Mechano-sensitivity of cardiac pacemaker function: Pathophysiological relevance, experimental implications, and conceptual integration with other mechanisms of rhythmicity

T. Alexander Quinn\textsuperscript{a,b,*,} Peter Kohl\textsuperscript{a,b}

\textsuperscript{a} National Heart and Lung Institute, Imperial College London, London, UK
\textsuperscript{b} Department of Computer Science, University of Oxford, Oxford, UK

\textbf{A B S T R A C T}

Cardiac pacemaker cells exhibit spontaneous, rhythmic electrical excitation, termed automaticity. This automatic initiation of action potentials requires spontaneous diastolic depolarisation, whose rate determines normal rhythm generation in the heart. Pacemaker mechanisms have been split recently into: (i) cyclic changes in trans-sarcolemmal ion flows (termed the ‘membrane-clock’), and (ii) rhythmic intracellular calcium cycling (the ‘calcium-clock’). These two ‘clocks’ \textit{undoubtedly} interact, as trans-sarcolemmal currents involved in pacemaking include calcium-carrying mechanisms, while intracellular calcium cycling requires trans-sarcolemmal ion flux as the mechanism by which it affects membrane potential. The split into separate ‘clocks’ is, therefore, somewhat arbitrary. Nonetheless, the ‘clock’ metaphor has been conceptually stimulating, in particular since there is evidence to support the view that either ‘clock’ could be sufficient in principle to set the rate of pacemaker activation.

Of course, the same has also been shown for sub-sets of ‘membrane-clock’ ion currents, illustrating the redundancy of mechanisms involved in maintaining such basic functionality as the heartbeat, a theme that is common for vital physiological systems.

Following the conceptual path of identifying individual groups of sub-mechanisms, it is important to remember that the heart is able to adapt pacemaker rate to changes in haemodynamic load, even after isolation or transplantation, and on a beat-by-beat basis. Neither the ‘membrane-’ nor the ‘calcium-clock’ do, as such, inherently account for this rapid adaptation to circulatory demand (cellular Ca\textsuperscript{2+} balance changes over multiple beats, while variation of sarclemmal ion channel presence takes even longer). This suggests that a third set of mechanisms must be involved in setting the pace. These mechanisms are characterised by their sensitivity to the cyclically changing mechanical environment, and – in analogy to the above terminology – this might be considered a ‘mechanics-clock’.

In this review, we discuss possible roles of mechano-sensitive mechanisms for the entrainment of membrane current dynamics and calcium-handling. This can occur directly via stretch-activation of mechano-sensitive ion channels in the sarcolemma and/or in intracellular membrane compartments, as well as by modulation of ‘standard’ components of the ‘membrane-’ or ‘calcium-clock’. Together, these mechanisms allow rapid adaptation to changes in haemodynamic load, on a beat-by-beat basis.

Additional relevance arises from the fact that mechano-sensitivity of pacemaking may help to explain pacemaker dysfunction in mechanically over- or under-loaded tissue. As the combined contributions of the various underlying oscillatory mechanisms are integrated at the pacemaker cell level into a single output – a train of pacemaker action potentials – we will not adhere to a metaphor that implies separate time-keeping units (‘clocks’), and rather focus on cardiac pacemaking as the result of interactions of a set

\textit{Abbreviations: AP, action potential; BR, beating rate; Ca\textsuperscript{2+}, calcium; DD, diastolic depolarisation; HCN, hyperpolarisation-activated, cyclic nucleotide-gated; I\textsubscript{Ca,L}, L-type calcium current; I\textsubscript{Ca,T}, T-type calcium current; I\textsubscript{f}, funny current (hyperpolarisation-activated depolarising current); I\textsubscript{K}, outward potassium currents; I\textsubscript{Na}, sodium–calcium exchanger current; K\textsuperscript{+}, potassium; MDP, maximum diastolic potential; MSP, maximum systolic potential; Na\textsuperscript{+}, sodium; E\textsubscript{rev}, reversal potential; RyR, ryanodine receptor channel; SACNS, cation non-selective stretch-activated channel; SAN, sino-atrial node; SERCA, sarco-/endoplasmic reticulum calcium-ATPase; SR, sarcoplasmic reticulum; V\textsubscript{m}, membrane potential.}

* Corresponding author. National Heart and Lung Institute, Imperial College London, Heart Science Centre, Harefield UB9 6JH, UK. Tel.: +44 1895 453 807.

E-mail address: t.quinn@imperial.ac.uk (T.A. Quinn).
of coupled oscillators, whose individual contributions vary depending on the pathophysiological context. We conclude by considering the utility and limitations of viewing the pacemaker as a coupled system of voltage-, calcium-, and mechanics-modulated oscillators that, by integrating a multitude of inputs, offers the high level of functional redundancy that is vitally important for cardiac automaticity.

## Contents

1. Introduction ............................................................................................................................ 258
2. Direct mechanical effects on cardiac pacemaker activity .................................................... 259
   2.1. Whole animal observations .................................................................................................. 259
   2.2. Tissue level insight ............................................................................................................. 260
   2.3. Cell level findings .............................................................................................................. 260
3. Mechanical effects on components of the V_m/Ca^{2+}-oscillator ........................................... 262
   3.1. Effects on the V_m-oscillator ............................................................................................... 262
   3.2. Effects on the Ca^{2+}-oscillator ......................................................................................... 262
4. The mechanics-oscillator and its pathophysiological relevance ........................................... 263
   4.1. Physiological loading ......................................................................................................... 263
       4.1.1. Chamber loading dynamics .......................................................................................... 263
       4.1.2. Atrial effects ................................................................................................................. 263
       4.1.3. Ventricular effects ....................................................................................................... 264
   4.2. Pathophysiological loading ............................................................................................... 264
       4.2.1. Atrial effects ................................................................................................................. 264
       4.2.2. Ventricular effects ....................................................................................................... 265
6. Conclusion .............................................................................................................................. 265

Editors’ note ............................................................................................................................ 266
Acknowledgements .................................................................................................................. 266
References .................................................................................................................................... 266

## 1. Introduction

Spontaneous mechanical activity of the heart is reported to have been recognised at least as far back as the second century, when Greek physician Claudius Galenus is credited with having noted that the heart continues to beat for an extended period of time when extracted from the chest (as cited by Anglo, 1537). It was not until the 19th century, however, that the seminal work of Gaskell (1882) confirmed the ‘myogenic theory’ of cardiac rhythm generation, originally proposed by Harvey (1651), and later supported by the work of von Haller (1757). Exploring the tortoise heart, Gaskell demonstrated rhythmicity as an intrinsic property of (in his view not fully differentiated) cardiac tissue and he proposed ‘laws’ of cardiac rhythm generation, in which he presaged that rhythmicity decreases with distance from the sinus, and that conductivity is inversely related to rhythmicity. This was followed by recognition of specific anatomical locations of cardiac automaticity in the mammalian heart, which are now known as the sino-atrial node (SAN, the primary structure responsible for initiation of the heartbeat (Keith and Flack, 1907)), and the atrio-ventricular node and Purkinje fibres (secondary and tertiary pacemaker regions (Tawara, 1906)).

A hundred years on, the cellular mechanisms of spontaneous pacemaker activity, and their relative contribution to pacemaking, are still a matter of debate (Brown et al., 1984; Lakatta and DiFrancesco, 2009; Rosen et al., 2012). It is clear, though, that cardiac pacemaking represents a robust and flexible system, integrating multiple contributors that allow adaptation to changes in circulatory demand.

In contrast to working cardiomyocytes in the atria and the ventricles, the action potential (AP) of cardiac pacemaker cells exhibits spontaneous diastolic depolarisation (DD; see Fig. 1). The rate of DD determines the time needed to take the trans-membrane potential (V_m) to the threshold for AP generation. Since V_m is an expression of the charge balance between the in- and out-side of the cell, changes in V_m require an imbalance between inward (depolarising) and outward (re- or hyperpolarising) trans-sarcolemmal currents, i.e., a net-flux of charge across the membrane. DD will result from a ‘surplus’ in inward currents which, in a dynamic setting, may arise either from a relative increase in inward currents, or a decrease in outward currents (in the presence of sufficient inward current).

Early DD is a result of both, as outward potassium (K^+) currents (I_K) are reducing, in the presence of increasing inward currents, such as the electrogenic sodium–calcium (Na^+–Ca^{2+}) exchanger current (I_{NaC}), the hyperpolarisation-activated (i.e., increasing in early diastole) ‘funny’ current (I_f; DiFrancesco, 1985), and any background conductances that carry inward currents, for instance the sodium (Na^+) background current (Noble et al., 1992). In contrast to the Na^+ background current, whose molecular nature is still under investigation, it is known that I_f is conducted by hyperpolarisation-activated cyclic nucleotide-gated (HCN) proteins, of which four isoforms have been identified in cardiac and neuronal tissue (Moosmang et al., 2001). Cardiac I_f is composed primarily of HCN4, and to a lesser degree of HCN2 and HCN1, in a species- and cell-type dependent manner (Barbuti et al., 2011; Scicchitano et al., 2012). Quantitative simulation has shown that these channels, even if one considers them as conductors of monovalent cations only (i.e., K^- and Na^+), although there is now evidence that they also conduct calcium, Ca^{2+}; Michels et al., 2008; Yu et al., 2007), are sufficient to give rise to cyclic changes in V_m (Noble et al., 1992). Their importance for pacemaking has been experimentally confirmed as HCN expression in non-pacemaking cells confers spontaneous activity (Li, 2012; Robinson et al,
2006), while pharmacological block significantly slows beating rate (BR; Bucchì et al., 2002; Koncz et al., 2011).

Later on during DD, $I_{\text{f}}$ is reduced (as $V_m$ increases), and trans-sarcolemmal Ca$^{2+}$ fluxes become more dominant, involving the transient (T-type) Ca$^{2+}$ ($I_{\text{CaT}}$) current and increasing $I_{\text{NCX}}$. This raises DD rate. Once $V_m$ crosses the threshold for activation of the long-lasting (L-type) Ca$^{2+}$ current ($I_{\text{CaL}}$), a new AP is initiated (Irisawa et al., 1993).

As the combined action of this ensemble of trans-sarcolemmal currents appears sufficient to rhythmically generate spontaneous APs (Noble et al., 1989), it has been dubbed the ‘membrane-clock’ ($'V_m$-clock’; Maltsev et al., 2006).

Interestingly, interventions that target intracellular Ca$^{2+}$ cycling (such as the application of ryanodine to inhibit Ca$^{2+}$ release from the sarcoplasmic reticulum, SR) affect DD rate in primary (Rigg and Terrar, 1996) and secondary (Rubenstein and Lipsius, 1989) pacemaker cells. This suggests that additional mechanisms are involved, which are thought to require spontaneous cytosolic Ca$^{2+}$ release from the SR, followed by re-uptake through the sarco-/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) and extrusion to the cell exterior, primarily via $I_{\text{NCX}}$ (Ju and Allen, 1998). Since Na$^+$–Ca$^{2+}$ exchanger activity is electrogenic (one Ca$^{2+}$ ion leaving the cell is ‘swapped’ for three Na$^+$ ions entering), this gives rise to increased inward $N_{\text{CCX}}$. More recently, SR Ca$^{2+}$ cycling has been identified as rhythmical, even after clamping sarcolemmal $V_m$ (Vinogradova et al., 2004). This has been conceptualised as representing a pacemaker mechanism whose origin is independent of trans-sarcolemmal ion fluxes (its mode of action is not, however, as it requires $I_{\text{NCX}}$ to cause the membrane depolarisation that drives DD (Sanders et al., 2006)), and has been referred to as the ‘Ca$^{2+}$-clock’ (Maltsev et al., 2006).

Both of these ‘clocks’ could theoretically drive pacemaker activity on their own. In reality, of course, they do not operate in isolation (as mentioned, the ‘Ca$^{2+}$-clock’ relies on a trans-sarcolemmal ion flux to alter $V_m$, and the ‘$V_m$-clock’ includes Ca$^{2+}$ cycling). It is more appropriate, therefore, to think of them as coupled oscillators, rather than separate ‘clocks’. This is especially true as their interaction at the level of normal whole-cell pacemaker function involves mutual entrainment (Noble et al., 2010), resulting in a robust and stable system (the $V_m$/Ca$^{2+}$-oscillator; see Fig. 1). This finds representation in conceptual and computational models, and can explain a vast range of experimentally observed aspects of cardiac pacemaker function (Maltsev and Lakatta, 2008; Severi et al., 2012).

Of course, the mere plausibility of an explanation does not necessarily confirm that the mechanisms identified as causal are indeed major contributors, or even involved at all (Hunter et al., 2001; Kohl et al., 2010). To avoid falling victim to this ‘plausibility trap’ (Quinn and Kohl, 2011) requires continuous questioning, even of what may seem ‘obvious’, and for pacemaker function this continues to generate interesting and productive discussion (DiFrancesco and Noble, 2012; Maltsev and Lakatta, 2012; Rosen et al., 2012).

One shortfall of our current understanding of pacemaker function is that it has been developed based largely on investigations of molecular mechanisms in isolated cells. Pacemaking in vivo is influenced by factors not generally represented in these preparations, including biochemical influences, such as from the metabolic and autonomic systems, para- and endocrine signalling, as well as biophysical parameters, such as temperature and cyclically changing levels of stretch (Quinn et al., 2011). This review identifies the relevance of mechanical modulation of pacemaker function, explores putative mechanisms underlying adaptation of pacemaker activity to the mechanical environment, and presents the foundations for conceptual consideration of cardiac pacemaking as a coupled $V_m$, Ca$^{2+}$-, and mechanics-oscillator, whose integration is relevant for heart function in health and disease. Other aspects of pacemaker control will not be covered, as they have been discussed extensively in numerous recent reviews (Boyd et al., 2010; DiFrancesco and Noble, 2012; Dobrzynski et al., 2007; Maltsev and Lakatta, 2012; Mighiu and Heximer, 2012; Rosen et al., 2012; Stewart et al., 2012).

2. Direct mechanical effects on cardiac pacemaker activity

2.1. Whole animal observations

Effects of stretch on pacemaker activity were recognised soon after the identification of the SAN. In 1915, Francis Arthur Bainbridge observed that intravenous fluid injection in anaesthetised dogs resulted in right-atrial distension and an increase in BR (Bainbridge, 1915). He described this as an “Acceleration of heart rate […] caused by impulses arising within the heart.” Similar effects have since been observed in various vertebrate phyla, including Amphibia, Reptilia, Aves, and Mammals (Pathak, 1973). Confirming this finding in humans proved difficult, initially, as most non-invasive interventions that raise central venous pressure in human (such as tilt-table studies) also increase arterial pressure. The latter triggers
a (dominant) baroreceptor response, the so called ‘depressor reflex’ (von Bezold and Hirt, 1867; Jarisch and Richter, 1939), which gives rise to a reduction in BR. It was not until 1978 that David Donald and John Shepherd resolved this experimental problem by passively elevating the legs of volunteers in the supine position (Donald and Shepherd, 1978). This favours venous return to the trunk of the body, raising central venous pressure with no concurrent rise on the arterial side. As a result, BR was seen to increase, establishing the presence of the ‘Bainbridge response’ in humans.

The positive correlation between right atrial volume loading and BR was originally attributed to a predominantly vagal autonomic reflex (Bainbridge, 1915). More recent research showed, however, that near instantaneous increases in BR occur in open-chest, anaesthetised dogs upon SAN-specific stimulation (Brooks et al., 1966), in a response that is insensitive to denervation, as well as adrenergic and cholinergic blockade. It has been confirmed that a positive chronotropic response to stretch can be observed in a host of denervated preparations, such as isolated hearts (Blinks, 1956), right atrial tissue (Blinks, 1956), and the SAN (Deck, 1964b), as well as isolated Purkinje fibres (Kaufmann and Theophile, 1967) and even single SAN cells (Cooper et al., 2000). This demonstrates that intracardiac ‘myogenic’ mechanisms contribute to mechanical modulation of pacemaker rate, beyond the originally implied vagal reflex response.

An interesting physiological phenomenon, which may in part be caused by stretch-induced changes in pacemaker rate, has been well documented in humans: heart rate variability synchronised with the respiratory cycle (in which BR rises during inspiration, and declines during expiration). This phenomenon is known as ‘respiratory sinus arrhythmia’ (even though it is a physiological fluctuation in BR). While generally considered to be a consequence (and, hence, useful indicator) of autonomic nervous input, variability continues to exist, albeit at a reduced magnitude, in the transplanted and, hence, denervated heart (Bernardi et al., 1989). This supports a role of intracardiac mechanical BR modulation. This may be explained by the fact that during inspiration, low intrathoracic pressure aids venous return (loading the right atrium), while during expiration, the opposite is true. Observed changes in BR are, thus, compatible with stretch effects on SAN function.

These early observations of stretch-effects on pacemaker electrophysiology have been followed up by studies examining underlying mechanisms at the tissue level.

2.2. Tissue level insight

Electrical and mechanical activity in the heart are linked via an intrinsic regulatory loop (mechano-electric coupling (Kohl et al., 2011)), involving both a feed-forward flow of information from electrical excitation to mechanical contraction (excitation-contraction coupling (Bers, 2002)) and feed-back from the mechanical environment to the origin and spread of excitation (mechano-electric feedback (Lab, 1982)). This can be observed at all levels of cardiac structural integration, from (sub-)cellular and tissue levels, to whole organ and body, including stretch effects on pacemaker electrophysiology.

In canine isolated hearts and atria isolated from various mammalian species subjected to progressive increases in atrial pressure at constant flow, BR responses similar to those in the intact animal have been observed (Blinks, 1956). In canine (Chiba, 1977) and rat (Wilson and Bolter, 2002) isolated right atrial preparations, and feline SAN tissue (Lange et al., 1966), it has been shown that adrenergic and cholinergic blockade with propranolol and atropine, respectively, do not abolish stretch-induced changes in BR, excluding intramural neural plexi as necessary contributors. Similarly, application of tetrodotoxin (a blocker of fast Na⁺ channels required for neuronal activation (Chiba, 1977; Wilson and Bolter, 2002)) or neonatal capsaicin injections (to ablate intracardiac neurons (Wilson and Bolter, 2002)) do not remove the positive chronotropic response to stretch. There is some evidence that application of dichloroisoproterenol, a non-selective β-adrenergic antagonist (Lange et al., 1966) can reduce stretch-induced changes in BR, but the mechanisms of action for this have not been identified. Finally, the near-instantaneous response of pacemaker rate to stretch, as well as the high solution flow-rates used in isolated heart preparations, suggest that humoural factors are also unlikely to be key contributors to the mechanical modulation of pacemaking.

Combined, these results suggest that neither humoral, nor extra- or intracardiac neuronal signalling, are pre-required for the intrinsic BR response to stretch. There is evidence, however, supporting an interaction between mechanical and autonomic BR modulation. In intact rabbit (Bolter, 1994; Bolter and Wilson, 1999), as well as isolated atria of rabbit (Bolter, 1996), guinea-pig (Wilson and Bolter, 2001) and rat (Barrett et al., 1998), an increase in right atrial pressure induces both BR acceleration and a significant reduction in the percentage-response to vagal stimulation, effectively reducing the influence of ‘vagal tone’ on the heart. Vice versa, when BR is reduced by vagal stimulation, the BR response to stretch is augmented. This effect may be mediated by the repolarising muscarinic K⁺ current, as an increase in pressure in rat isolated right atria has been shown to close these channels, and application of Tertiapin-Q appears to eliminate their mechano-sensitivity (Han et al., 2010). It is speculated that mechanical regulation of muscarinic K⁺ current may at least partly be explained by a rapid change in RGS4 (regulator of G protein signalling 4) activity via a stretch-induced increase in intracellular Ca²⁺ (Mighiu and Heximer, 2012). Thus, parasympathetic control of BR may be continuously modulated by atrial loading, contributing to efficient stretch-induced adjustment of BR to fluctuations in venous return. Similarly, when BR is reduced by carbamylcholine (a cholinergic agonist), the BR response to stretch is amplified, and when increased with isoprenaline (a non-selective β-adrenergic agonist), the response is diminished (and in the extreme case reversed, such that stretch decreases already elevated BR, similar to the response seen in smaller mammals with a normally high resting BR (Cooper and Kohl, 2005)). The extent to which this is a consequence of basal BR per se, however, is a matter of continued investigation.

In contrast, it has been observed that stretch, caused by increased pressure in isolated rabbit right atria, potentiates the dose-dependent decrease in BR upon application of the muscarinic agonist β-homobetaine methylester, a structural isomer of acetylcholine (Rossberg et al., 1985). Thus, intrinsic and systemic BR modulations occur in conjunction, and may amplify, or dampen, each other’s effect (part of this may be indicative of possible differences between neural and humoral effects). In addition, as previously mentioned, haemodynamic changes that affect both venous return and arterial pressure will give rise to potentially competing regulatory responses (e.g., stretch-induced acceleration vs. the ‘depressor reflex’).

2.3. Cell level findings

In 1964, Klaus Deck reported intracardiac ‘sharp electrode’ recordings of SAN pacemaker cell Vr during linear and equi-biaxial stretch of feline and rabbit isolated atrial tissue containing the SAN (Deck, 1964b). Along with an instantaneous increase in BR, he found a reduction in the absolute values of both maximum diastolic and maximum systolic potentials (MDP and MSP, respectively). The extent of positive chronotropic change was inversely related to background BR (altered by acetylcholine, pH, temperature, or hypoxia). At about the same time, similar effects were
demonstrated in Rhesus-monkey isolated Purkinje fibres, where stretch moved MDP in a positive direction and increased DD rate (Kauffman and Theophile, 1967). These electrophysiological observations help in narrowing the range of plausible molecular mechanisms involved in the V_m response to stretch, as the most intuitive explanation would invoke a trans-sarcolemmal whole-cell net current with a reversal potential (E_rev) somewhere between the MDP and MSP of primary or secondary pacemaker cells.

Early single cell studies into the mechanisms underlying mechanical modulation of pacemaker BR used positive pressure cell inflation as a mechanical stimulus. In rabbit isolated SAN cells, this activates a cell swelling related chloride current with an E_rev near 0 mV, which could in theory account for observed changes in pacemaker electrophysiology (Hagiwara et al., 1992). However, this current does not activate instantaneously upon cell swelling (lag times usually exceed 1 min in cardiac cells), rendering it too slow for acute beat-by-beat changes. Furthermore, cell inflation is micro-mechanically different from stretch, being associated with an increase in cell diameter and negligible changes in length (which may even reduce). In contrast, stretch causes cell lengthening and (as cell volume remains constant) a reduction in diameter. Indeed, subsequent studies using hypo-osmotic swelling of spontaneously beating rabbit isolated SAN cells showed a reduction, rather than the anticipated increase, in BR (Lei and Kohl, 1998). Computational modelling of SAN cell swelling effects suggested that the reduction in BR was driven by dilution of the cytosol which, via a decrease in intracellular K⁺ concentration, reduced rapid delayed rectifier I_k, shifting MDP towards more depolarised levels and reducing I_k. The proposed swelling-induced reduction in rapid delayed rectifier I_k (also seen in guinea-pig isolated ventricular myocytes (Rees et al., 1995)) has been confirmed in rabbit SAN cells (Lei and Kohl, 1998).

Cell volume manipulation has been shown to affect other pacemaking-relevant currents in isolated myocytes, such as I_bKCa, I_Ca,L, I_Ca,T, slow delayed rectifier I_k, the Na⁺–K⁺ pump, and muscarinic K⁺ currents (for a review, see (Baumgarten et al., 2011)). Differences in micro-mechanical characteristics, pathophysiological context, response times, and electrophysiological outcomes suggest, however, that cell volume manipulation is not a suitable intervention to experimentally explore the effects of stretch, such as encountered by pacemaker tissue in situ on a beat-by-beat basis during the cardiac cycle.

Just over a decade ago, spontaneously beating SAN cells were axially stretched using a modified version (Iribe et al., 2007) of the carbon fibre technique (Le Guennec et al., 1990), while simultaneously collecting patch-clamp recordings of their V_m dynamics (Cooper et al., 2000). In this study, a significant increase in BR during 5–10% stretch was observed, which was accompanied by a reduction in the absolute values of MDP and MSP, as previously reported in native SAN tissue (Deck, 1964b). V_m-clamp studies revealed that this response was caused by a stretch-activated whole-cell current with an E_rev of ~11 mV (Cooper et al., 2000).

This current is similar to that carried by cation non-selective stretch-activated channels (SACNS), reported in many other eukaryotic cells. SACNS are rapidly activating, with an E_rev between 0 and ~20 mV in cardiac myocytes (Craelius et al., 1984; Guharay and Sachs, 1984). SACNS opening will therefore depolarise diastolic, and repolarise systolic, V_m, potentially explaining the observed changes in SAN BR during stretch. Over a slightly slower time scale, SACNS may also affect intracellular Ca²⁺ dynamics, such as through direct Ca²⁺ influx, or effects on I_{NCX} that are secondary to Na⁺ influx (Gannier et al., 1996).

Interestingly, BR responses to stretch can differ between species. Most medium- and large-sized mammals (with inherently slow background BR) respond to sustained stretch of the SAN with BR acceleration, while smaller mammals (with resting BR exceeding 200 bpm) show BR deceleration (Cooper and Kohl, 2005). It would appear, nonetheless, that both responses are caused by SACNS activation. Larger animals with slower baseline BR have SAN AP shapes that are characterised by a relatively slow AP upstroke and a relatively prominent plateau-like early repolarisation phase, while smaller mammals with high BR exhibit faster upstrokes (often carried by a mix of Na⁺ and Ca²⁺ currents (Lei et al., 2007)) and swift initial repolarisation, giving rise to a more spike-like AP shape (see Fig. 2). As a consequence, ‘slow’ SAN APs spend the majority of each cycle moving their V_m towards the E_rev of SACNS, while, in contrast, ‘fast’ SAN APs spend a larger proportion of time moving their V_m away from the E_rev of SACNS. This can explain why activation of SACNS (which will act to pull V_m in the direction of E_rev) may increase slow, yet reduce fast, BR (Cooper and Kohl, 2005).

The plausibility of SACNS as a key contributor to the chronotropic response of SAN pacemaking to stretch is corroborated by experimental studies which have shown reversible block of stretch-induced BR changes in guinea pig and murine SAN tissue using GSMTx-4 (Grammostola spatulata mecanotoxin-4), the most specific blocker of SACNS available to date (Cooper and Kohl, 2005). This now requires confirmation in mechanically loaded isolated primary and lower order pacemaker cells.

Interestingly, SACNS might also affect BR if they are located not in pacemaker cells, but in electrically coupled non-myocytes, such as fibroblasts. These usually are relatively depolarised cells, possess SACNS (Stockbridge and French, 1988), and their heterotopic electrical coupling to cardiac myocytes has been confirmed in rabbit SAN tissue (Camelliti et al., 2004). Stretch has been shown to cause fibroblast depolarisation (Kohl et al., 1994; Kohl and Noble, 1996),
a response that is sensitive to pharmacological depletion or buffering of fibroblast intracellular Ca\(^{2+}\) (Kiseleva et al., 1996). In computational models, this is quantitatively sufficient to increase BR in electrically coupled SAN cells on a beat-by-beat basis (Kohl et al., 1994). Experimental validation of the importance of fibroblast- and other non-myocyte-mediated stretch-effects on BR is another important target for further research.

Therefore, stretch of pacemaker tissue and cells has a pronounced effect on their electrophysiology, acting at least in part via activation of specific mechano-sensitive ion channels in the sarcolemma of pacemaker (and possibly other) cells. Mechanically induced changes demonstrably affect pacemaking rate, and they do so with a time-course sufficient to explain beat-by-beat responses. This may tune the \(V_{m}/Ca^{2+}\)-oscillator, in keeping with circulatory system requirements. However, quantitative plausibility is no substitute for validation. For instance, SACNS-based computational models do not currently reproduce certain cycle-dependent effects on BR (such as deceleration upon systolic stretch in murine SAN models). This suggests that other mechanisms must be involved. In fact, mechano-sensitivity is known to be wide-spread in cardiac electrophysiology and Ca\(^{2+}\)-handling. The evidence for direct effects of the mechanical environment on components of the \(V_{m}/Ca^{2+}\)-oscillator will be considered next.

3. Mechanical effects on components of the \(V_{m}/Ca^{2+}\)-oscillator

3.1. Effects on the \(V_{m}\)-oscillator

Effects of mechanical stimulation on components involved in driving the \(V_{m}\)-oscillator have been reported (see Fig. 1). For instance, it has been shown in oocytes that current through HCN2 channels is reversibly increased by hypo-osmotic swelling and by intracellular fluid injection (Calloe et al., 2005). In addition, in cell-attached oocyte patches, it has been demonstrated that membrane deformation accelerates both hyperpolarisation-induced activation and depolarisation-induced deactivation of HCN2 channels, essentially ‘accelerating the dynamics’ of this channel (Lin et al., 2007). To determine how this might affect automaticity, oocyte membrane patches were AP-clamped to cyclic SAN and Purkinje cell AP waveforms. Whether membrane deformation caused an increase or a decrease in HCN2 current depended on BR: at higher rates HCN2 current was increased by stretch, and at lower rates it was decreased. If HCN mechano-sensitivity is also present in axially stretched pacemaker cells, which is yet to be tested, it could have complex and potentially species-dependent effects on BR responses to stretch.

Mechano-sensitivity has been demonstrated as well in recombinant human L-type (but interestingly not T-type) Ca\(^{2+}\) channels expressed in HEK cells (Gamble et al., 2002; Lyford et al., 2002), as well as for various \(V_{m}\)-gated Na\(^{+}\) (Na\(_{h}\)L,1.5 and Na\(_{v}\)1.6) and K\(^{+}\) (K\(_{h}\), K\(_{s}\), K\(_{v}\), K\(_{Ca}\)) channels (for review, see (Morris, 2011)). Thus, several components of the \(V_{m}\)-oscillator are directly mechano-sensitive, at least in various expressions systems.

3.2. Effects on the \(Ca^{2+}\)-oscillator

Stretch has been shown to directly affect intracellular Ca\(^{2+}\) handling in cardiac cells (Allen and Kentish, 1985; Calaghan and White, 1999; ter Keurs, 2012). The pacemaker BR response to stretch, in particular, is reduced by lowering extracellular Ca\(^{2+}\) concentration, and by interference with normal SR Ca\(^{2+}\) handling using ryanodine or thapsigargin (Arai et al., 1996), but it is insensitive to \(i_{Ca,L}\) block with either nifedipine (Arai et al., 1996) or verapamil (Chiba, 1977). As BR responses are decreased by interventions which either reduce SR Ca\(^{2+}\) content (lowered extracellular Ca\(^{2+}\) or block of SERCA activity), or reduce SR Ca\(^{2+}\) release (by interfering with ryanodine receptor channels, RyR), this suggests an involvement of intracellular Ca\(^{2+}\) mobilisation in the stretch-induced enhancement of pacemaker activity. Of note, stretch has been shown to acutely increase SR Ca\(^{2+}\) release in guinea-pig (Iribe and Kohl, 2008) and rat (Gamble et al., 1992; Iribe et al., 2009) ventricular myocytes (see Fig. 3). This appears to occur by an acute and transient increase in Ca\(^{2+}\) spark rate (attributed to augmented RyR open probability (Iribe et al., 2009)). In rat (which shows diastolic loss of SR Ca\(^{2+}\) content, RyR open probability (Iribe et al., 2009)). In rat (which shows diastolic loss of SR Ca\(^{2+}\) content), stretch enhances the rate of loss of SR Ca\(^{2+}\) — i.e., in both cases, stretch favours Ca\(^{2+}\) extrusion from otherwise well-loaded SR. This may point towards a more general applicability of stretch-induced increase in RyR open probability. At the same time, the mechanism of the acute stretch-induced increase in Ca\(^{2+}\) spark rate is unknown: RyR may themselves be mechanosensitive, or they may be locally activated due to release of Ca\(^{2+}\) from mitochondria upon mechanical stimulation (possibly involving mitochondrial \(\Delta N\)) (Belmonte and Morad, 2008)). The fact that mitochondrial Ca\(^{2+}\) flux can alter BR by affecting spontaneous SR Ca\(^{2+}\) release has recently been corroborated in rabbit isolated SAN cells (Yaniv et al., 2012).

![Fig. 3.](image-url) **Fig. 3.** (A) Confocal images of intracellular Ca\(^{2+}\) concentration showing an acute increase in Ca\(^{2+}\) spark activity with axial stretch (change in sarcomere length of 8% from resting) applied by carbon fibres to only one half (right side) of a rat isolated myocyte. (B) Time course of relative intracellular Ca\(^{2+}\) concentration signal intensity, illustrating dynamic local changes in spatially resolved Ca\(^{2+}\) concentration during half-cell stretch (time progressing from back to front) and an acute increase in spark rate in the distended part of the cell only (individual spark dynamics remain unchanged). From Iribe et al. (2009), with permission.
Stretch, maintained over several minutes, additionally increases Ca\(^{2+}\) spark rate via a nitric oxide-mediated pathway (Petroff et al., 2001). This mechanism is not involved, however, in the acute stretch-induced increase in Ca\(^{2+}\) spark rate seen in rat ventricular myocytes (Iribe et al., 2009).

Intracellular free Ca\(^{2+}\) concentration in contracting cardiac myocytes is also affected by stretch-induced changes in Ca\(^{2+}\) buffering capacity, due to length-dependent modulation of the affinity of troponin-C to Ca\(^{2+}\) (Allen and Kentish, 1988). As a result, mismatched electrical and mechanical activity can give rise to mechanically induced Ca\(^{2+}\) waves, for example in ventricular myocardium (ter Keurs et al., 2008).

If mechanisms similar to those described above for acute and sustained changes in Ca\(^{2+}\) handling in response to stretch are present in pacemaker cells, they would be relevant for modulation of pacemaker activity through effects on the Ca\(^{2+}\)-oscillator. The potential importance of this is supported by recent evidence showing that acute changes in SAN intracellular Ca\(^{2+}\) concentration can result in immediate changes in BR (Yaniv et al., 2011). In addition, altered Ca\(^{2+}\) handling may affect the store-operated Ca\(^{2+}\) channel current (an inward Ca\(^{2+}\) current which depends on the level of depletion of intracellular Ca\(^{2+}\) stores). While not generally included as a ‘standard’ component of the Ca\(^{2+}\)-oscillator, this current has been implicated in modulation of pacemaker BR (Ju et al., 2007). This is thought to occur either directly via a background inward current that is modulated by beat-by-beat changes in SR Ca\(^{2+}\) content (Ju et al., 2007), or through interactions with NCX (Ju and Allen, 1998). Moreover, it has been suggested that this channel may be accounted for by transient receptor potential cation channel expression (Ju et al., 2007), and since type 1 of this protein may underlie SACOC (Maroto et al., 2005), it could also play a role in direct mechanical effects on cardiac pacemaker activity.

It is evident, then, that stretch of pacemaker cells alters automaticity, not only by activation of specialised mecano-sensitive ion channels in the sarcolemma and in sub-cellular membrane compartments, but also by affecting components of the V\(_{\text{m}}\) and Ca\(^{2+}\)-oscillators.

### 4. The mechanics-oscillator and its pathophysiological relevance

#### 4.1. Physiological-oscillator

##### 4.1.1. Chamber loading dynamics

The cardiac cycle involves a complex sequence of atrial and ventricular electro-mechanical activity. During atrial diastole, the tissue containing the SAN is stretched, initially by ventricular contraction that pulls the atrio-ventricular plane in an apical direction, promoting ventricular ejection by the development of significant positive pressure gradients compared to outflow arteries, and atrial filling at negative pressure gradients relative to inflow veins. This atrio-ventricular valve plane shift is a crucial determinant of the dual pressure-suction pump activity of ventricular contraction. Upon ventricular relaxation (and still during atrial diastole), the ventricles are passively re-filled to about 85% of their end-diastolic volume, simply by the return of the atrio-ventricular valves to their more basal resting position, resulting in stretch of the Purkinje network. After ‘diastasis’ (the pause between the end of ventricular, and the onset of atrial, activity), atrial contraction increases ventricular volume to its final end-diastolic level, prior to the next ventricular activation (thanks to the atrio-ventricular conduction delay).

As chamber dimensions are determined by volume (largely a function of venous return in the healthy heart), the above gives rise to loading dynamics which differ for the atria and ventricles (and, less prominently in terms of timing, for the right and left side of the heart). This is associated with different stretch cycles of pacemaker regions, such as the SAN and Purkinje system. Stretch effects on V\(_{\text{m}}\) dynamics of pacemaker tissue will therefore vary with time and with location. Pre-load-induced effects are maximal in the respective chamber-specific diastole — i.e., at the very time when V\(_{\text{m}}\) in pacemaker cells is moving towards initiation of the next AP. This means that physiological stretch will predominantly affect MDP and DD rate (rather than MSP, which is often altered during experimental application of stretch, such as during inter-ventions sustained throughout multiple cardiac cycles). Any additional change in driving forces for venous return (such as alterations in posture, or modulation of thorax-abdomen pressure gradients caused by respiratory activity, coughing, or defecation straining) will modulate the effect on DD. This has been employed, for example by using the Valsalva manoeuvre in heart transplant recipients (Ambrosi et al., 1995), to demonstrate the link between reduction in pre-load and termination of ventricular tachycardia.

Mechanical ‘priming’ of pacemaker cell electrophysiology by mechanical factors (the mechanics-oscillator) would act, therefore, in addition to and in combination with the V\(_{\text{m}}$/Ca\(^{2+}\)-oscillator, to adjust the pacemaking system on a beat-by-beat basis to diastolic load.

#### 4.1.2. Atrial effects

An increased right-atrial preload can accelerate SAN excitation, contributing to the matching of cardiac output (which is the product of stroke volume and RR) to venous return. Interestingly, as mentioned above, mechanically induced increases in BR are proportionally greater when stretch is applied at low rates, such as during vagus nerve activation, or pharmacological cholinergic stimulation (Barrett et al., 1998; Bolter, 1996; Deck, 1964b; Wilson and Bolter, 2001). This may help to prevent excessive slowing and diastolic (over-)distension, while maintaining cardiac output and adequate circulation (Brooks and Lange, 1977).

In contrast, appropriately maintained ‘minimal’ diastolic distension may be important for stabilisation of rhythm. In feline isolated SAN tissue preparations with irregular or no rhythm, it has been demonstrated that application of physiological levels of stretch can restore regular activity (Lange et al., 1966), an effect accompanied by a shift of MDP towards less negative values (see Fig. 4). In this setting, it was apparent that missed beats were due to the failure of other pacemaker oscillators to sustain spontaneous DD in the absence of a sufficient mechanical preload. As shown in Fig. 4A, stretch restored function by decreasing the gap between MDP and AP threshold. Likewise, stretch was seen to initiate spontaneous firing in quiescent SAN tissue by progressively reducing MDP, which resulted initially in the appearance of sub-threshold oscillations of V\(_{\text{m}}\) followed by spontaneous AP generation (see Fig. 4B). This pre-requirement for a minimum mechanical background stimulus is also apparent from observations on ontogenetic initiation of the first heartbeat. This has been linked to fluid pressure build-up in the quiescent cardiac tube, which is required not only for structurally competent pacemaker formation (Sankova et al., 2010), but even for the very initiation of spontaneous activity (Rajala et al., 1977).

These data demonstrate that a physiological mechanical environment is necessary for the initiation and maintenance of normal pacemaker function. Absence of physiological preloads affects pacemaking and can result in abnormal activity. Such lack of mechanical control is, however, common for experimental models used to investigate cardiac pacemaker mechanisms ex vivo.

Apart from the technical challenges associated with controlling the mechanical environment of isolated cardiac cells and tissue, it is not entirely clear what determines a physiological mechanical...
environment. Aspects that need to be addressed include: (i) whether the key mechanical parameter for changes in pacemaker function is stretch (Kamiyama et al., 1984; Sanders et al., 1979), tension (Arai et al., 1996; Brooks et al., 1966; Chiba, 1977), or a combination of both (Lange et al., 1966); (ii) whether there is an effect of the application rate of mechanical load (Brooks et al., 1966; Lange et al., 1966) or not (Chiba, 1977); and (iii) whether particular stretch patterns (linear, biaxial, concentric) are more appropriate than others (Deck, 1964b). Additionally, as stretch-responses (at least in ventricular myocytes) are AP phase-dependent (Calkins et al., 1991; Franz et al., 1992; Hansen et al., 1990; Nishimura et al., 2008), the timing of load application must be controlled to mimic desired patho/physiological states. Conceptual models of pacemaker function suggest that, late diastolic stretch (e.g., mimicking physiological preload) should speed-up BR, while systolic/early diastolic stretch (e.g., such as during pathophysiologically increased afterload or in dysynchronous contraction) should cause slowing. Thus far, responses have been studied largely with non-physiological (sustained, often excessive) stretch. In this setting, responses may be inconclusive, in part because of background BR-dependent effects (Cooper and Kohl, 2005).

4.1.3. Ventricular effects

In the ventricles, physiologically increased preload may support faster spread of excitation via the Purkinje system by a stretch-induced increase in Purkinje fibre conduction velocity (Deck, 1964a; Dominguez and Fozzard, 1979; Rosen et al., 1981) and enhanced automaticity (Sanders et al., 1979). That Purkinje fibres are indeed stretched during diastole (and buckle in systole) has been confirmed in sheep hearts fixed in the two different mechanical states (Canale et al., 1983). At the level of the whole heart, increased conduction velocity in the Purkinje system may be important in counter-acting the stretch-induced reduction in ventricular tissue conduction velocity reported upon increased ventricular filling (Sung et al., 2003), maintaining rapid and coordinated activation of the ventricles as preload is raised.

4.2. Pathophysiological loading

In diseases associated with atrial or ventricular volume- or pressure-overload, pathophysiological stretch-related effects may unbalance pacemaker mechanisms.

4.2.1. Atrial effects

In isolated SAN preparations, it has been observed that after an initial acceleration in BR, excessive stretch induces irregular rhythms (Lange et al., 1966) and multifocal activity (Hoffman and Cranefield, 1960), which can lead to the induction and sustenance of cardiac arrhythmias. In the whole body, SAN stretch (such as in patients with pulmonary arterial hypertension (McGowan et al., 2009), or in anesthetised pigs with targeted right-atrial distension (Horner et al., 1996)) reduces normal heart rate variability. This has been correlated with an increased risk of cardiac arrhythmias and sudden cardiac death (Chattipakorn et al., 2007; Sandercock and Brodie, 2006). Currently, relative contributions of stretch to heart rate variability per se, or of the stretch-induced increase in BR (which, in its own right, is associated with reduced variability), are unknown. This constitutes another important target of further investigation, especially in diseases associated with right atrial overload.

Pathophysiological states such as chronic atrial volume overload (Morton et al., 2003; Sanders et al., 2003; Sparks et al., 1999) and atrial fibrillation (Elvan et al., 1996; Kurmaga et al., 1991), or advanced age (Mohler and Anderson, 2008; Rubenstein et al., 1972), are often associated with fibrosis and tissue remodelling, and may be accompanied by SAN dysfunction. The validation of causal links in this setting is difficult, so the following discussion is largely hypothetical, to guide consideration of further investigations in this area. Changes in tissue characteristics will alter tissue mechanical properties and, potentially, restrict changes in chamber volume (while possibly increasing tension, a potentially important modulator of the stretch response (Arai et al., 1996; Brooks et al., 1966; Chiba, 1977; Lange et al., 1966)) during diastolic filling. At the same time, as overload of the atria increases chamber radii, similar absolute changes in volume will result in smaller changes in surface area, if the chambers are already enlarged (as a consequence of the surface area : volume ratio, which for a sphere is $3\times{\text{radius}}^2$ (Humphrey, 2002)). Alterations in tissue stiffness may be heterogeneous, introducing regional stretch differences, and thus the extent of changes in mechanical load experienced by cells in different areas of the tissue. The importance of tissue mechanics for the stretch-response is demonstrated by the observation that, for similar levels of stretch, changes in BR are greater in SAN preparations from younger animals (Deck, 1964b), where generally less fibrosis is present. The possible relevance of mechanical heterogeneity is corroborated by the observation that the central SAN is less extensible than the perinodal region, while changes in BR during externally applied force correlate best with maximum tissue strain in the periphery of the SAN (Kamiyama et al., 1984). This might be explained by the fact that the SAN periphery is where the (stretch-sensitive) $I_f$ is thought to play the largest role in pacemaker activity (due to a more negative MDP (Kreitner, 1985; Nikamaram et al., 1997), which will also increase the driving force for $SAC_{NS}$). Both $I_f$ and $SAC_{NS}$ furthermore affect transmission of electrical activity from SAN to atrium (which opposes SAN periphery depolarisation (Garny et al., 2003)). This could make electrical invasion of the atria more secure or faster during physiological levels of stretch (while disease-related changes in regional tissue mechanics could have deleterious effects). This will depend greatly on the pre-existing structural complexity of the SAN, which will result in
regional differences in stretch sensing, and variation across species (Nikolaidou et al., 2012). An additional mechanism worth considering is a possible change in the inherent mechano-sensitivity of SAN cells that may occur with disease or age, due to changes in expression and activity of mechano-sensitive channels (Boyett, 2009; Stones et al., 2008; Tellez et al., 2011). To determine the role and relative influence of the various factors potentially contributing to mechanically induced changes in SAN function will require experiments examining responses in isolated cells under mechanical load, from chronically volume- or pressure-overloaded hearts, as well as studies at different developmental stages.

In cases where disease-related changes in mechanics are accompanied by myocardial ischaemia, an additional current may be important for mechanical modulation of pacemaker function, the ATP-sensitive K\textsuperscript{+} current (Billman, 2008). This current, whose activity is increased by stretch in atrial and ventricular myocytes (Van Wagoner, 1993), has been shown to be present in rabbit isolated SAN cells (Han et al., 1996). By allowing K\textsuperscript{+} to exit the cell, activation of this current should hyperpolarise V_{m}, and therefore slow BR in the ischaemic heart.

### 4.2.2. Ventricular effects

Excessive loading of the ventricles may over-stretch Purkinje fibres, which, in isolated preparations, has been shown to result in reduced conduction velocity (Rosen et al., 1981) and eventual loss of AP activation (Dudel and Trautwein, 1954; Kaufmann and Theophile, 1967). In addition, as a consequence of increased DD rate and reduced MDP, focal ectopy (see Fig. 5A; Dudel and Trautwein, 1954; Hoffman and Cranefield, 1960) and tachycardia (see Fig. 5B; Dudel and Trautwein, 1954) have been observed. These effects have been proposed as mechanisms of ventricular bigeminy (Reynolds et al., 1975). So, like excessive stretch of the SAN, Purkinje fibre overload may play an important role in the induction and sustenance of cardiac arrhythmias.

The pathophysiological effects of Purkinje fibre network stretch are further complicated in chronic disease settings. Changes in chamber dimensions and tissue composition, structure, electrophysiology, and cellular coupling (such as are associated with heart failure, cardiomyopathies, ischaemia, and infarction) alter ventricular geometry, regional mechanical properties, and electromechanical function of myocardial tissue. This can result in altered regional ventricular stretch distributions, which may lead to increased dispersion of Purkinje fibre excitation. Dispersion of mechanically inactive regions by contraction of healthy myocardium will stretch attached and intramural Purkinje fibres, which may result in sub-threshold after-depolarisations (Ferrier, 1976), potentially promoting focal arrhythmias (Dudel and Trautwein, 1954; Hoffman and Cranefield, 1960). In patients with coronary artery disease, for instance, an increase in systolic aortic pressure with metaraminol infusion causes ventricular ectopy, which shows a strong correlation with the presence of segmental wall motion abnormalities (Stiogas et al., 1998). Similarly, focal stretch of the Purkinje fibre network has been implicated as a cause of idiopathic left ventricular tachycardia in patients with Purkinje system branches interconnecting the interventricular septum and posterior-inferior free wall (Thakur et al., 1996).

### 4.2.3. Suggested mechanisms

Thus, cardiac disease is often associated with changes in the magnitude and distribution of stretch and tension in the heart. This can lead to mechanically induced pathophysiological effects on primary and lower order pacemaker function. Abnormal activation leads to non-physiological excitation rates, reduction in heart rate variability, and increased dispersion of pacemaker electrophysiology, all of which may increase the risk of arrhythmias. These clinically relevant effects may occur directly via SACNS, or through V_{m}/Ca\textsuperscript{2+}-oscillator pathways. For instance, both I_{Ca,L} (Lyford et al., 2002) and the Na\textsuperscript{+} window current (Beyder et al., 2012) have been shown to be mechano-sensitive, and they can be involved in the generation of early after-depolarisations and ectopy in the SAN (Matsuda and Kurata, 1999; Miyamae et al., 1991) and Purkinje fibres (Fedida et al., 2006; January and Riddle, 1989; Nattel and Quantz, 1988; Orth et al., 2006). Importantly, both of these currents become increasingly relevant with depolarisation of MDP, a well-established consequence of SACNS activation.

### 5. Conclusion

The cardiac pacemaker control system is sensitive to the beat-by-beat changes in the mechanical environment that are present in vivo (the mechanics-oscillator). This affects pacemaking directly via stretch-activation of specialised mecano-sensitive ion channels in sarcolemmal and in intracellular membrane compartments, as well as by interaction with ‘standard components’ of the V_{m} and Ca\textsuperscript{2+}-oscillators.

The pacemaker mechanisms that are currently conceptualised as forming the V_{m}/Ca\textsuperscript{2+}-oscillator do not, as such, account for beat-by-beat adaptation in pacemaker activity: cellular Ca\textsuperscript{2+} balance changes measurably over the course of multiple beats (Vinogradova et al., 2005), while modification of sarcolemmal ion channel...
expression takes even longer. This is a dynamic system, with pronounced circadian dynamics, such as was shown initially for Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger surface-to-internalised ratio that doubles during the night/day activity cycle of mice (Shen et al., 2007), and more recently for a whole host of key players underlying cardiac cellular electrophysiology (Jeyaraj et al., 2012). The ability to adjust BR to diastolic filling, however, is required to adapt primary and lower order pacemaker function to varying loading conditions on a beat-by-beat basis, and involves contributions by mechanosensitive mechanisms to DD. While stretch is associated with the very beginning of cardiac rhythmic activity (Rajala et al., 1977) and remains a regulator of BR throughout life, in disease this system can destabilise pacemaker function, promoting irregular rhythms and the induction or sustenance of arrhythmias. All this calls for an improved understanding of the mechanical modulation of pacemaker electrophysiology.

This is an area which deserves more attention in basic research, as isolated cells – the main source of insight into the molecular mechanisms underlying Vm/Ca\textsuperscript{2+}–oscillator function – are not normally subjected to controlled mechanical environments. In vivo, however, pacemaker cells are continuously subjected to cyclic variations in haemodynamic load. Therefore, improved experimental approaches are needed to explore the complex interrelations between Vm, Ca\textsuperscript{2+}, and mechanical oscillators.

As highlighted before, the notion of separate ‘clocks’ is not helpful for a systematic understanding of integrated pacemaker function, as under normal conditions none of them work in isolation. Cyclic stretch, Ca\textsuperscript{2+}–handling, and Vm\textsuperscript{2+}–dynamics represent coupled systems. Their interaction prevents uncontrolled changes in BR, even when individual mechanisms are impeded. Such reciprocity of mechanisms involved in cardiac pacemaking was first demonstrated on the example of individual components of the Vm-oscillator, where I\textsubscript{Kr} and Na\textsuperscript{+}–background current behave in an inversely related manner. This prevents major alteration in BR when one or the other of the currents is subjected to increasing levels of block (Noble et al., 1992).

Thus, the entire pacemaker system is probably best considered as a Vm/Ca\textsuperscript{2+}/mechanics-oscillator, which integrates at the cellular level a multitude of inputs with a high degree of redundancy, generating a single output – automatically. This integrative approach will improve our ability to conceptually interpret normal and disease-related changes in primary and lower order pacemaker function.

Editors’ note

Please see also related communications in this issue by Markhasin et al. (2012) and Morris et al. (2012).

Acknowledgements

Work of the authors is supported by the Engineering and Physical Science Research Council of the United Kingdom, the British Heart Foundation, the Magdi Yacoub Institute, and the European Commission FP7 Virtual Physiological Human Initiative (Network of Excellence, preDiCT, euHeart).

References


Deck, K.A., 1964b. Dehnungseffekte am spontanschlagenden, isolierten Sinus-
De Francesco, D., Noble, D., 2005. The funny current has a major pacemaking role in
the sinus node. Heart Rhythm 9, 299–301.
Dobrzynski, H., Boyett, M.R., 1991. Stretch-induced increase in Na+ current in
rabbit sino-atrial node cells. J. Physiol. 224, 367–387.
cycle length and membrane ion channel clock underlie robust initiation and
regulation of pacemaker frequency in the isolated rabbit sinoatrial node. Jpn. J.
Physiol. 34, 153–187.
impairs sinus node function in dogs. Physiological remodeling. Circulation 94,
295–360.
Fedida, D., Orth, P.M., Hesketh, J.C., Ezrin, A.M., 2006. The role of late INa and
antiarrhythmic drugs in EAD formation and termination in Purkinje
Fedida, D., Orth, P.M., Hesketh, J.C., Ezrin, A.M., 2006. The role of late INa and
antiarrhythmic drugs in EAD formation and termination in Purkinje
February, K.A., 1964b. Dehnungseffekte am spontanschlagenden, isolierten Sinus-
knöchelchen. Pflugers Arch. Gesamte Physiol. 280, 120–130.
De Francesco, D., 1985. The cardiac hyperpolarizing-activated current, i, Origins and
Kamiyama, A., Niumara, I., Sugi, H., 1984. Length-dependent changes of
pacemaker frequency in the isolated rabbit sinoatrial node. Jpn. J. Physiol. 34,
131–141.
component of muscarinic control of the sinoatrial pacemaker in the rat. Clin.
Pharmacol. Ther. 88, 25–33.
Jarisch, A., Richter, H., 1939. Die afferenten bahnen des veratrine effektes in den
Jenner, T.A., Quinn, P., Kohl / Progress in Biophysics and Molecular Biology 110 (2012) 257–268