

Species- and Preparation-Dependence of Stretch Effects on Sino-Atrial Node Pacemaking

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ABSTRACT: Acute dilation of the right atrium (e.g., via increased venous return) raises spontaneous beating rate (BR) of the heart in many species. Neural mechanisms contribute to this behavior *in vivo*, but a positive chronotropic response to stretch can also be observed in isolated right atrial tissue preparations and even at the level of single sino-atrial node (SAN) cells. The underlying mechanism has previously been reported to be compatible with stretch-activation of cation nonselective ion channels (SAC). This review reports species peculiarities in the chronotropic response of isolated SAN tissue strips to stretch: in contrast to guinea pig, murine SAN preparations respond to distension with a reduction in spontaneous BR. This differential response need not necessarily involve disparate (sub-)cellular mechanisms, as SAC activation would occur against the background of very different SAN electrophysiology in the two species. On the basis of single SAN cell action potential recordings, this review illustrates how this may give rise to potentially opposing effects on spontaneous BR. Interestingly, streptomycin (a useful SAC blocker in isolated cells) has no effect on stretch-induced chronotropy *in situ*, and this is interpreted as an indication of protection of SAC, in native tissue, from interaction with the drug.

KEYWORDS: guinea pig; *in situ*; mouse; streptomycin; stretch activated channels; volume activated channels

INTRODUCTION

Changes in venous return to the heart do not only affect the volume available for rapid ventricular filling and subsequent atrial contraction, but also diastolic atrial dimensions. Increased right atrial filling distends the atrial wall, including the sino-atrial node (SAN), which impinges on pacemaker function. Francis Bainbridge showed in 1915 that injection of fluids into the jugular vein of anaesthetised dogs elevates venous return and increases cardiac beating rate (BR).¹ This was attributed to a vagal response and is referred to as the *Bainbridge Reflex*. Several investigators repeated Bainbridge's experiments, and the majority indeed observed tachycardia upon increased venous return (although some found little change, or even bradycardic responses; for reviews, see Refs. 2 and 3).

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Ann. N.Y. Acad. Sci. 1047: 324–335 (2005). © 2005 New York Academy of Sciences.
doi: 10.1196/annals.1341.029

Subsequent studies in isolated hearts⁴ and SAN tissue⁵ revealed that—even in denervated preparations—stretch of the SAN pacemaker may give rise to a positive chronotropic response. More recent work, involving acute and chronic pharmacological blockade of intrinsic cardiac autonomic pathways, illustrated that this intrinsic component of the positive chronotropic response to stretch is not mediated via the activity of cardiac neurones, either.⁶ Furthermore, experiments in isolated, spontaneously active SAN pacemaker cells confirmed that axial stretch may reliably and reversibly increase their spontaneous BR via a mechanism that involves stretch activation of an ion current with the properties of cation non-selective stretch activated channels (SAC).⁷ This data confirms that Bainbridge's observation is encoded, in part at least, at the level of SAN pacemaker cells.

The stretch-induced increase in BR observed in single cells (+5% of initial spontaneous BR⁷) is significantly smaller than that seen in multicellular preparations (+15% to +40%^{4,5}) and whole animal studies (+32% in dog;¹ +23.5% in man⁸). Clearly, a multitude of factors—such as the level of investigation, biological preparation, amplitude of stretch, experimental techniques and tools—affect SAN mechanosensitivity and subsequent electrophysiological behavior.

There are, however, similarities in the electrophysiological responses reported from SAN preparations at various levels of functional integration. Thus, the electrophysiological signature of the SAN's positive chronotropic response to stretch is (1) a reduction in maximum diastolic potential (less negative), (2) a reduction in maximum systolic potential (less positive), and (3) an increase in BR.

Also, at each level of investigation, the response to stretch is affected by the characteristics of mechanical stimulation (the larger the rate of rise and/or amplitude of stretch, the more pronounced the BR response) and by background BR (the lower the initial BR, the larger the potential effect of stretch within a given species). The latter has been investigated in some depth by Coleridge and Linden,³ who studied the influence of anaesthetics employed in whole animal experiments (dog) to replicate the observations of Bainbridge.¹ They found that the type of anaesthetic influenced background BR of the heart, and that bolus fluid injections could increase BR at low initial BR (50–100 bpm) or, conversely, decrease BR at high initial BR (150–200 bpm). This may help to explain some of the variability of findings reported by researchers who attempted to reproduce Bainbridge's observations *in vivo*.

Here, we investigate whether in addition to (or instead of) variations in background BR, species differences in action potential (AP) characteristics may affect SAN responses to mechanical stimulation. This is assessed using SAN tissue isolated from species with very different cardiac electrophysiological background characteristics: guinea pig and mouse.

METHODS

Right atrial tissue containing the SAN was dissected from Langendorff-perfused hearts of either guinea pig ($n = 46$) or mouse ($n = 25$), after 5 min of coronary perfusion with physiological saline solution. Standard solution contained (in mM): NaCl 118, KCl 4.8, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, Glucose 11, CaCl₂ 1.8; it was bubbled with 95% O₂/5% CO₂, maintained at 37°C, and checked for correct osmolarity (297–305 mOsm, A0300; Knauer, Berlin, Germany).

The SAN region was identified as the area on the posterior wall of the right atrium between the superior and inferior *venae cavae*, *Crista terminalis* (*CT*) and atrial septum. One to three SAN tissue strips were cut perpendicular to the *CT*, near the leading pacemaker site. Silk suture loops (7/0, Ethicon Johnson and Johnson; St-Stevens Woluwe, Belgium) were tied to either end of the strips (*CT* and atrial septum), and attached to miniature hooks on a vertical force transducer (FORT10; WPI, Stevenage, UK) and a micrometer. Manual adjustment of the micrometer allowed setting of resting diastolic tension (0.1 g) and permitted length changes for experimental intervention (usually between 25%–60% of strip length, except for FIGURE 1 where length was increased by up to 123% of strip length for reference purposes). The tension developed by the preparation was recorded online using Acknowledge 3.7 software and Biopac MP150 data acquisition equipment (Biopac Systems Inc., Goleta, CA). BR was determined in real-time as the inverse of the peak-to-peak interval of active force recordings.

Sino-atrial node tissue preparations were placed in a water-jacketed organ bath containing 50 mL of physiological saline solution (at 37°C, unless otherwise stated) and allowed to equilibrate for 30 min for stabilization of BR. Pharmacological agents, when used, were added directly to the superfusate and allowed to equilibrate for at least 10 min (in some cases this was subsequent to coronary perfusion with the same drug for 5 min prior to SAN dissection). Complete exchange of the organ bath solution occurred every 15 min.

All stretch protocols were performed on the background of a stable BR. Based on previous data in the literature on rabbit SAN stretch,⁹ passive tension was adjusted (via the micrometer screw) to the following levels relative to control diastolic tension: +0.5 g, +1.0 g, +1.5 g, and +2.0 g. Tissue strips were held at the new length for about 30 s (during which passive tension would, after an initial tissue-viscosity related peak, decline to a quasi-steady state), before length was returned to the original (pre-stretch) level. A recovery period of at least 5 min followed each intervention.

Within this range of passive diastolic tension increases, the BR response rose linearly with the amplitude of stretch (see FIG. 1B). It is important to note that these tension steps, modelled after a prior report,⁹ cause very significant strain of the SAN tissue strips (e.g., +28 to +64% at +0.5 g, and +85 to +123% at +2.0 g). This extent of SAN tissue lengthening clearly exceeds expected (patho-)physiologically relevant changes *in situ* (a rise in right atrial pressure by 10 mmHg, observed in heavy exercise,¹⁰ has been related to a 30% change in SAN strip length).⁵

Subsequent investigations, therefore, employed the smallest tension level at which a rate response could be reliably observed (+0.5 g, unless otherwise stated), to avoid non-physiological strain and possible injury-related artefacts.

All drugs (except GsMTx-4, courtesy of Dr. Fred Sachs) used in this study were sourced from Sigma-Aldrich Ltd. (Poole, UK) and dissolved appropriately to prepare stock solutions on the day of the experiment. Final concentrations used were as follows: 1 mM 9-anthracene carboxylate (9-AC); 40 to 500 μ M streptomycin sulphate; 0.1 to 2 μ M acetylcholine.

Background BR was determined by averaging the rate of contractions during 15 s prior to application of stretch. During stretch, both peak BR (as in Arai *et al.*⁹) and sustained changes (averaged over 30 s) were assessed. Recovery BR at original tissue length was monitored for 5 min after return to control length. Analysis of peak

BR responses to stretch did not provide qualitatively different results to the sustained responses reported in the following text (although both the overall amplitude and the variability of the response were larger).

Where data is presented as a group's mean, the error is expressed as the standard error of the mean (mean \pm SEM). Statistical analysis was performed in Prism (v4.0; GraphPad, San Diego, CA), using Student's t-test or ANOVA, where appropriate, and significance of null-hypothesis rejection was accepted at $p < 0.05$.

RESULTS

Effect of Stretch

In order to describe the effect of stretch characteristics on SAN pacemaking rate in guinea pig tissue strips, mechanical stimulation was initially applied at a number of tension levels. Usually, BR peaked with maximum passive tension and then declined during the subsequent reduction in tissue stress (attributed to gradual lengthening of tissue viscous elements; see example in FIG. 1A). Upon release of stretch, BR recovery was not necessarily instantaneous and could require a period of up to 15 s.

FIGURE 1B illustrates that the stretch-induced increase in guinea pig SAN BR is linearly related, within the tested range of mechanical stimuli, to the extent of passively applied tension, and reaches 5.0% to 14.4% of initial BR values ($p = 0.006$). This is similar (although slightly less pronounced) to the increase in peak BR observed by Kodama *et al.*¹¹ and Arai *et al.*,⁹ who used rabbit SAN tissue strips. Dif-

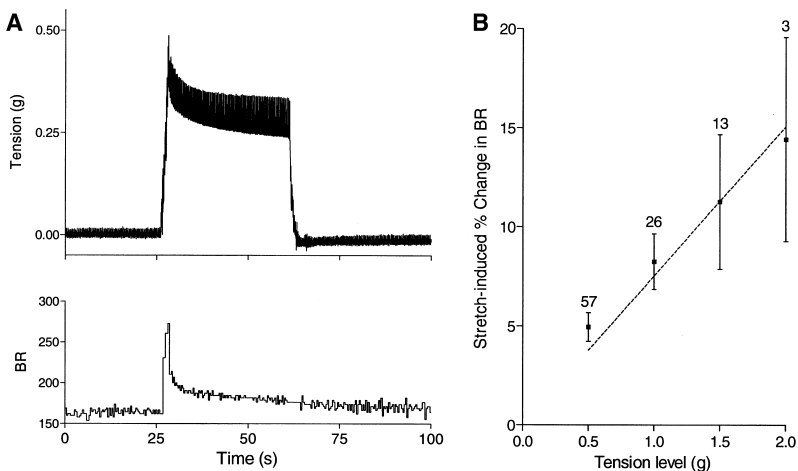


FIGURE 1. Effect of stretch on guinea pig sino-atrial node tissue. (A) *Upper panel* shows stretch-induced change in tissue tension (by $<+0.5$ g), and the *lower panel* shows the concomitant increase in beating rate (BR). (B) Mean (\pm SEM) percentage increase in BR recorded at different tension levels ($p = 0.006$, ANOVA, across all groups; numbers above columns refer to n values).

ferences may be species dependent or related to minor differences in experimental procedures or data analysis (e.g., peak vs. maintained response).

In comparison, axial stretch of single rabbit SAN cells (by up to 8% of cell length, using carbon fibers) causes an increase in spontaneous BR of approximately 5%,⁷ which is quite compatible with the tissue response observed in this investigation at the lower levels of mechanical stimulation.

Mechanisms

It has been suggested that mechano-dependent ion channels may underlie the stretch-induced increase in heart rate.¹² There are at least two possible candidates for this response: cation non-selective SAC⁷ and cell-volume activated chloride channels (VAC).⁹

Arai and colleagues^{9,11} found that application of various VAC blockers caused a reduction in the stretch-induced increase in BR of rabbit isolated SAN tissue (which was significant only at high levels of tissue distension). To reassess this, we applied mechanical stimuli at the lower, more physiological range reported in the literature (+0.5 g).⁹ Even this intervention causes strains that potentially exceed the levels normally observed *in situ*.⁵ Application of 1 mM 9-AC (a blocker of VAC) has *no effect* on the stretch-induced increase in BR (FIG. 2), supporting the notion that VAC do not underlie the SAN response to (near-) physiological stress or strain.

As in the study by Arai *et al.*,⁹ pharmacological block of VAC reduces background BR (i.e., in the absence of mechanical stimulation). This is different from observations made in single isolated SAN pacemaker cells, where a 9-AC induced reduction in spontaneous BR was only observed subsequent to hyposmotic cell swelling.¹³ It is likely that tissue dissection and/or experimental conditions (i.e., colloid-free perfusion) will cause cell swelling in multicellular preparations (despite checking osmotic pressure of solutions). In any case, the observed reduction in BR in the presence of 9-AC would, if anything, favor the identification of a stretch-induced increase in BR. Importantly, it confirms that 9-AC is likely to have reached

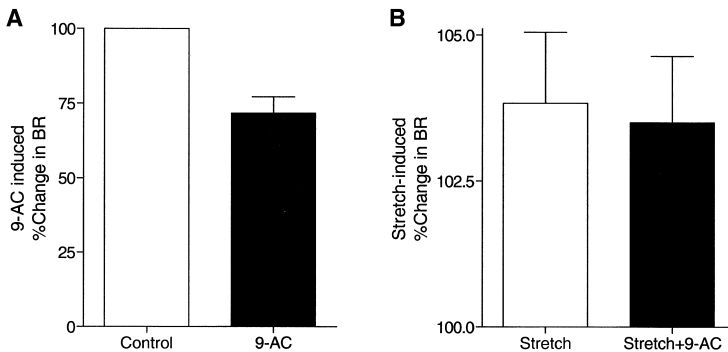


FIGURE 2. Effect of 9-AC (1 mM) on guinea pig sino-atrial node. **(A)** 9-AC reduced initial heart rate to $71.7 \pm 5.5\%$ ($n = 3$, $p = 0.035$). **(B)** 9-AC had no significant effect on the stretch-induced increase in beating rate ($n = 3$, $p = 0.865$).

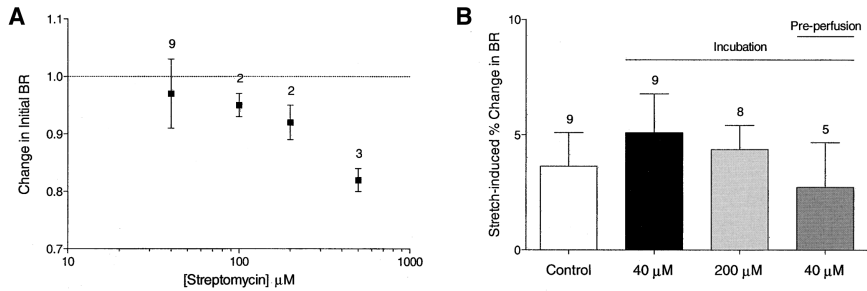


FIGURE 3. Effect of streptomycin on guinea pig sino-atrial node (SAN) tissue. (A) Negative chronotropic effect of high streptomycin concentrations (100–500 μM , $p = 0.006$) on spontaneous beating rate (BR) in guinea pig SAN tissue strips. (B) Streptomycin (40 and 200 μM) did not affect the stretch-induced increase in spontaneous BR ($p = 0.76$), regardless of whether the drug was applied to the superfusate only (Incubation), or additionally by 5 min coronary perfusion prior to SAN tissue extraction (Pre-perfusion). Numbers above columns indicate n values for each group.

VAC inside the SAN tissue strip, thereby making it less probable that the lack of a 9-AC effect on stretch-induced chronotropy is a false-negative finding.

To investigate the alternative possibility that activation of SAC might underlie the stretch-induced increase in BR, we applied streptomycin at concentrations from 40 to 500 μM .¹⁴ At the lowest concentration (40 μM), streptomycin blocks SAC relatively selectively in isolated cardiac cells. At higher concentrations the drug is known to affect Ca^{2+} transients (200 μM or more) and to inhibit the L-type Ca^{2+} current (by 50% at 2 mM).¹⁵ In keeping with the latter, high concentrations (100–500 μM) of streptomycin reliably and reversibly reduced spontaneous BR ($p = 0.006$; FIG. 3A) and contractility ($p = 0.02$; not shown) of guinea pig SAN tissue strips. This observation lends credibility to the suggestion that streptomycin reaches pacemaker cells inside the isolated SAN tissue strips. The streptomycin-induced reduction in background BR was observed regardless of whether the drug was applied by coronary perfusion prior to tissue dissection, or via the superfusate only (where the effect occurred within 5 min).

Interestingly, streptomycin did *not* affect the ability of SAN tissue to respond to distension by an increase in spontaneous BR, neither at low concentrations that are known to block SAC in isolated cells (40 μM), nor at levels that are high enough to affect background BR in the present multi-cellular preparation (200 μM ; see FIG. 3B). This was, again, observed regardless of the drug application protocol, including coronary pre-perfusion and incubation for durations of up to 50 min.

Species Differences

The overall response to stretch, observed in the guinea pig preparations, is similar to previous observations in other slow-beating species, such as the rabbit. To assess the effect of species differences, we compared stretch effects on BR in guinea pig to those in mouse hearts, which show a much higher average BR (240 bpm and 450 bpm, respectively). FIGURE 4 shows a summary of stretch responses in guinea pig and mouse SAN tissue strips. Some of the experiments were performed at a reduced tem-

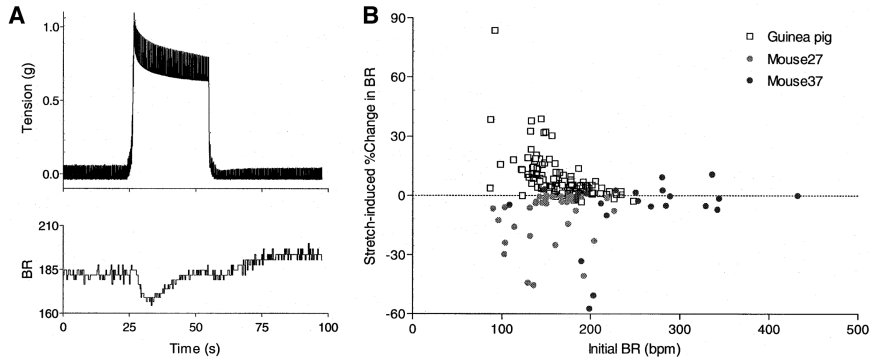


FIGURE 4. Species differences in sino-atrial node (SAN) beating rate (BR) response to stretch. **(A)** *Upper panel* shows change in passive tension level (here to $<+1.0$ g) with a stretch intervention, and the *lower panel* shows the concomitant BR reduction in a mouse SAN strip, recorded at 27°C . **(B)** Summary of stretch responses in guinea pig at 37°C and mouse SAN tissue strips recorded at either 37°C (Mouse37) or 27°C (Mouse27), plotted as a function of initial BR.

perature (27°C instead of 37°C) or after application of acetylcholine ($0.1\text{--}2\ \mu\text{M}$) in order to extend the range of background BR and their overlap between the two species.

In line with earlier suggestions, reduced background BR appears to enhance the stretch-induced effects on chronotropy if studied *within* a species. Thus, in guinea pig the average stretch-induced increase in BR was $+13.8\%$ from a control BR of 58 ± 5 bpm (at 27°C), yet only $+9.8\%$ from a control BR of 150 ± 12 bpm (at 37°C , $n = 4$, $p = 0.0004$, paired observations; not shown).

More striking, however, is the observation that the BR-dependence of stretch effects on BR cannot be extrapolated between species. Thus, murine SAN tissue strips overwhelmingly responded to stretch with a *reduction* in BR—a negative chronotropic response (FIG. 4)—which is also more pronounced at lower background BR. The stretch-induced negative chronotropy was observed over a wide range of background BR levels, which overlapped completely with the BR range of guinea pig (FIG. 4B) or rabbit⁹ (two species that show a positive chronotropic response to stretch).

These responses (either positive or negative chronotropy) are independent of neuronal influences, as experiments conducted in the presence of $1\ \mu\text{M}$ atropine and $1\ \mu\text{M}$ propranolol did not alter the response (guinea pig: $n = 6$, $p = 0.67$; mouse: $n = 12$, $p = 0.77$; not shown).

DISCUSSION

In many species employed in classical physiology studies (including man⁸), the heart responds to stretch of the SAN region with an increase in spontaneous BR. This has been observed in whole animals, isolated hearts, SAN tissue strips, and single SAN cells. However, the mechanisms underlying this response have not yet been comprehensively identified.

In this study, we used tissue strips from the right atrium, including the SAN region, from guinea pig and mouse. In order to keep the strain of SAN preparations closer to physiological limits, we employed changes in tissue tension at the low end of those used in previous experimental investigations. Additionally, stretch interventions were applied only up to three times per strip (during control, drug application, and washout, for analysis of paired observations). This is different from previous work, where multiple stretch interventions across large tension ranges (from +0.5 g, over +1.0 g, and +1.5 g, to +2.0 g) were repeated 8 to 12 times per strip.⁹

We found in this study that repeated mechanical stimulation—in particular after high levels of stretch—required larger length changes in order to elicit matching tension levels, suggesting irreversible (or at least long-lasting) changes in visco-elastic properties of the tissue strip. This is not surprising if one considers that a +2.0 g stress causes tissue strain by 73% to 123%. Such distension is very likely to cause cell and/or tissue damage. This may potentially be associated with stretch-induced swelling, which may have contributed to some of the controversy on the effects of VAC block on the chronotropic response of the heart to stretch, which has been observed only at non-physiologically high levels of tissue distension.⁹

We focused our analysis on the average BR response over a 30 s period of stretch. The sustained response to stretch, both in the presence and absence of pharmacological agents, shows the same *qualitative* behavior as the peak BR, albeit at a smaller amplitude. Hence, we are reporting conservative values for stretch-induced responses, compared to other published experimental work that focussed solely on peak changes in BR.

Based on the previous identification, in rabbit single SAN pacemaker cells, of VAC (activated by cell inflation via a pipette in ruptured patch mode),¹⁶ it had been suggested that VAC might underlie the positive chronotropic response of the heart to right atrial distension. Mechanical activation of VAC has also been demonstrated using centrifugal magnetic bead stretching of β 1-integrins in ventricular myocytes,¹⁷ and underlying channel properties have been reported in atrial cells.¹⁸

In contrast, Lei and Kohl showed that hyposmotic swelling of *spontaneously* beating rabbit SAN pacemaker cells *slows* their spontaneous BR.¹³ This suggests that cell swelling may be an inappropriate intervention to mimic effects caused by changes in diastolic wall stress or strain. In addition, Sasaki *et al.*¹⁹ found no activation of VAC in ventricular myocytes by direct cell membrane manipulation; only in the presence of cell volume changes was VAC activation observed. As there is no evidence to suggest that SAN cell volume changes during the *normal* cardiac cycle of contraction and relaxation, it is probable that other mechanisms must be involved. These mechanisms could be related to mechanical activation of SAC.

In order to identify a potential contribution by SAC, we applied streptomycin. This antibiotic has been shown to inhibit stretch-mediated effects in isolated ventricular myocytes at concentrations of 40 μ M.^{14,20} At higher concentrations (2 mM), voltage-dependent ion channels (e.g., the L-type Ca^{2+} channel and the delayed rectifier K^{+} channel¹⁵) are affected in isolated cardiac cells. In whole heart preparations, however, varied results have been obtained. Salmon *et al.* found that 200 μ M (but not 50 μ M) streptomycin causes a significant reduction in wall-stress induced arrhythmias in working rat heart.²¹ In contrast, Sung *et al.* saw no effect of 200 μ M streptomycin on acute load-induced changes in conduction velocity and AP duration in rabbit whole heart preparations.²²

In our superfused SAN tissue preparations, high concentrations of streptomycin (100–500 μM) caused a reduction in control BR (and contractility), suggesting an inhibitory effect on the L-type Ca^{2+} channels. However, streptomycin had no effect on the stretch-induced positive chronotropic response, neither at 40 μM (where it blocks single cell SAC), nor at 200 μM (where it reduces BR in our multi-cellular preparations, compatible with the previously reported blocking action on L-type Ca^{2+} channels).

This observation may be interpreted in two principally different ways: either SAC play no role in stretch-induced changes in SAN electrophysiology, or streptomycin does not succeed in blocking SAC *in situ*. Given that (1) the axial stretch induced changes in single SAN pacemaker cell electrophysiology are *fully consistent* with SAC activation, (2) implementation of matching SAC currents in mathematical models of SAN cell and tissue activity is sufficient to *fully reproduce* experimentally observed stretch effects, and (3) the signature of single cell stretch effects (reduced maximum diastolic potential, reduced AP amplitude, increased BR) is *fully compatible* with isolated SAN tissue electrophysiology responses,^{5,7} we believe that the first of the previously listed interpretations (lack of SAC effect in SAN tissue) is unlikely to be correct. Alternatively, SAC may be protected, *in situ*, from the blocking action of streptomycin. Such an interpretation would be in keeping with the observation that vestibular inhibition in patients taking aminoglycoside antibiotics (such as streptomycin) is usually observed only after long-term exposure to the drug.²³

To assess the principal plausibility of this suggestion, we conducted a pilot experiment using the novel peptide blocker of SAC, GsMTx-4.²⁴ This drug can be used in multi-cellular preparations²⁵ and has been reported to be highly selective for cationic SAC.²⁴ In our guinea pig SAN preparation, 400 nM GsMTx-4 caused full and reversible inhibition of the stretch-induced increase in BR. Using a +1.0 g mechanical stimulus, the stretch-induced change in BR was: +11.1% during control conditions (initial BR 140 bpm), -2.0% after 5 min exposure to GsMTx-4 (initial BR 119 bpm), and +11.9% after 6 min wash-out (initial BR 121 bpm).

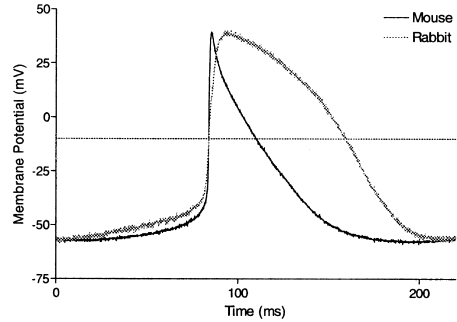
It should be noted, therefore, that streptomycin—while being a useful drug to dissect cation non-selective SAC effects in single cells—is likely to give false-negative results at higher levels of functional integration, including native tissue preparations, isolated heart, and whole animal studies.

In our experiments, the magnitude of stretch-induced BR changes was correlated to background BR (and mechanical stimulus amplitude) within each species. The direction of change, however, was opposite, with guinea pig SAN tissue showing a positive chronotropic response to stretch, and murine preparations overwhelmingly a negative chronotropic response (see FIG. 4B; note that only 1% of 322 individual stretch applications caused negative chronotropy in guinea pig SAN tissue, even in tissue maintained at 27°C [not shown]).

This difference in stretch-induced changes in BR may be a consequence of the very different underlying electrophysiology of species with moderate (guinea pig, rabbit) and very high BR (mouse). Thus, murine pacemaking involves a significant contribution of the fast Na^+ current²⁶ and has much faster repolarization dynamics (no plateau), giving its repolarization a “convex,” rather than “concave,” appearance.

To illustrate this point, FIGURE 5 shows a superimposition of pacemaker AP, recorded from rabbit and mouse single SAN cells. Overlaying this with an indication of the SAC reversal potential, one can appreciate that rabbit SAN pacemaking is

FIGURE 5. Superimposition of isolated sino-atrial node (SAN) cell action potentials from rabbit (*gray*) and mouse (*black*). The dashed line at -10 mV indicates the expected reversal potential for cation non-selective stretch-activated currents (as identified in rabbit SAN cells⁷).



dominated by periods of time during which the SAN AP moves *toward* the stretch-activated reversal potential. The corresponding potential change would be *accelerated* by activation of SAC, thereby shortening cycle length. In contrast, in murine cells the duration of time during which the SAN AP moves *away* from the SAC reversal potential is more pronounced and often exceeds 50% of the cardiac cycle.²⁷ Thus, SAC activation would have the potential of *slowing* murine pacemaking.

The same mechanisms—SAC activation by mechanical stimulation—may therefore be expected to have differential effects on overall BR in different species and could underlie both the positive chronotropic response to stretch in hearts with low to moderate BR *and* the negative chronotropy seen in species with very high BR.

Finally, in order to resolve any potential contribution of differences in cardiac intrinsic nerve system activity, we performed experiments in the presence of atropine and propranolol ($1 \mu\text{M}$ each), which had no effect on the initial BR. Stretch-induced responses in both guinea pig and mouse SAN tissue strips were not affected by this autonomic block (as previously reported for rat SAN tissue).⁶ This underscores the suggestion that intracardiac neurons are unlikely to have a significant effect on mechanical modulation of BR in isolated tissue.

CONCLUSION

The chronotropic response of the heart to stretch can be explained on the basis of SAC activation, while VAC do not appear to play a significant role under physiological conditions. Likewise, the response does not require a contribution of the intrinsic cardiac autonomous nervous system.

Streptomycin, a suitable blocker of SAC in isolated cells, appears ineffective for experimental identification of acute SAC effects *in situ*.

Background BR affects stretch responses, with lower BR generally promoting more pronounced (relative and absolute) stretch-induced changes in chronotropy, whether positive or negative. There is not an absolute relation between background BR and stretch-induced changes, and extrapolation across species boundaries may be misleading.

The principally different effects of stretch on SAN BR in guinea pig (acceleration) and mouse (deceleration) do not necessarily need to involve disparate underlying mechanisms, but may be a consequence of species-dependent differences in SAN

electrical activity. Further studies, involving axial distension of isolated SAN cells from species with moderate and fast BR, are required to verify this hypothesis.

ACKNOWLEDGMENTS

We thank Drs. Fred Sachs (for kindly providing us with a sample of GsMTx-4), Alan Garry (for help with modeling studies), and Ming Lei (who contributed to mouse SAN cell electrophysiology work). Peter Kohl is a Royal Society Research Fellow. This work was supported by the British Heart Foundation and the UK Medical Research Council.

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