velocity of shock-induced break excitations, and an increase in the complexity of post-shock VF patterns. This leads to a flattening of the defibrillation dose-response curve and an increase in the defibrillation threshold (210, 615).

Again, as for the examples of computational modelling of arrhythmia triggering above, for the case of sustained arrhythmia termination by precordial thump, 3D results are similar to the 2D case, demonstrating that in the whole ventricle mechanical stimulation can obliterate excitable gaps, thus terminating the arrhythmia, and that the efficacy of this intervention is reduced by ischaemia (356). Similarly, the reduced efficacy of electrical defibrillation due to
SACNS activation shown with 2D modelling has been replicated in a 3D electro-mechanical model of the acutely volume overloaded ventricle (354).

3. Summary

Multi-cellular computational models have been an effective means for quantitative theoretical exploration of integrated (patho-)physiological MEC responses, especially mechanisms of mechanically-induced and -terminated tachyarrhythmias, such as in the setting of Commotio cordis, acute regional ischaemia, and precordial thump. These simulations have predicted the critical importance of the relation of mechanical stimulation to underlying electrical activity for (anti-)arrhythmic outcomes, generating novel hypotheses that have since been experimentally verified. Moving forward, continually increasing computational power, combined with advances in experimental insight, will make 2D and 3D simulations an increasingly powerful tool for studies of MEC.

C. Knowledge Gaps and Future Directions

- Computational models continue to increase in complexity and sophistication, but they often do not include MEC; this should become standard practice for relevant studies of cardiac structure and function.
- Much of the fundamental information needed for the effective development and parameterisation of computational models that include MEC is still lacking; nonetheless, simulations of known MEC effects have be informative in identifying key knowledge gaps.
- Experimental and computational research should be conducted in close collaboration, using computational simulations to interpret and integrate experimental data, to generate novel experimentally testable hypotheses and predictions, and to guide experimental investigations that test these theories and suggestions, providing novel input data for computational model refinement.

V. SUMMARY AND CONCLUSIONS

In this review, we hope first and foremost to have conveyed the critical role of cardiac mechano-sensitivity in the auto-regulation of the heart’s electro-mechanical activity, and the vital importance of MEC in the acute adaptation of cardiac electrical behaviour to changes in the mechanical environment. The feedback from mechanics to electrics is essential for the maintenance of normal HR and rhythm. In cardiac pathologies, however, MEC can contribute to the initiation and maintenance of arrhythmias, both in acute and chronic disease states. Finally,
and very much like electrical current delivery to the heart, MEC can have not only arrhythmogenic effects, but also contribute to heart rhythm restoration.

Almost all clinical and experimental observations of MEC in the heart can be explained quantitatively by invoking no other mechanism besides SACNS in cardiac myocytes. However, one should be wary of the plausibility trap: simply because one mechanism may explain a response does not mean that said mechanism is the major driver of a response, or even involved at all. The mechanical modulation of other ion channels, as well as of intra-cellular Ca\(^{2+}\) handling and other signalling mechanisms (e.g., ROS) matter for MEC in healthy and diseased myocardium. In addition, mechano-sensitive non-myocytes in the heart may play important roles in MEC effects. This complexity, along with our lack of understanding of the role and interplay of many molecular sensors and information pathways involved in MEC, leaves much to be discovered.

MEC studies are hampered by several experimental limitations, such as our inability to measure tension inside cardiac tissue, and by the lack of selective pharmacological probes that can block or activate individual relevant players in native tissue, ideally in a cell-type specific manner. This restricts our ability to link discrete islands of insight at molecular, cellular, tissue, organ, and organism levels, and to project between species. At times, the setting reminds one of the famous 'street-light effect' (284), where one looks for missing items not where they are most likely to be found, but where it is most convenient to search for them. That said, bridges can be built between experimentally-intractable questions and theoretical explanations using computational models to quantitatively assess the plausibility of interpretations and novel hypotheses, and to design new experimental research.

Finally, even based on our current partial understanding of mechanisms and importance of cardiac MEC in health in disease, it is clear that exciting potential for therapeutic interventions exists, for instance in the use of mechanical stimulation for heart rhythm management, in controlling mechanical modifiers of electro-mechanical structure and function, and in pharmacological targeting of sub-cellular players as novel means for anti-arrhythmic therapies. Overall, the current state of cardiac MEC research warrants further investigation into acute mechanical effects on HR and rhythm, to help us come closer to a comprehensive understanding of the integrated electro-mechanical crosstalk that makes the heart tick in health and disease.
VI. REFERENCES


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