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## Review article

# Molecular candidates for cardiac stretch-activated ion channels

Alistair Reed<sup>1</sup>, Peter Kohl<sup>2,3,4</sup>, Rémi Peyronnet<sup>2,3,\*</sup>**ABSTRACT**

The heart is a mechanically-active organ that dynamically senses its own mechanical environment. This environment is constantly changing, on a beat-by-beat basis, with additional modulation by respiratory activity and changes in posture or physical activity, and further overlaid with more slowly occurring physiological (e.g. pregnancy, endurance training) or pathological challenges (e.g. pressure or volume overload). Far from being a simple pump, the heart detects changes in mechanical demand and adjusts its performance accordingly, both via heart rate and stroke volume alteration. Many of the underlying regulatory processes are encoded intracardially, and are thus maintained even in heart transplant recipients. Over the last three decades, molecular substrates of cardiac mechanosensitivity have gained increasing recognition in the scientific and clinical communities. Nonetheless, the processes underlying this phenomenon are still poorly understood. Stretch-activated ion channels (SAC) have been identified as one contributor to mechanosensitive autoregulation of the heartbeat. They also appear to play important roles in the development of cardiac pathologies – most notably stretch-induced arrhythmias. As recently discovered, some established cardiac drugs act, in part at least, via mechanotransduction pathways suggesting SAC as potential therapeutic targets. Clearly, identification of the molecular substrate of cardiac SAC is of clinical importance and a number of candidate proteins have been identified. At the same time, experimental studies have revealed variable – and at times contrasting – results regarding their function. Further complication arises from the fact that many ion channels that are not classically defined as SAC, including voltage and ligand-gated ion channels, can respond to mechanical stimulation. Here, we summarise what is known about the molecular substrate of the main candidates for cardiac SAC, before identifying potential further developments in this area of translational research.

*Keywords:* heart, mechanotransduction, mechano-electric feedback

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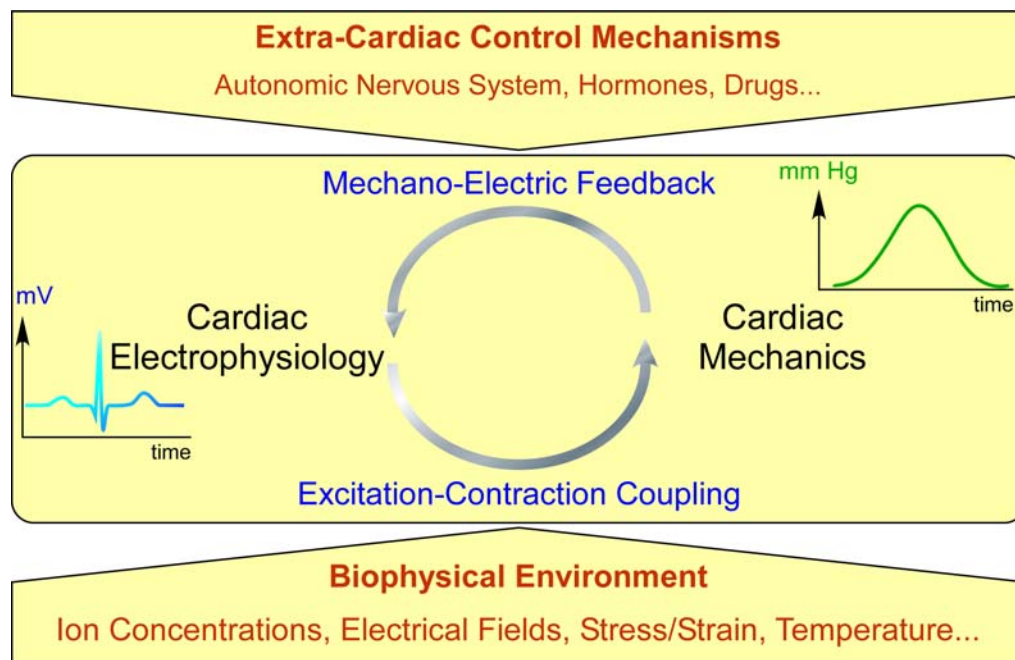
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## INTRODUCTION

Mechanosensitivity is an intrinsic property of cardiac functional autoregulation (Figure 1), affecting mechanical activity (e.g. via the Frank-Starling effect<sup>1,2</sup>: an acute stretch-induced increase in cardiac contractility in the absence of raised intracellular calcium) and electrical behaviour (e.g. through the Bainbridge effect<sup>3</sup>: the stretch-induced increase in spontaneous pacemaker rate) of the heart. These mechano-electric feedback (MEF) responses are sustained in denervated (e.g. isolated<sup>4-6</sup> or transplanted<sup>7-9</sup>) hearts, in isolated tissue<sup>10,11</sup> and even single cells – in both cardiac myocytes<sup>12-16</sup> and non-myocytes.<sup>17-19</sup>

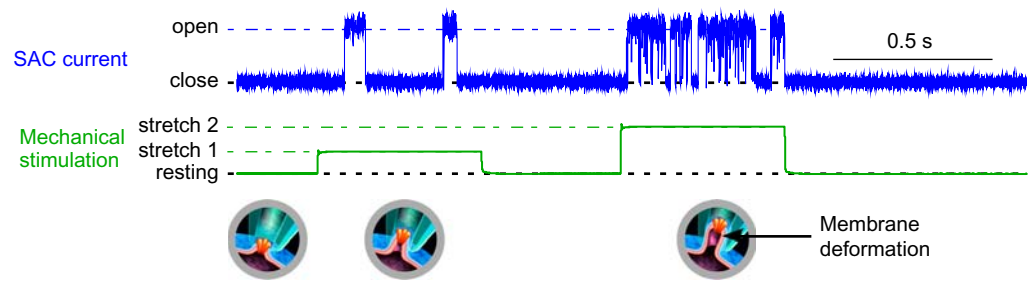


**Figure 1.** Simplified diagram of cardiac electromechanical integration. Cardiac electrophysiology controls cardiac mechanics via excitation-contraction coupling. Changes in the heart's mechanical environment from contraction or external interventions affect electrophysiology via mechano-electric feedback. This intra-cardiac regulatory loop (middle) is subject to modulation by extra-cardiac control (top) and environmental parameters (bottom).

Adaptation to a highly dynamic mechanical environment is a crucial feature of normal cardiac function. It is involved in the regulation of beat-by-beat physiology,<sup>4,20,21</sup> and implicated in the progression of cardiac diseases, including rhythm disturbances.<sup>22-25</sup> For a compendium of current insight into cardiac mechano-electric coupling and arrhythmias, from pipette to patient, see<sup>26</sup>. Although the mechanisms underlying cardiac mechanotransduction are not completely understood, key players are thought to include mechanosensitive ion channels (MSC). MSC are defined in the broadest sense by their ability to change ion channel open probability in response to mechanical stimuli, thereby converting mechanical energy into the modification of an electrochemical signal.<sup>27</sup> MSC have been demonstrated to act as functional mechanotransducers in a number of different tissues, including the heart, and their block is capable of preventing or terminating certain mechanically-induced arrhythmias.<sup>28,29</sup>

MSC can be subcategorised by the type of mechanical stimulation required for channel activation. Although these boundaries are far from clear-cut, it is useful to make this conceptual distinction. In this review we shall focus on *stretch*-activated ion channels (SAC), which are those MSC whose switching from 'closed' to 'open' state can be driven over their full dynamic range by stretch alone, for example through direct mechanical membrane deformation (Figure 2).

Another subcategory of MSC, *cell volume*-activated ion channels (VAC), are also considered to respond to mechanical stimuli,<sup>30</sup> but these differ in their micro-mechanical deformation properties (cell swelling increases cell diameter with less dominant effects on length, while axial stretch increases cell



**Figure 2.** SAC current recording (top) and patch pipette suction (middle) used for membrane deformation (bottom). Using the patch clamp technique, the most common way to cause mechanical stimuli is to apply negative pressure to the inside of the pipette; this induces membrane patch deformation (inserts). At the beginning of the protocol, after seal formation but in the absence of additional suction, channels are closed (no current). If negative pressure is applied (first pulse), channels open (here two openings of a single SAC). After a brief release, a higher level of suction is applied. This increases the open probability of the SAC (second pulse), but not the single channel conductance (same current amplitude). The original current trace shown here was taken from a potassium selective SAC; insets adapted from F. Aguila (CNRS), with permission.

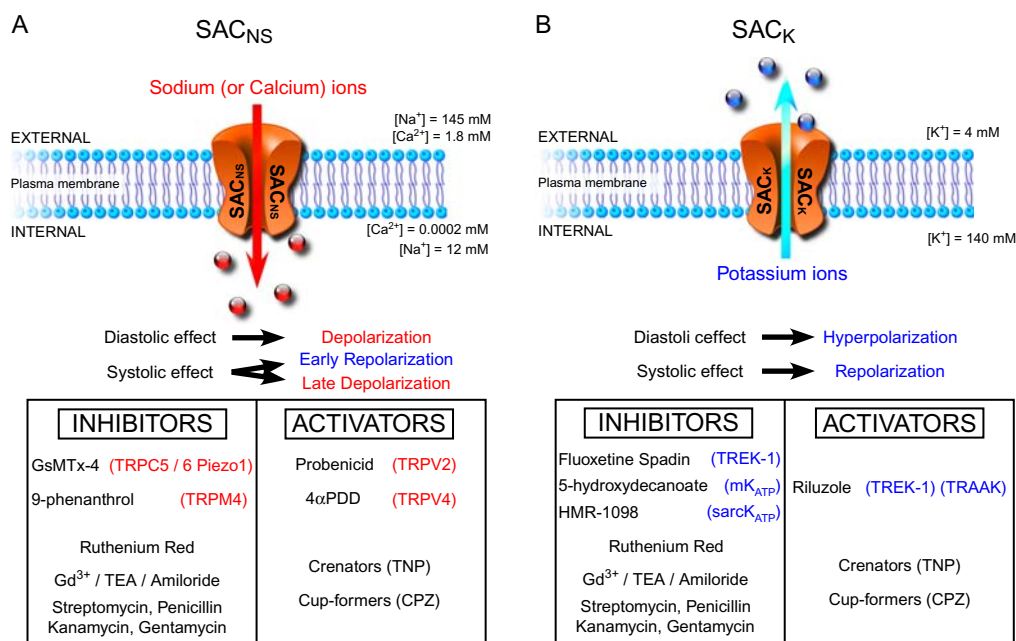
length and reduces diameter), time course of response (VAC usually show a lag-time of tens of seconds to minutes between the onset of cell swelling and channel opening, while SAC activate instantaneously), and pathophysiological context (it is assumed that – in contrast to ischaemia or hypertrophy – the normal cycle of contraction and relaxation is not associated with cell volume changes). For these reasons, VAC are unlikely to be main contributors to acute and/or beat-by-beat responses of the heart to mechanical stimuli, and they will not be considered in detail here (for a review on VAC, see<sup>30</sup>).

SAC were discovered in 1984 in embryonic chick skeletal myocytes by Guharay and Sachs.<sup>31</sup> In subsequent years, SAC have been identified in many other cell types<sup>32,33</sup> including cardiomyocytes.<sup>34</sup> Cardiac SAC can be either cation non-selective (SAC<sub>NS</sub>)<sup>34</sup> or potassium-selective (SAC<sub>K</sub>)<sup>35</sup> (Figure 3). The development of the patch clamp technique was vital for the study of cardiac SAC, and it revealed, in addition to stretch-activated whole-cell currents, evidence of single-channel activity in atrial myocytes,<sup>35</sup> foetal<sup>34</sup> and (for SAC<sub>K</sub> at least) adult ventricular myocytes,<sup>36</sup> as well as cardiac non-myocytes.<sup>19</sup> That said, formation of membrane patches is associated with significant alterations in local mechanical and structural properties, especially in complex and densely ‘crowded’ cells such as cardiac myocytes. This leaves the potential for false-positive (e.g. channels that would normally be protected from opening, such as by cytoskeletal interaction) and false-negative observations (channels that are constitutively activated by patch formation may not be identified as mechano-sensitive upon additional patch deformation). This highlights the importance of multi-level investigations, combining a range of electrophysiological recording techniques, from lipid bilayers to sub-cellular and cellular studies in expression systems and native cells, to cultures, tissue slices, native tissue and organs, right through to whole animal or patient research. As pointed out elsewhere, much of this hinges on the availability of improved pharmacological agents, and it requires quantitative structure-based integration, such as by computational modelling.

First insights into the structure and possible mechanisms of operation of these channels were provided by the cloning and crystallization of two bacterial SAC.<sup>37,38</sup> However, even after an exhaustive search, no sequence homologues of these particular ion channels were found in mammals. The first cloned mammalian SAC was the TREK channel (a ‘tandem of two-pore K<sup>+</sup> domains in a weak inwardly rectifying K<sup>+</sup> channel’ = TWIK-related potassium channel).<sup>39</sup> Despite these significant steps, the molecular identities of mammalian *cardiac* SAC have yet to be determined.

In spite of a lack of firm molecular identification, there are several prominent candidates for mammalian cardiac SAC, and these will be reviewed here. For SAC<sub>NS</sub> these include transient receptor potential (TRP) channels, and Piezo1. SAC<sub>K</sub> candidates are TREK-1, the large-conductance calcium-activated K<sup>+</sup> channel (BK<sub>Ca</sub>; a member of the ‘Big K<sup>+</sup>’ channel family), and the ATP-sensitive potassium channel (K<sub>ATP</sub>); see Table 1.

In the following, we evaluate the available evidence for presence and contributions of these main cardiac SAC candidates, including their sensitivity to pharmacological interventions, highlight some of the present experimental challenges, and conclude with a consideration of anticipated further



**Figure 3.** Overview of cation non-selective (SAC<sub>NS</sub>) and potassium-selective (SAC<sub>K</sub>) channel function, effects, and pharmacological modulators. A. SAC<sub>NS</sub> opening leads to sodium and possibly calcium entry (in addition to also present potassium fluxes); this depolarises the plasma membrane during diastole, repolarises it during early systole, and may cause after-depolarisation-like behaviour in later stages of action potential repolarisation, depending on the current's reversal potential (usually in the mid-region between systolic and diastolic peak potentials). B. SAC<sub>K</sub> opening leads to potassium exit from the cytoplasm; this acts to hyperpolarize the plasma membrane during diastole, and to repolarize it during systole. SAC modulators are summarized in boxes, with the more selective ones at the top of each table. Channels: mK<sub>ATP</sub>: mitochondrial K<sub>ATP</sub>, sarK<sub>ATP</sub>: sarcolemmal K<sub>ATP</sub>, Gd<sup>3+</sup>: gadolinium ions, TEA: tetraethylammonium, TNP: trinitrophenol, CPZ: chlorpromazine.

developments in this exciting and dynamic field of translational heart research. We will not discuss alternative mechano-sensors, detailed signalling pathways, or protein-protein interactions, all of which form deserving topics for separate reviews.

### SAC<sub>NS</sub>

Whole-cell currents with a linear current-voltage relationship attributed to SAC<sub>NS</sub> ( $I_{SAC,NS}$ ), were first identified in cardiac cells by Craelius et al.,<sup>34</sup> using whole-cell patch clamp recordings from neonatal rat ventricular myocytes. By applying a voltage clamp, Zeng et al.<sup>40</sup> later described the properties of this current further, including a lack of inactivation and a pronounced sensitivity to block by gadolinium ions (Gd<sup>3+</sup>). The channel's reversal potential is positive to the resting potential of working cardiomyocytes, so that activation of SAC<sub>NS</sub> will depolarise resting cells.<sup>27</sup> In contrast to SAC<sub>K</sub>, SAC<sub>NS</sub> are distinctly sensitive to a peptide, isolated by Sachs et al. from Chilean tarantula venom: GsMTx-4 (Grammostola spatulata Mechano-Toxin 4<sup>41</sup>). The use of GsMTx-4 has allowed researchers to extend the evidence on whole-cell  $I_{SAC,NS}$  towards identification of SAC<sub>NS</sub> effects at the tissue and whole organ levels. At the same time, no SAC<sub>NS</sub> single-channel recordings from freshly-isolated adult ventricular cardiomyocytes have been reported. This has led to the suggestion that SAC<sub>NS</sub> may be localised in membrane regions that are difficult to access in patch clamp studies, such as transverse tubules (T-tubules<sup>42</sup>), caveolae (which, themselves, form a mechanosensitive structural domain that may be integrated into the surface sarcolemma by excess stretch<sup>43</sup>), or at intercalated discs.<sup>44</sup> The main molecular candidates for cardiac SAC<sub>NS</sub>, TRP channels<sup>45</sup> and the recently discovered Piezo1 protein,<sup>46</sup> will be discussed in more detail.

### TRP CHANNELS

TRP proteins form a family of widely expressed cation channels, responsible for a variety of cellular functions. Polymodal regulation is a distinct feature of TRP (<http://www.ncbi.nlm.nih.gov/gene/724608>).

**Table 1. Summary of the currently known main molecular candidates for stretch-activated ion channels in cardiac myocytes. TT: T-tubules, S: sarcolemma; N/S: not specified.**

Ion channels	Species	Location	
		Cellular	Sub-cellular
SAC <sub>NS</sub> <i>TRPC1</i>	Human <sup>48</sup>	Myocardium	N/S
	Rat <sup>42</sup>	Atrial myocyte	S
<i>TRPC6</i>	Human <sup>48</sup>	Ventricular myocyte	TT
	Mouse <sup>58</sup>	Myocardium	N/S
	Mouse <sup>62</sup>	Ventricular myocyte	TT
<i>TRPV2</i>	Mouse <sup>62</sup>	Myocardium	N/S
<i>TRPV4</i>	Neonatal rat <sup>64</sup>	Ventricular myocytes	Nucleus
<i>TRPM4</i>	Rat <sup>158</sup>	Myocardium	N/S
<i>Piezo1</i>	Rat <sup>74</sup>	Myocardium	N/S
SAC <sub>K</sub> <i>TREK-1</i>	Rat <sup>80</sup>	Atrial myocyte	S
		Ventricular myocyte	N/S
<i>BK<sub>Ca</sub></i>	Chick embryo <sup>94,129</sup>	Ventricular myocyte	S
<i>K<sub>ATP</sub></i>	Neonatal rat <sup>159</sup>	Atrial myocyte	S

Known activators of TRP channels include chemical stimuli, temperature elevation, and mechanical interventions ranging from local patch deformation to membrane stretch and shear strain.<sup>47</sup> In particular, the so-called 'canonical' TRP channels TRPC1 (<http://www.ncbi.nlm.nih.gov/gene/7220>) and TRPC6 (<http://www.ncbi.nlm.nih.gov/gene/7225>) have been implicated as candidates for cardiac SAC<sub>NS</sub>.

**TRPC1:** Analysis of mRNA expression suggested that TRPC1 is present in the human heart.<sup>48</sup> Using immuno-histochemical labelling and confocal imaging, TRPC1 protein was found to colocalise with phalloidin stain in rat ventricular myocytes.<sup>42</sup> This suggests that TRPC1 may be located in T-tubules and is consistent with the hypothesised spatial distribution of endogenous SAC<sub>NS</sub> in adult ventricular cardiomyocytes.

Mechanosensitivity of TRPC1 was first noted by Maroto et al.<sup>49</sup> in *Xenopus* oocytes. In their experiments,  $I_{SAC,NS}$  was measured after membrane protein fractionation and reconstitution of individual proteins in liposomes. A particularly mechanosensitive fraction was found to contain an 80-kDa protein which was immunoreactive to TRPC1 antibody, indicating the presence of a TRPC1 homologue. Further expression of the human TRPC1 (hTRPC1) isoform in *Xenopus* oocytes and Chinese hamster ovary (CHO) K1 cells increased  $I_{SAC,NS}$  tenfold, whereas microinjection of antisense hTRPC1 RNA greatly reduced  $I_{SAC,NS}$  in both cell types.

Since publication, these findings have been challenged by several studies, including one by some of the authors of the original report. They found that transfection of hTRPC1 into COS cells (a fibroblast-related cell line, originally derived from kidney tissue of monkey) had no discernible effect, while transfection of a different putative SAC (the SAC<sub>K</sub> TREK-1; see below), induced an increase by three orders of magnitude in mechanosensitive whole-cell currents. This result puts into question the significance of the less pronounced (ten-fold) increase seen in the earlier experiments.<sup>50</sup> The authors of the later study found limited ion channel expression at the sarcolemma, which is in agreement with a more recent report showing predominantly intracellular expression of transfected TRPC1 in a skeletal muscle cell line, unless co-expressed with Cav3 (<http://www.ncbi.nlm.nih.gov/gene/859>).<sup>51</sup> Thus, even if TRPC1 is successfully transfected, it may require associated molecular machinery for a correct subcellular localization and/or proper function. In addition, TRPC1 may require other TRPC isoforms to form a functional heteromeric channel.<sup>52</sup>

The conflicting results reported above highlight problems that can be associated with the use of heterologous expression systems to study cardiac ion channels. Clearly, the intracellular environment of stable cell lines differs significantly from that of cardiomyocytes, while additional transfection with exogenous ion channels can alter the structure and function of recipient cells.<sup>50</sup> Given the dependence of SAC gating properties on micro-mechanical and structural properties of a cell, it is difficult to establish suitable control protocols,<sup>50</sup> or to arrive at definitive conclusions from these experiments.

Interestingly, mice in whom TRPC1 has been knocked out (TRPC1<sup>-/-</sup>) exhibit no significant difference in ventricular slow force response (SFR, initially characterised in cat papillary muscle,<sup>53</sup> is a gradual,

calcium-related increase in muscle contractility upon exposure to maintained stretch), compared to wild-type controls.<sup>54</sup> This suggests that TRPC1 may not be an obligatory and/or exclusive component of the SFR (similar findings were reported for TRPC3 (<http://www.ncbi.nlm.nih.gov/gene/7222>)<sup>55</sup>). However, as with all knockout experiments, there is always the possibility of compensatory changes in expression of other genes. One way of assessing this would be to use acute knockdown experiments, ideally involving tissue-specific drivers of protein expression. It would also be instructive to explore acute MEF responses that would be expected to precede the SFR in cardiac myocytes or tissue preparations of TRPC1<sup>-/-</sup> mice.

**TRPC6:** Mammalian TRPC6 was initially identified as a mechanosensitive ion channel by Spassova et al.,<sup>56</sup> who found that overexpression of TRPC6 in human embryonic kidney cell line 293 (HEK293) cells induced I<sub>SAC,NS</sub>. However, a subsequent study by Gottlieb et al.<sup>59</sup> found that TRPC6 overexpression in CHO and COS cells had no significant effect. More recently, it has been suggested that TRPC6 is not mechanosensitive, unless co-expressed with the angiotensin II type 1 (AT1) receptor.<sup>45,47</sup> Data, more directly relevant for cardiac mechanosensitivity, came from Dyachenko et al.,<sup>58</sup> who used mouse ventricular myocytes, as opposed to heterologous expression systems. Their whole-cell patch clamp experiments identified a robust I<sub>SAC,NS</sub> in response to shear stimuli, which was inhibited by pore-blocking TRPC6 antibodies. TRPC6 knockout blunts the SFR in wild-type murine models, while its genetic down-regulation or pharmacological block returns 'hyper-responsive' murine models of Duchenne muscular dystrophy back to normal SFR levels,<sup>55</sup> highlighting the potential clinical relevance of targeted TRPC6 manipulation.

TRPC6 is among a small number of SAC candidates that is highly expressed in human heart homogenates.<sup>48</sup> In murine heart, TRPC6 appears to be localised to T-tubules.<sup>58</sup> In agreement with this observation, detubulation inhibits I<sub>SAC,NS</sub> in murine cardiomyocytes.<sup>58</sup> Interestingly, a recent paper has suggested that the localization of TRPC6 shows marked plasticity in response to sympathetic stimulation via  $\alpha$ 1A receptors, and that these channels can translocate from T-tubules to the sarcolemma.<sup>59</sup> Whether this occurs physiologically is unclear; however, pre-treatment with  $\alpha$ 1A-agonists might serve as a useful experimental intervention to facilitate single-channel recordings of TRPC6, and potentially other channels localised in T-tubules, in adult ventricular myocytes.

**Other TRP channels:** Several other members of the TRP family are mechanosensitive and are expressed in the heart. The TRPC3 protein has been identified in rat ventricular myocytes, also located in T-tubules.<sup>60</sup> Mechanical stretch of neonatal cardiomyocytes in a mouse model displays TRPC3 overexpression induced ROS production, similar to TRPC3-activation by 1-oleoyl-2-acetyl-sn-glycerol (OAG). However, OAG is a fairly unspecific activator of TRPC channels, so effects cannot be attributed with confidence to TRPC3.<sup>60</sup> The vanilloid TRP channel 2 (TRPV2 (<http://www.ncbi.nlm.nih.gov/gene/51393>)) has been reported to be mechanosensitive (studied using cell-volume changes and patch pipette suction<sup>61</sup>) and is expressed in the mouse heart.<sup>44,62</sup> Using the TRPV2 agonist probenicid in wild-type and TRPV2<sup>-/-</sup> constitutive knockout mice, it was shown that this channel appears to contribute to baseline cardiac function, participating in the calcium-handling machinery of heart cells.<sup>62</sup>

TRPV4 (<http://www.ncbi.nlm.nih.gov/gene/59341>) mRNA is weakly expressed in cardiac muscle,<sup>63</sup> and TRPV4 protein was located in cultured neonatal rat ventricular myocytes only in the nucleus.<sup>64</sup> However, caution is warranted here, regarding mRNA or protein expression in tissue. Firstly, mRNA and protein expression levels do not necessarily correlate<sup>65</sup> and secondly, while high expression levels are confirmatory of a significant presence and indicative of functional relevance, low expression in whole tissue homogenates should be interpreted with care. If a protein is present in minority cell populations of the heart (such as Purkinje fibres), it could be of immense functional relevance, even if it was only detected at trace levels. In addition, some SAC, such as TREK, may be expressed at the membrane, but can be strongly inhibited in resting conditions,<sup>66</sup> making the assessment of availability of functional channels even more difficult.

Finally, the melastatin TRP channel 4 (TRPM4) is expressed in cardiomyocytes,<sup>45</sup> and has been implicated in stretch-activated responses of vasculature smooth muscle cells.<sup>67</sup> Overexpression of TRPM4 may be involved in an inheritable form of progressive familial heart block type I,<sup>68</sup> and the identification of a possible stretch-activated component of this disease – mediated by TRPM4 – would be of pronounced clinical relevance. Thus, in addition to TRPC1 and TRPC6, the ion channels TRPC3, TRPV2, TRPV4 and TRPM4 form translationally-relevant targets for further basic and applied research.

### Piezo1

The discovery of Piezo1 and Piezo2 (<http://www.ncbi.nlm.nih.gov/gene/63895>) by Patapoutian's group in 2010<sup>46</sup> represents one of the most important breakthroughs in the field of mechanotransduction in recent years. Piezo1 was initially identified in the neuro-2a neuronal cell line by siRNA knockdown of the expression of membrane proteins with unknown function. Knockdown of the FAM38A (<http://www.ncbi.nlm.nih.gov/gene/415849>) gene inhibited  $I_{SAC,NS}$  and the gene product was named Piezo1. Mechanosensitivity was confirmed by heterologous expression of Piezo1 in HEK cells, which induced a robust  $I_{SAC,NS}$ . Piezo1 is huge, containing approximately 2,500 amino acids, arranged in 24–32 transmembrane domains that assemble as a tetramer.<sup>69</sup> Intuitively, this massive structure, and the associated large surface area, could be well-adapted for sensing changes in bilayer curvature and/or stretch. In line with this hypothesis, it has been shown that Piezo1 gating is associated with dimensional changes.<sup>70</sup>

Currently, no data has been published directly addressing Piezo1 mechanosensitivity in the heart. However, Piezo1 channel electrophysiological properties are similar to that of endogenous cardiac  $SAC_{NS}$ , including weak voltage dependency, comparable single channel conductance, inactivation, and sensitivity to GsMTx-4.<sup>71–73</sup> Furthermore, Piezo1 mRNA is expressed in the murine heart<sup>46,74</sup> albeit at low levels (see comment on whole-tissue expression levels, above). Piezo1 is involved in erythrocyte volume homeostasis. Morpholino-mediated knockdown of Piezo1 results in swelling and lysis of red blood cells and consequent anemia.<sup>75</sup> Interestingly, this function is close to that of bacterial mechanosensitive channels of large and small conductance (MscL and MscS).<sup>76</sup>

Undoubtedly this is an exciting and dynamic area of development. Basic science questions concerning structure, protein partners, and regulation of Piezo1 need to be addressed,<sup>77</sup> as does the question of whether Piezo1 is present in, and relevant for, the human heart.

### $SAC_K$

Whole-cell  $SAC_K$  currents ( $I_{SAC,K}$ ) were first described by Kim et al.<sup>35</sup> in rat atrial myocytes. In contrast to  $SAC_{NS}$ ,  $SAC_K$  are outwardly rectifying, and as such, allow potassium ions to move more easily out of the cell than into it. Compared to  $SAC_{NS}$ ,  $SAC_K$  tend to have larger single channel conductances. They also inactivate in a time-dependent manner and are generally insensitive to GsMTx-4.<sup>78</sup> Being potassium-selective, their reversal potential lies negative to the resting membrane potential of cardiac cells, so activation of  $SAC_K$  will generally cause membrane repolarisation or hyperpolarisation.<sup>27</sup> To date, single-channel recordings of  $I_{SAC,K}$  in adult mammalian cardiac myocytes have been obtained from atrial<sup>35</sup> and ventricular myocytes,<sup>36,79,80</sup> suggesting that their subcellular compartmentalization differs from  $SAC_{NS}$ . Primary molecular candidates for cardiac  $SAC_K$  are TREK-1,  $BK_{Ca}$  and  $K_{ATP}$ .

### TREK-1

TREK-1 is a member of the two-pore domain potassium channel family, which is associated with a 'leak' potassium ion conductance in cardiomyocytes.<sup>81</sup> TREK-1, however, displays more complex permeation and gating properties than a simple 'leak' channel, and is regulated by a number of factors including pH, temperature, second messenger systems, and membrane deformation/stretch.<sup>82</sup> Mechanosensitivity was attributed to TREK-1 by Patel et al.<sup>39</sup> based on single-channel patch clamp recordings from transfected COS cells. Subsequently, Terrenoire et al.<sup>83</sup> demonstrated that  $I_{SAC,K}$  (endogenous to rat atrial myocytes) displays a number of properties that bear striking similarities to recombinant TREK-1 channels. This includes their single-channel conductance, lack of voltage dependency, 'burst mode' activity, and sensitivity to pharmacological interventions, and, in particular, a unique sensitivity to volatile anaesthetics not shared by other cardiac potassium ion channels.<sup>81</sup>

A number of studies have identified TREK-1 mRNA expression in rat atria, as well as in left, right, and septal ventricular myocytes.<sup>83–86</sup> The protein appears to be arranged in longitudinal stripes at the surface of cardiomyocytes: a pattern that might support directional stretch sensing.<sup>80</sup> At the tissue level, TREK-1 expression is distinctly heterogeneous, with a gradient of mRNA expression that increases, transmurally, from epicardial to endocardial cells.<sup>86</sup> This heterogeneity appears to correlate with transmural changes in MEF sensitivity, where stretch causes the most pronounced action potential shortening in the sub-endocardium.<sup>87</sup> To our knowledge, mRNA analysis thus far has failed to identify TREK-1 expression in the human heart.<sup>88,89</sup> It has been suggested that the TWIK-related arachidonic acid-activated potassium channel (TRAAK (<http://www.ncbi.nlm.nih.gov/gene/50801>)) or TWIK-related

acid-sensitive potassium channel (TASK-1 (<http://www.ncbi.nlm.nih.gov/gene/3777>)) of the K2P family, which appear to be expressed in the human heart,<sup>90</sup> may act as TREK-1 homologues. Indeed, when TRAAK is expressed in heterologous model systems, it forms a channel with very similar properties to TREK-1.<sup>91</sup> Characterisation of both the presence and functional relevance of these ion channels in human requires further elucidation.

### **BK<sub>Ca</sub>**

BK<sub>Ca</sub> channels have large conductances, and they respond to voltage changes and alterations in intracellular calcium ion concentration in a manner that allows them to contribute to repolarisation.<sup>92</sup> Functionally, BK<sub>Ca</sub> channels have been suggested to control heart rate and to offer cardioprotection during ischaemia.<sup>93</sup> Kawakubo et al.<sup>94</sup> identified mechanosensitivity of BK<sub>Ca</sub> channels in membrane patches excised from cultured embryonic chick ventricular myocytes. Attempts to measure single-channel activity in post-hatch chick cardiomyocytes have been unsuccessful, although Iribe et al.<sup>95</sup> characterised a whole-cell I<sub>SAC,K</sub> which was sensitive to iberiotoxin (a BK<sub>Ca</sub> inhibitor). Interestingly, this I<sub>SAC,K</sub> was also sensitive to extracellular sodium. The authors suggest that BK<sub>Ca</sub> activation could occur secondary to mechanical modulation of sodium ion influx (e.g. via SAC<sub>NS</sub>), and consequentially shift sodium-calcium exchanger activity towards preservation of intracellular calcium. If this is the case, BK<sub>Ca</sub> might not be directly stretch sensitive.

Whether or not BK<sub>Ca</sub> channels are responsible for I<sub>SAC,K</sub> in embryonic chick cardiomyocytes, there is little evidence to suggest that BK<sub>Ca</sub> channels form cardiac SAC<sub>K</sub> in other species. In the human heart, BK<sub>Ca</sub> channels are sparsely expressed,<sup>92</sup> and they may be confined to cardiac fibroblasts (where BK<sub>Ca</sub> was detected using Western blot<sup>96</sup>). However, functional fibroblast-myocyte electrical coupling can occur (at least in some areas of the heart<sup>97,98</sup>) so it is possible that stretch-activation of BK<sub>Ca</sub> channels in cardiac fibroblasts could still affect myocyte function via a heterocellular coupling pathway.

### **K<sub>ATP</sub>**

Although classically considered to be ligand-gated, Van Wagoner et al.<sup>99</sup> obtained single-channel inside-out patch clamp recordings from neonatal rat atrial myocytes which revealed that patch pipette negative pressure increased K<sub>ATP</sub> channel ATP-sensitivity.<sup>99</sup> Synergistically, ATP-reduction potentiated stretch sensitivity.<sup>99</sup> Ischaemia, simulated in adult guinea pig ventricular myocytes by application of a metabolic uncoupler, also uncovered K<sub>ATP</sub> mechanosensitivity that was absent in control conditions,<sup>27</sup> an observation that was more recently confirmed in rat cardiomyocytes.<sup>100</sup> The synergism for K<sub>ATP</sub> channel activation by metabolic and mechanical stress might explain differences in quantitative aspects of ATP reduction needed to activate K<sub>ATP</sub> in isolated cells, compared to the organ level (where K<sub>ATP</sub> open at less depleted ATP levels). A reason for this difference could be the fact that isolated cells are not normally subjected to mechanical co-activation, while at the organ level ischaemia is usually associated with decreased shortening, or even stretch, of the tissue affected by reduced availability of ATP.

In keeping with this notion, 'stretch-preconditioning' has been reported to reduce ischaemia-reperfusion injury, an effect that disappears when K<sub>ATP</sub> channels are blocked.<sup>68</sup> Moreover, cardiac fibroblasts progressively express functional K<sub>ATP</sub> channels in scar and border zone tissue following infarction, suggesting that we must consider the effect of cells other than cardiomyocytes in pre-/post-conditioning of the heart, and the role of SAC in these processes.<sup>101–103</sup>

Although there is little evidence to suggest that K<sub>ATP</sub> are responsible for mechanosensitivity of the heart in normal beat-by-beat physiology (in healthy cells and tissue, diastolic mechanical stimulation depolarizes cardiomyocytes), the potential role of these ion channels in ischaemic or other disease conditions warrants further research.

### **SAC MODULATORS**

Several pharmacological compounds have been identified to modulate SAC activity (Figure 3),<sup>104,105</sup> and their potential role as pharmacological tools for heart rhythm management has been previously reviewed by White.<sup>106</sup>

Most of the known SAC-modulators are non-specific inhibitors, such as gadolinium ions, amiloride and cationic antibiotics (streptomycin, penicillin, kanamycin). Among the very few specific SAC inhibitors<sup>107</sup> reported so far is the peptide GsMTx-4. It inhibits TRPC<sub>5</sub> when activated by hypo-osmotic and receptor stimulation,<sup>108,109</sup> as well as TRPC<sub>6</sub>,<sup>56</sup> and Piezo1 channels when applied to the external



face of the membrane.<sup>72,73</sup> GsMTx-4 is active both in its D and L enantiomers, showing the mechanism of action is not stereospecific or chiral.<sup>110</sup> Instead, the mode of action of GsMTx-4 is thought to involve insertion into the outer membrane leaflet in the proximity of the channel, relieving lipid stress and favouring the closed state of SAC.<sup>110</sup> Counterintuitively, GsMTx-4 sensitizes the bacterial channels, MscS and MscL, to tension,<sup>111</sup> while it has no effect on TREK-1 channels,<sup>72</sup> so that overall the mode of action of GsMTx-4 still requires further elucidation.

TREK-1 is poorly responsive to classic potassium channel blockers,<sup>112</sup> but its functions are modified by a variety of pharmacologic agents such as volatile anaesthetics,<sup>112</sup> riluzole,<sup>113</sup> fluoxetine<sup>114</sup> and spadin.<sup>115,116</sup> Recently, new modulators of TREK-1 were identified by Bagriantsev et al.<sup>117</sup> They characterized inhibitors and, importantly, activators (which are very rare for SAC channels). Known openers for SAC are amphipathic substances which insert selectively into one membrane leaflet only, locally inducing either concave or convex curvature, which may induce SAC-activating tension.<sup>118</sup>

Other useful substances include probenidic, which is a TRP agonist that is fairly specific to TRPV2,<sup>119</sup> and 9-phenanthrol, which blocks TRPM4.<sup>120</sup>

It is important to note, though, that not all SAC blockers that work at the level of isolated or cultured cells are equally efficient in native tissue. Streptomycin, for example, may not be able to easily access SAC in whole cardiac tissue,<sup>121</sup> even though it is an efficient SAC<sub>NS</sub> blocker in single cardiomyocytes (an important consideration for cell-culture based work, which often employs media containing streptomycin by default). This will be one of the reasons for which antibiotics, such as streptomycin, can be prescribed to patients without instantaneous side effects on stretch-sensing. Another compound, Gd<sup>3+</sup>, is used clinically in a chelated formulation, which explains the lack of pronounced immediate SAC-effects in radio-contrast studies. As an aside, Gd<sup>3+</sup> precipitates in physiologically buffered solutions.<sup>122</sup> Clearly caution is called for when assessing (potentially false-) negative results on Gd<sup>3+</sup> effects in physiological solutions, or on streptomycin effects *in vivo*.

## DISCUSSION

The heart is a superbly mechanosensitive organ. SAC are thought to provide one of the mechanisms underlying cardiac MEF,<sup>20,123,124</sup> the process by which changes in the mechanical environment of the heart lead to altered cardiac electrical activity. Identification of molecular substrates for cardiac SAC will not only provide novel insight into processes that underlie MEF, but also support the long-term aim of developing SAC-specific drugs for the treatment of mechanically induced cardiac pathologies.<sup>106</sup>

In terms of physiological beat-by-beat effects, activation of SAC has been shown to underlie the stretch-induced increase in spontaneous sino-atrial node (SAN) cell pacemaking rate.<sup>16</sup> Block of SAC using GsMTx-4 terminates this positive chronotropic response in SAN tissue<sup>121</sup> which, in its guise of a respiratory sinus arrhythmia, persists at whole body level – even in heart transplant recipients after additional pharmacological denervation.<sup>125</sup>

At the same time, sustained pressure and/or volume overload favour arrhythmogenesis.<sup>25,124,126,127</sup> Application of SAC-blockers such as GsMTx-4 has been shown to reversibly reduce the preload dependent increase in both incidence and duration of burst-pacing induced atrial fibrillation in isolated heart experiments.<sup>28</sup> In patients, it can be difficult to distinguish stretch-induced changes in electrophysiology from other chronically occurring aspects of structural and functional remodelling. However, an impressive illustration of acute effects of ventricular loading has been provided by Waxman et al.,<sup>128</sup> who showed that performing the *Valsalva manoeuvre* may terminate ventricular tachycardia by temporary reduction of ventricular filling. The *Valsalva manoeuvre*, an attempt to forcefully exhale against the closed glottis, increases intrathoracic pressure, favouring a net reduction of intravascular volume in the chest (i.e. impeding venous return and favouring arterial drainage to other parts of the body). In this study, the reduction in cardiac dimensions was confirmed radiographically. Cessation of ventricular tachycardia coincided with removal of ventricular strain, while arrhythmia resumption occurred upon refilling after the end of the manoeuvre. Since this type of response can be seen not only in neurologically intact, but also in pharmacologically<sup>128</sup> or surgically<sup>7</sup> denervated patients (transplant recipients), it is not attributable to a nervous reflex. This highlights how removal of strain may unmask the presence of stretch-induced arrhythmias, even in a chronic setting.

Various SAC have been implicated in the heart's (patho-)physiological responses to mechanical stimuli, but in the absence of firm identification of molecular substrates for cardiac SAC, successful mechanistic exploration of cardiac mechanosensitivity is a challenging task.

Conceptually, it is pragmatic to subdivide SAC into two categories, SAC<sub>NS</sub> and SAC<sub>K</sub>. For both, there are several candidate proteins. SAC<sub>NS</sub> were initially thought to be formed by TRP proteins and, most convincingly, TRPC6 antibodies inhibit whole-cell I<sub>SAC,NS</sub> in mouse ventricular myocytes.<sup>58</sup> However, subsequent heterologous expression studies yielded conflicting results.<sup>50,56</sup> More recently, attention has turned towards the newly discovered Piezo1 channels.<sup>46</sup> Although there is no published data yet on specific electrophysiological effects of Piezo1 in cardiomyocytes, comparative kinetic analysis suggests that these proteins may function as cardiac SAC<sub>NS</sub>. In as far as cardiac SAC<sub>K</sub> are concerned, recombinant TREK-1 has remarkably similar properties to endogenous SAC<sub>K</sub>,<sup>78</sup> but the protein has yet to be identified in human heart.<sup>88</sup> BK<sub>Ca</sub> channels have also received considerable interest, but their stretch-sensitivity, if present,<sup>95</sup> may be limited to immature<sup>129</sup> and/or non-mammalian cardiomyocytes,<sup>94</sup> or to cardiac connective tissue.<sup>96</sup> Finally, K<sub>ATP</sub> channels display metabolic and mechanical co-activation,<sup>99</sup> which may help explain some of the differences between experimental and clinical findings on the extent of ATP-reduction needed to activate them. In addition, this insight could shed light on hitherto ill-explored links between ischaemic and mechanical preconditioning.

As will be apparent from the above, the currently available information on the molecular substrates of cardiac SAC poses more questions than it answers. A number of reasons contribute to this. It is notoriously difficult to control and/or quantify the extent and quality of local mechanical stimuli that an individual ion channel is exposed to.<sup>130</sup> Tools to apply strain at whole-cell, tissue, and organ levels exist (including the application of shear stress, axial stretch, or cell volume changes), but there is no commonly implemented 'gold standard' for the stimulation of SAC.<sup>27</sup> Furthermore, these techniques have been used with a wide variety of cellular models from different species and developmental stages, making cross comparison of results challenging. In addition, it is difficult to interrelate macroscopic interventions and observations at cell and tissue levels with molecular substrates: in part because there is no 'zero-strain' reference even in patch clamp studies. Attempts to explore causal links from low-level mechanism to integrated response, and back, include changes in gene expression,<sup>87</sup> pharmacological interventions,<sup>131</sup> and computational modelling.<sup>132–135</sup>

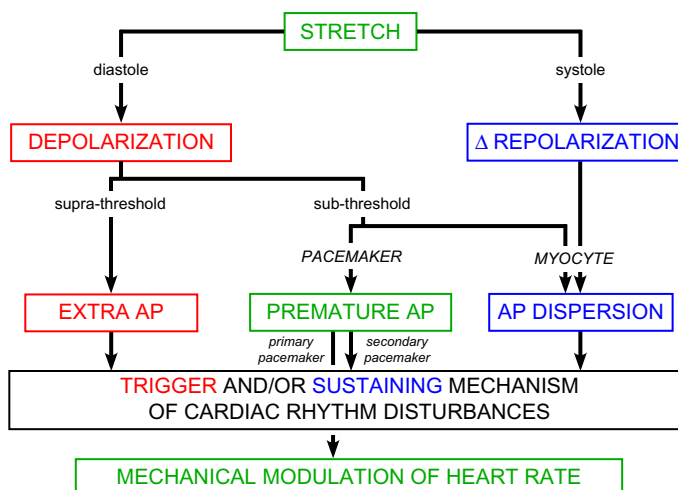
Further challenges arise from the possibility that ventricular SAC may be localised in T-tubules, caveolae, or intercalated discs. This is thought to explain why patch clamping of single SAC is so rare in freshly isolated ventricular cardiomyocytes from adult mammals.<sup>130</sup> One possible way around this problem may be to use pre-exposure to  $\alpha$ 1A receptor stimulation, to aid SAC translocation from T-tubules to the sarcolemma.<sup>59</sup> Another would be pre-stretching of the cardiac tissue prior to cell isolation, as this can cause surface membrane incorporation of caveolae.<sup>43</sup> Thirdly, one could isolate the T-tubules using sequential centrifugation of homogenised cardiomyocytes followed by purification of T-tubule membranes by vesicle immuno-isolation and reconstitution into a continuous membrane.<sup>136</sup> It might then be possible to directly patch clamp SAC on the isolated T-tubule membrane. Less invasively, scanning ion conductance microscopy, which generates a three-dimensional topographical map of the cell surface prior to patch clamping, has been suggested as a means to directly target the T-tubule ostium where SAC are more likely to be present.<sup>137</sup> On the other hand, there is evidence to suggest that SAC may activate indirectly via second messenger signalling cascades.<sup>138</sup> Therefore patch clamping single ion channels (where SAC activation probably occurs as a direct result of bilayer deformation<sup>130</sup>) may provide only a partial picture of patho-physiological function.

In addition to SAC in the outer cell membrane, there may be non-sarcolemmal SAC in the sarcoplasmic reticulum<sup>12</sup> or mitochondria.<sup>139,140</sup> Cardiac non-myocytes are also mechanosensitive and exhibit electrophysiological properties modulated by mechanical stimuli.<sup>18,141–143</sup> Channels, such as Nav1.5 and TRPM7, that were initially identified as stretch-modulated in non-cardiac cell types,<sup>144,145</sup> have now been found in cardiac fibroblasts.<sup>146,147</sup>

Finally, there is a growing body of evidence to suggest that many cardiac ion channels, even those that are not classically considered as SAC (e.g. voltage- or ligand-gated channels), are sensitive to mechanical modulation of their gating behaviour.<sup>148</sup> Future research should therefore focus on characterising the mechanical stimuli experienced by cardiomyocytes *in vivo*, so that they can be more closely replicated *in vitro*. This can be aided greatly by high-resolution imaging of the beating heart,<sup>149</sup> followed by whole heart histological reconstruction<sup>150,151</sup> and subsequent computational re-integration of tissue deformation<sup>152,153</sup> with a granularity that allows identification of local stress-strain dynamics<sup>154</sup> and prediction of microstructural effects on electrophysiology.<sup>155</sup> Direct measurement, and validation of modelling predictions, currently suffers from a number of technical limitations, in particular the inability to measure locally acting forces *in situ*. The recent development of Förster

Resonance Energy Transfer (FRET)-based force sensors that can be genetically inserted into intracellular proteins,<sup>156</sup> may open up a treasure chest of novel insight if they can be applied to heart research. These force sensors are based on the energy transfer between two compatible fluorophores. The efficacy of the energy transfer is inversely proportional to the distance between the donor and the acceptor, multiplied by  $10^6$ , making the FRET signal very sensitive to small distance changes. Meng and Sachs<sup>157</sup> have calibrated their probe using DNA to be able to quantify forces from fluorescent signal changes. These sensors constitute a very powerful tool for the assessment of the mechanical state in components of single cells or tissues. Until now little is known about forces within the cell/cytoskeleton, both when cells are at rest, or while mechanically stimulated. In addition, intracellular force reporters would be very useful to improve our understanding of the interplay of SAC with other mechanosensors, like integrins and the cytoskeleton.

Armed with a more thorough understanding of physiological mechanical stimuli, and novel techniques, we expect to improve our understanding of the molecular substrate of cardiac SAC, and to better predict their pathophysiological roles for the regulation of heart rate and rhythm in the mechanosensitive heart (Figure 4).



**Figure 4.** Timing-, amplitude-, and target-dependent stretch effects on heart rhythm. AP: action potential,  $\Delta$ : change in. Adapted from Kohl *et al.*, 1998, with permission.

## CONCLUSION

The heart is an integrated electro-mechanical system (Figure 1). SAC (Figure 2) are key contributors to cardiac mechanosensitivity.  $SAC_K$  generally cause re- (or even hyper-) polarisation, while  $SAC_{NS}$  depolarise resting tissue and have differential effects on activated cells (speeding up early, delaying late, repolarisation; Figure 3). Systemic effects of SAC activation therefore depend on timing (relative to the cardiac cycle), intensity (e.g. relative to excitation threshold), and location (relative to the different components of cardiac tissue; Figure 4). Thus, diastolic stretch may accelerate heart rate, or even trigger extra beats, depending on whether pacemaker cells or working myocardium drive the response. This is believed to underlie mechanical pacing by pre-cordial percussion, and the occasionally observed termination of re-entrant arrhythmias upon application of pre-cordial thump.<sup>160–162</sup> Systolic stretch will alter repolarisation dynamics which, in particular in the context of spatially heterogeneous mechanical stimuli and/or regionally varying expression of SAC, may act as an arrhythmia-sustaining mechanism for mechanically-induced rhythm disturbances.

We are on the brink of obtaining an improved understanding of the molecular substrates of cardiac SAC, and of the way in which macroscopic mechanical interventions translate into stimuli at subcellular levels. This is hoped to lead to improved insight into the physiological relevance of SAC, their involvement in acute and chronic diseases, and to guide the development of novel means to targeting cardiac SAC for therapeutic benefit.

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## COMPETING INTERESTS

None.

## LIST OF ABBREVIATIONS

BK<sub>Ca</sub>: Large-conductance calcium-activated potassium channel  
 CHO: Chinese hamster ovary cell line  
 GsMTx-4: Grammostola spatulata Mechano-Toxin 4  
 HEK: Human embryonic kidney cell line  
 I<sub>SAC,K</sub>: Potassium-selective stretch-activated current  
 I<sub>SAC,NS</sub>: Cation non-selective stretch-activated current  
 K<sub>ATP</sub>: ATP-sensitive potassium channel  
 MEF: Mechano-electric feedback  
 MSC: Mechanosensitive ion channels  
 OAG: 1-oleoyl-2-acetyl-sn-glycerol  
 SAC: Stretch-activated channels  
 SAC<sub>K</sub>: Potassium-selective SAC  
 SAC<sub>NS</sub>: Cation non-selective SAC  
 SFR: Slow force response  
 TASK: TWIK-related acid-sensitive potassium channel  
 TRAAK: TWIK-related arachidonic acid-activated potassium channel  
 TREK: TWIK-related potassium channel  
 TRP: Transient receptor potential  
 TRPC: Canonical TRP channel  
 TRPM: Melastatin TRP channel  
 TRPV: Vanilloid TRP channel  
 TWIK: Tandem of two-pore K<sup>+</sup> domains in a weak inwardly rectifying K<sup>+</sup> channel  
 VAC: Volume-activated ion channels

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