Effects of gadolinium on cardiac mechanosensitivity in whole isolated swine hearts

Hanyu Zhang1, Gregory P. Walcott2 & Jack M. Rogers1

Mechanical stimulation can elicit electrical activation of the heart. This mechanosensitivity can start life-threatening arrhythmias (commotio cordis) or terminate them (precordial thump). Mechanosensitivity may also be involved in arrhythmogenesis in other settings. Stretch-activated ion channels (SACs) are thought to be important in mechanosensitivity and a number of agents that block them have been identified. Such agents could potentially be used as tools in experimental investigation of mechanosensitivity. However, studies using them in intact-heart preparations have yielded inconsistent results. In the present study, we used isolated, perfused hearts from 25–35 kg pigs and a computer-controlled device that repeatably delivered focal mechanical stimuli. The concentration-dependent ability of the SAC blocker gadolinium to suppress mechanical activation was assessed by the success rate of mechanical stimulation and by the delay between successful mechanical stimulation and electrical activation. In six hearts, perfusate was recirculated. In an additional six hearts, perfusate was not recirculated to prevent gadolinium from forming complexes with metabolic waste and possibly precipitating. Gadolinium did not suppress mechanically-induced activation. Although gadolinium has been shown to be an effective SAC blocker in isolated cells, using it to probe the role of mechanical stimulation in whole heart preparations should be done with great caution.

Mechanical function and electrical activity are bidirectionally coupled in the heart1. Cardiac electrical activity can be modulated by mechanical stimulation, which is known as cardiac mechanosensitivity. Generally, cardiac mechanosensitivity includes change of heart rhythm or initiation of ectopic electrical excitation in response to mechanical stimulation1. Both phenomena have been documented for decades.

Mechanosensitivity is an intrinsic property of the heart and has been documented in many heart preparations, including isolated heart4,5, isolated cardiac tissue6, and isolated cardiac cells7. Mechanosensitive ion channels (MSCs) have been thought to play a key role in cardiac mechanosensitivity. MSCs were first discovered by Guharay and Sachs in chick skeletal muscle cells in 19848. Since then, several MSCs have been identified from a wide range of cells, including cardiac cells (see the review by Reed et al.9).

Stretch-activated ion channels (SACs) are a subcategory of MSCs that switch from closed state to open state in response to mechanical stretch. Among SACs, non-selective cation SACs (SACNS) have been viewed as important contributors to mechanically induced electrical excitation. Those SACs conduct Na⁺, K⁺ and Ca²⁺ currents and have a linear voltage-current relationship with reversal potential ranging from −30 mV to 0 mV10,11. At resting potential, when activated by mechanical stretch, they depolarize the membrane potential by carrying inward Na⁺/Ca²⁺ currents and thus may trigger ectopic electrical excitation12. Potassium-selective SACs (SACKs) also have been found in cardiac cells. These channels generally have a larger conductance than that of SACNS13,14. Being selective to potassium and having reversal potential about −70 mV, SACK channels tend to cause repolarization/hyperpolarization when activated15.

The potential role of SACs in arrhythmogenesis is an active area of research16. Pharmacologic agents that modulate the gating behavior of SACs17 are a potentially powerful tool in this research and are therefore of great interest. Streptomycin is commonly used as an SAC blocker. However, while there is some inconsistency in the literature, it is typically reported to be effective in blocking SACs in isolated cells, but not in intact tissue18,19,20. Gadolinium (Gd³⁺) is another commonly used SAC blocker; it has been reported to modulate stretch-induced

1University of Alabama at Birmingham, Department of Biomedical Engineering, Birmingham, 35294, United States of America. 2University of Alabama at Birmingham, Division of Cardiovascular Disease, Department of Medicine, Birmingham, 35294, United States of America. Correspondence and requests for materials should be addressed to J.M.R. (email: jrogers@uab.edu)
two silver wire electrodes hooked into the subepicardium at the LV apex. Whenever Gd^3+ precipitation of Gd^3+

be a specific SAC blocker24, but is not currently practical for this application because of the large quantity that

should have a substantial SAC-blocking effect. We did not evaluate a third agent, GsMtx-4, which is reported to

whole, isolated large animal hearts subjected to localized stretch. To be an experimentally useful tool, gadolinium

is to evaluate the ability of gadolinium to inhibit mechanically induced electrical activation in the specific case of

KCl 3, CaCl_2 2.6, MgCl_2 0.6, HEPES 10, Glucose 12. The perfusate was oxygenated with 100% O_2 and the pH

To prevent this, we used HEPES-buffered perfusate in this study. The perfusate contained (mmol/L): NaCl 140,

from domestic farm pigs of either sex, weighing 25–35 kg were studied. Anesthesia, heart excision, and perfusion

and Use of Laboratory Animals. Hearts were excised from animals in a surgical plane of anesthesia. Twelve hearts

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arrhythmias in intact myocardium, but again, the literature is inconsistent21–23. The purpose of the present study

to evaluate the ability of gadolinium to inhibit mechanically induced electrical activation in the specific case of

were similar to our previous publication25. The hearts were retrogradely perfused in a Langendorff apparatus with

warm (37 ± 1 °C), oxygenated perfusate at 100–200 mL/min. Hearts were suspended in air by the aortic cannula.

A piece of concave-shaped polypropylene foam was placed against the posterior side of the heart for support
during mechanical stimulation of the anterior left ventricular (LV) epicardium. Electrical activation of the heart

was monitored and recorded using a silver wire unipolar electrode hooked into the subepicardium of the lateral

LV wall. The reference electrode was immersed in perfusate in the aortic cannula.

Gd^3+ strongly binds with anions such as bicarbonate and phosphate26 and quickly precipitates when added to

commonly used perfusate solutions (such as Krebs-Henseleit or Tyrodes) that use bicarbonate as the buffer.

To prevent this, we used HEPES-buffered perfusate in this study. The perfusate contained (mmol/L): NaCl 140,

KCl 3, CaCl_2 2.6, MgCl_2 0.6, HEPES 10, Glucose 12. The perfusate was oxygenated with 100% O_2 and the pH

was adjusted to 7.4 ± 0.1 with NaOH. In six hearts, 3 L of perfusate was used. The coronary effluent was filtered

with a 20 μm filter and recirculated. Gadolinium(III) chloride hexahydrate (Sigma-Aldrich) was dissolved in

perfusate solution on the day of experiment. This solution was added to the perfusate in a serial manner with

final Gd^3+ concentrations of 0, 25, 50 and 100 μmol/L. Gd^3+ has been reported to block SACs at concentrations

of 1 to 50 μmol/L 22,23,27. In the six remaining hearts, the coronary effluent was not recirculated to avoid possible

precipitation of Gd^3+ caused by metabolic wastes that the hearts produced. In those experiments, perfusate with

different Gd^3+ concentration was made and stored in four individual reservoirs. During the experiment, Gd^3+

concentration was changed by switching the reservoir connected to the perfusion system. As before, concentra-
tions of 0, 25, 50, and 100 μmol/L were tested. For analysis, 0 μmol/L data were used as the baseline group.

In all hearts, 2,3-Butanedione monoxime (BDM) (Sigma-Aldrich) was added to the perfusate (20 mmol/L) to

suppress mechanical contraction while preserving electrical propagation. BDM also suppresses spontaneous elec-
trical excitation of isolated hearts28. Thus, in this preparation, all cardiac wall stretch was induced experimentally

and all electrical activations were either electrically paced or mechanically induced.

Once warm, hearts were defibrillated and electrically paced (twice diastolic threshold current) at 60 bpm by
two silver wire electrodes hooked into the subepicardium at the LV apex. Whenever Gd^3+ concentration was
changed, 10 minutes of perfusion was allowed for equilibration.

Mechanical Stimulation. We elicited mechanically induced beats with a custom-built, computer-controlled
piston that contacted the anterior LV epicardium midway between apex and base. The impact head at the end
of the piston was approximately hemispherical with 6 mm radius (Fig. 1(a)). The mechanical stimulus strength
was adjusted by altering the piston's stroke length (with longer strokes inducing greater tissue deformation).
Before each stimulus, the impact head was placed in an initial position that provided gentle contact with the
epicardial surface. To provide for consistent initial contact force, the impact head was mounted on a spring.
Figure 2. Example unipolar electrogram and electrical/mechanical stimulation trigger signals from a stimulation protocol. (a) The 1st, 2nd and 4th mechanical stimuli successfully elicited electrical activation; the 3rd mechanical stimulus failed. (b) A localized view of the signals near 4th mechanical stimulus in (a) shows the delay ($D$) between mechanical stimulation onset and electrical activation.

Every few stimuli, the locking screw was loosened, and if necessary, the piston’s initial position was adjusted to compress the spring to a consistent length. The locking screw was then tightened before stimulus delivery. The mechanical stimulation threshold strength (i.e. the threshold stroke length) under baseline conditions ($0 \mu$mol/L $\text{Gd}^{3+}$) was found by gradually increasing the piston’s stroke. Stimulation success was noted when electrical activation was observed in the unipolar electrogram recorded from the lateral LV (5–10 cm from the mechanical stimulation site). Because the expected effect of $\text{Gd}^{3+}$ was to reduce the likelihood of stimulation success, the stimulation stroke was adjusted until the majority of ($\geq 70\%$), but not all, stimulation attempts at baseline were successful. Once the near-threshold stimulation strength was found for a heart, stimulus stroke was unchanged for the remainder of the experiment. The threshold stroke length varied from heart-to-heart, but was in the range 15–25 mm.

**Experimental Protocol.** For each $\text{Gd}^{3+}$ concentration level, a stimulation protocol containing ~50 (51 ± 8) mechanical stimuli (Fig. 1(b)) was performed. During the protocol, electrical pacing rate was reduced to 30 bpm and mechanical stimuli were delivered 1 s after every third or more electrical pacing pulse (Fig. 1(c)). The ~1 s delay between electrical and mechanical stimuli allowed myocardium to recover electrically before mechanical stimulus delivery. The unipolar electrogram and the trigger signals for electrical and mechanical stimulation were continuously recorded during the protocols. Electrical pacing resumed at 60 bpm between stimulation protocols.

**Data Availability.** Data listing the outcome of each individual mechanical stimulus are listed in the supplementary data. Raw recordings are available from the corresponding author upon request.

**Results**

The successful and unsuccessful mechanical stimuli were identified from recordings of the left ventricular electrogram and the stimulus triggers. An example is shown in Fig. 2. The success rate for a mechanical stimulation protocol was defined as $R = (\text{number of mechanically-induced electrical activations})/(\text{number of mechanical stimuli})$. We also measured the delay between the onset of mechanical stimulation and cardiac electrical activation (which we defined as the time of the steepest downslope of the unipolar electrogram signal$^{29}$) for every successful mechanical stimulus (e.g., the interval $D$ in Fig. 2(b)). Effective suppression of cardiac mechanosensitivity by SAC blockade should reduce $R$ from its baseline value in a concentration-dependent manner and also increase the stimulation-activation delay $D$ due to the decreased depolarizing current$^{30}$. For analysis, we averaged $D$ over the successful stimuli in each stimulus protocol. The results of all individual stimuli are shown in the supplementary data.

We used linear mixed models (SPSS MIXED procedure) to evaluate the effects of concentration. $\text{Gd}^{3+}$ concentration was a repeated measure. Because mechanical stimuli were held constant for each trial in an animal and the status of the heart was approximately constant throughout an experiment, we considered as plausible the assumption that variance and covariance of residuals among levels of concentration were equal. We therefore used the “compound symmetry” covariance structure when fitting models$^{31}$.

The effect of $\text{Gd}^{3+}$ for the hearts in which perfusate was recirculated is shown in Fig. 3. To be experimentally useful, SAC blockade should have a substantial effect. However, the linear mixed model analysis did not indicate any significant differences in $R$ or $D$ among the 4 levels of $\text{Gd}^{3+}$ concentration ($p = 0.614, p = 0.821$, respectively).

We observed that when $\text{Gd}^{3+}$ was added to fresh perfusate with high concentration ($7.5 \mu$mol/L), it remained dissolved. However, when a similar concentration was added to perfusate that had been recirculated through a heart during an experiment, a visible precipitate formed, possibly through interaction with metabolic wastes that had washed into the solution. To avoid this potential for loss of $\text{Gd}^{3+}$, we also tested the effect of $\text{Gd}^{3+}$ in six hearts without perfusate recirculation. These data are shown in Fig. 4. The $100 \mu$mol/L data from one heart was lost due to a technical issue. Mixed model analysis in this case indicated that there was a significant difference in $R$ among the levels of $\text{Gd}^{3+}$ concentration ($p = 0.006$). Examination of the pairwise comparisons between baseline
(0 μmol/L Gd³⁺) and the other three levels showed significant differences with 25 and 50 μmol/L Gd³⁺ but not with 100 μmol/L Gd³⁺ (p = 0.003, p = 0.015, p = 0.227, respectively with Sidak adjustment for multiple comparison). However, in all 6 animals, R was increased, rather than decreased, by 25 and 50 μmol/L Gd³⁺, which is the opposite of the hypothesized effect. Mixed model analysis did not indicate a significant effect of Gd³⁺ concentration on D (p = 0.286).

To investigate possible loss of mechanical stimulation efficacy with repeated delivery (“mechanical run-down”)32,33, we compared the stimulation-activation delay D between the first and second mechanical stimuli in each stimulation protocol and also between the two stimuli immediately preceding each failed mechanical stimulus. D was significantly shorter for the first stimulus compared to the second (154 ± 43 vs. 202 ± 48 ms, mean ± stdev, p < 0.001, paired t-test, n = 46 pairs), but there was not a significant difference between D of the two stimuli preceding failure (209 ± 54 vs. 211 ± 56 ms, mean ± stdev, p = 0.62, paired t-test, n = 214 pairs).

Discussion
The major finding of the present study is that gadolinium (Gd³⁺) does not reliably suppress electrical excitation due to focal mechanical stimulation in whole isolated swine hearts.

Gadolinium (Gd³⁺) is frequently used as a SAC blocker. It has been reported to block SACs at concentrations of 1 to 50 μmol/L.7-10,22,23,27 Other lanthanides (La³⁺ and Lu³⁺) also show SAC-blocking effects, but higher concentrations are required27. The mechanism of action of Gd³⁺ is not yet clear and multiple mechanisms and sites of action may be involved34. Gd³⁺ is not a specific SAC blocker. Within the effective concentration range, it also blocks many other currents in cardiac cells, including calcium, sodium and potassium currents17. However, some studies have reported an inability of Gd³⁺ to block SACs. Zhang et al. reported that Gd³⁺ (100 μmol/L) can...
block background non-selective cation current but failed to block stretch-induced non-selective cation current in rat atrial myocytes. In the same study, they also demonstrated that action potentials can still be elicited by mechanical stretch after Gd$^{3+}$ treatment$^{18}$. Similarly, Kim reported that SAC current in rat atrial cells cannot be blocked by 100μmol/L Gd$^{3+}$.$^{36}$ In the present study, Gd$^{3+}$ failed to suppress mechanically induced electrical excitation in whole isolated hearts (Figs 3 and 4), which is consistent with those findings. Furthermore, Gd$^{3+}$ did not significantly prolong the delay between the onset of mechanical stimulation and electrical activation. Stacy et al. reported that the delay between mechanical stimulation and electrical activation as well as the amplitude of mechanically induced depolarization depended on the strength of the stimulation$^{37}$. Thus, if Gd$^{3+}$ successfully attenuated depolarizing current resulting from mechanical stimulation, it would be expected that D would increase in a concentration dependent manner, even if the attenuation was insufficient to prevent electrical activation.

The efficacy of Gd$^{3+}$ in some studies but not others may suggest that there are sources of mechanosensitivity other than channels susceptible to block by Gd$^{3+}$. The lack of activity of Gd$^{3+}$ in our study could also have been due to failed transport of the agent to its site of action. This broad mechanism was suggested by Cooper and Kohl to explain similar inconsistency of streptomycin in suppressing mechanosensitivity$^{9}$. Gd$^{3+}$ is known to strongly bind with anions that commonly exist in physiological solutions (e.g. carbonate, phosphate, EGTA, etc.). Such binding effectively removes free Gd$^{3+}$ from the solution$^{39}$. This is why we used a phosphate-free HEPES-based perfusate solution, which lacks these anions. However, in a recirculating perfusion system, the possibility remains for Gd$^{3+}$ to bind with metabolites and other substances washed into the perfusate. Indeed, we observed that when Gd$^{3+}$ was added to a HEPES-based perfusate, visible precipitate formed. In a typical recirculating perfusion system, precipitate would be quickly filtered from the working solution. We therefore additionally tested Gd$^{3+}$ using non-recirculating perfusates. These experiments also showed a lack of effect, suggesting that anion-binding was responsible for reducing the efficacy of Gd$^{3+}$. It has been proposed that Gd-anion complexes rather than free Gd$^{3+}$ are responsible for blocking SACs and that this could partly explain the reported positive effects of gadolinium in the presence of suspect anions$^{27,28}$. If this is the case, it is possible that SAC block by gadolinium occurs under conditions in which Gd-anion complexes form, but are not rapidly removed from solution by precipitation and filtration.

Use-dependent decrease of mechanical stimulation efficacy (also called "mechanical rundown") has been reported$^{25,42}$ and is a potential cause for failed mechanical stimulation in repetitive protocols such as ours. The sustainability of successful mechanical stimulation depends on the presence of background electrical pacing and decreases as pacing rate increases$^{32}$. In our study, mechanical stimuli were interspersed with electrical stimuli at a ratio ≥3:1 (electrical:mechanical) and the hearts were paced at a rate lower than normal pig sinus rhythm$^{38}$. This pacing condition facilitates the sustainability of successful mechanical stimulation$^{52}$. In our experiments, we observed mechanical rundown at the beginning of each stimulation protocol (increased D for the second stimulus compared to the first), but the phenomenon was not observed as mechanical stimulation approached failure (no difference between D of the two stimuli preceding a failed mechanical stimulus). This is consistent with Quinn and Kohl’s observation that rundown was pronounced after the first mechanical stimulation but less prominent thereafter$^{32}$. It is therefore unlikely that mechanical rundown played an important role in our results.

Myocardial edema is common in isolated heart preparations as a consequence of interstitial fluid accumulation and cell swelling$^{29}$. It is known that cell swelling affects several ion channels, including volume-sensitive channels (e.g. CI-selective volume-sensitive channels)$^{30}$ as well as SACs$^{40}$ in cardiac cells. However, in this study, the most important effect of edema was likely on the heart’s mechanical properties (i.e., stiffness). Changing stiffness alters tissue deformation patterns resulting from the mechanical stimulus and could modulate the electrical response to stretch. Because swelling is most rapid immediately after starting perfusion, to minimize this variability, after isolating the hearts and initiating perfusion, we allowed the hearts to stabilize for ~40 min before proceeding to the remaining experimental protocols.

In our experiments, there was no visible tissue damage (e.g. tissue tearing, rupture) from mechanical stimulation; however it is possible that non-visible damage occurred. Cooper et al. reported that creatine kinase elevation, an indicator for muscle tissue damage, was observed when focal mechanical impact exceeded a certain energy level$^{41}$. However, the energy level required for triggering electrical activation generally does not cause such tissue damage$^{20,22}$. In the present study, the mechanical stimulation strength was maintained at a level just sufficient to elicit electrical activation.

We used BDM to prevent stretch from muscle contraction and to suppress spontaneous electrical activation. BDM is known to affect cardiac electrophysiology, including calcium and potassium currents, action potential duration, and ventricular fibrillation patterns$^{53,54}$. Although no evidence has been reported suggesting that BDM affects the gating behavior of SACs, it is possible that it interfered with SAC blockade.

Many studies have shown that cardiac mechanosensitivity can be arrhythmogenic, e.g., ventricular fibrillation initiated by non-penetrating impact on the chest$^{1}$ or ectopic beats elicited by mechanical stretch of the heart wall$^{9}$. It has been suggested that abnormal mechanical stretch at the interface of normally contracting and ischemic tissue following acute regional ischemia may play a role in arrhythmogenesis$^{55,56}$. Pharmacologic agents to suppress mechanosensitivity are therefore of great interest, both as experimental tools to probe the mechanisms of these phenomena, and as possible therapeutic agents to prevent arrhythmia. However, studies evaluating the ability of SAC blocking agents to prevent arrhythmia have yielded inconsistent results. Bode et al. showed that GS-Mtx-4, a more specific SAC blocker than that used in the present study, effectively inhibited atrial fibrillation following mechanical stretch$^{24}$. Similar antiarrhythmic effects have also been reported after streptomyacin and Gd$^{3+}$ treatment$^{8,22,23}$. On the other hand, antiarrhythmic effects of these SAC blockers were not observed in other studies$^{19-21,45}$. In the present study, we used a large animal heart preparation in which confounding sources of wall stretch and electrical activation were absent. We mechanically stimulated the heart with controlled, repeatable, focal stimuli. Our results indicate that Gd$^{3+}$ is not effective in eliminating ectopic beats due to local stretch of the ventricular wall and should not be relied upon in this role experimentally.
References

1. Kohl, P., Hunter, P. & Noble, D. Stretch-induced changes in heart rate and rhythm: clinical observations, experiments and mathematical models. Prog. Biophys. Mol. Biol. 71, 91–138 (1999).

2. Bainbridge, F. A. The influence of venous filling upon the rate of the heart. J. Physiol. 50, 65–84 (1915).

3. Maron, B. J. & Estes, N. A. M. Commotio Cordis. N. Engl. J. Med. 362, 917–927 (2010).

4. Franz, M. R., Cima, R., Wang, D., Profitt, D. & Kurz, R. Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. Circulation 86, 968–978 (1992).

5. Lerman, B. B., Burkhoff, D., Yue, D. T., Franz, M. R. & Sagawa, K. Mechanoelectrical feedback: independent role of preload and contractility in modulation of canine ventricular excitability. J. Clin. Invest. 76, 1843–1850 (1985).

6. Cooper, P. J. & Kohl, P. Species- and Preparation-Dependence of Stress on Sino-Atrial Node Pacemaking. Ann. N. Y. Acad. Sci. 1047, 324–335 (2005).

7. Ruknudin, A., Sachs, F. & Bustamante, J. O. Stretch-activated ion channels in tissue-cultured chick heart. Am. J. Physiol. 264, H960–972 (1993).

8. Guharay, F. & Sachs, F. Stretch-activated single channel currents in tissue-cultured embryonic chick skeletal muscle. J. Physiol. 352, 683–701 (1984).

9. Reed, A., Kohl, P. & Peyronnet, R. Molecular candidates for cardiac stretch-activated ion channels. Glob. Cardiol. Sci. Pract. 14, 9–25 (2014).

10. Craciulescu, W., Chen, V. & el-Shrief, N. Stretch activated ion channels in ventricular myocytes. Biosci. Rep. 8, 407–414 (1988).

11. Zeng, T., Bett, G. C. & Sachs, F. Stretch-activated whole cell currents in adult rat cardiac myocytes. Am. J. Physiol. Heart Circ. Physiol. 278, H548–557 (2000).

12. Zabel, M., Koller, B. S., Sachs, F. & Franz, M. R. Stretch-induced voltage changes in the isolated beating heart: importance of the timing of stretch and implications for stretch-activated ion channels. Cardiovasc. Res. 32, 120–130 (1996).

13. Hu, H. & Sachs, F. Mechanically activated currents in chick heart cells. J. Membr. Biol. 154, 205–216 (1996).

14. Kim, D. A mechanosensitive K⁺ channel in ventricular heart cells. Activation by arachidonic acid. J. Gen. Physiol. 100, 1021–1040 (1992).

15. Kohl, P., Bollensdorff, C. & Garry, A. Effects of mechanosensitive ion channels on ventricular electrophysiology: experimental and theoretical models. Exp. Physiol. 91, 307–321 (2006).

16. Peyronnet, R., Nerbonne, J. M. & Kohl, P. Cardiac Mechano-Gated Ion Channels and Arrhythmias. Circ. Res. 118, 311–329 (2016).

17. White, E. Mechanosensitive channels: therapeutic targets in the myocardium? Curr. Pharm. Des. 12, 3645–3663 (2006).

18. Salmon, A. Effect of streptomycin on wall-stress-induced arrhythmias in the working rat heart. Circ. Res. 34, 493–503 (1997).

19. Garan, A. R., Maron, B. J., Wang, P. J., Estes, N. A. M. & Link, M. S. Role of streptomycin-sensitive stretch-activated channel in chest wall impact induced sudden death (commotio cordis). J. Cardiovasc. Electrophysiol. 16, 435–438 (2005).

20. Quinn, T. A., Jin, H., Lee, P. & Kohl, P. Mechanically Induced Ectopy via Stretch-activated Cation-Nonselective Channels Is Caused by Local Tissue Deformation and Results in Ventricular Fibrillation if Triggered on the Repolarization Wave Edge (Commotio Cordis). Circ. Arrhythm. Electrophysiol. 10, e004777 (2017).

21. Barrabés, J. A. et al. Intracoronary infusion of Gd³⁺ into ischemic region does not suppress phase lb ventricular arrhythmias after coronary occlusion in swine. Am. J. Physiol. Heart Circ. Physiol. 290, H2344–2350 (2006).

22. Franz, M. R. & Bode, F. Mechano-electrical feedback underlying arrhythmias: the atrial fibrillation case. Prog. Biophys. Mol. Biol. 82, 163–174 (2003).

23. Hansel, D. E., Borganelli, M., Stacy, G. P., Jobe, R. L. & Hansen, D. E. Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine ventricles. Evidence for a unique mode of antiarrhythmic action. Circ. Res. 69, 820–831 (1991).

24. Bode, F., Sachs, F. & Franz, M. R. Tarantula peptide inhibits atrial fibrillation. Nature 409, 35–36 (2001).

25. Qin, H. & Bode, F. Effects of heart isolation, voltage-sensitive dye, and electromechanical uncoupling agents on ventricular fibrillation. Am. J. Physiol. Heart Circ. Physiol. 284, H1818–1826 (2003).

26. Caldwell, R. A., Clemo, H. F. & Baumgarten, C. M. Using gadolinium to identify stretch-activated channels: technical considerations. Am. J. Physiol. 275, C619–621 (1998).

27. Yang, X. C. & Sachs, F. Block of stretch-activated ion channels in Xenopus oocytes by gadolinium and calcium ions. Science 243, 1068–1071 (1989).

28. Jou, C., Spitzer, K. W. & Tristani-Firouzi, M. Blebbistatin effectively uncouples the excitation-contraction process in zebrafish embryonic heart. Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 25, 419–424 (2010).

29. Cantwell, C. D. et al. Techniques for automated local activation time annotation and conduction velocity estimation in cardiac mapping. Comput. Biol. Med. 65, 229–242 (2015).

30. Tacker, W. A. & Geddes, L. A. The laws of electrical stimulation of cardiac tissue. Proc. IEEE 84, 355–365 (1996).

31. West, B. T., Welch, K. B., Galecki, A. T. & Gillespie, B. W. Linear mixed models a practical guide using statistical software (CRC Press, 2015).

32. Quinn, T. A. & Kohl, P. Comparing maximum rate and sustainability of pacing by mechanical vs. electrical stimulation in the Langendorff-perfused rabbit heart. EP Eur. 18, i85–i93 (2016).

33. Dick, D. J. & Lab, M. J. Mechanical modulation of stretch-induced premature ventricular beats: induction of mechanoelectric adaptation period. Cardiovasc. Res. 38, 181–191 (1998).

34. Hamill, O. P. & McBride, D. W. The pharmacology of mechanogated membrane ion channels. Pharmacol. Rev. 48, 231–252 (1996).

35. Zhang, Y. H. et al. Stretch-activated and background non-selective cation channels in rat atrial myocytes. J. Physiol. 523, 607–619 (2000).

36. Kim, D. Novel cation-selective mechanosensitive ion channel in the atrial cell membrane. Circ. Res. 72, 225–231 (1993).

37. Stacy, G. P., Jobe, R. L., Taylor, I. K. & Hansen, D. E. Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine left ventricles. Am. J. Physiol. 263, H613–621 (1992).

38. Hannon, J. P., Bosson, C. A. & Wade, C. E. Normal physiological values for conscious pig used in biomedical research. Lab. Anim. Sci. 40, 293–298 (1990).

39. Arbel, E. R., Prabhhu, R., Ramesh, V., Pick, R. & Glick, G. A perfused canine right bundle branch-septal model for electrophysiological studies. Am. J. Physiol. 236, H379–384 (1979).

40. Vandenberg, J. I., Kees, S. A., Wright, A. R. & Powell, T. Cell swelling and ion transport pathways in cardiac myocytes. Cardiovasc. Res. 32, 85–97 (1996).

41. Cooper, P. et al. Soft tissue impact characterisation kit (STICK) for ex situ investigation of heart rhythm responses to acute mechanical stimulation. Prog. Biophys. Mol. Biol. 90, 444–468 (2006).

42. Liu, Y. et al. Effects of diacetylsulmonoxide on the electrical properties of sheep and guinea pig ventricular muscle. Cardiovasc. Res. 27, 1991–1997 (1993).

43. Iie, X., Gurev, V. & Trayanova, N. Mechanisms of mechanically induced spontaneous arrhythmias in acute regional ischemia. Circ. Res. 106, 185–192 (2010).

44. Coronel, R., Wilms-Schopman, F. J. G. & deGroot, J. R. Origin of ischemia-induced phase 1b ventricular arrhythmias in pig hearts. J. Am. Coll. Cardiol. 39, 166–176 (2002).

45. Barrabés, J. A. et al. Effects of the Selective Stretch-Activated Channel Blocker GsMtx4 on Stretch-Induced Changes in Refractoriness in Isolated Rat Hearts and on Ventricular Premature Beats and Arrhythmias after Coronary Occlusion in Swine. Plos One 10, e0125753 (2015).
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Author Contributions
H.Z. and J.M.R. designed the experiments, H.Z. G.P.W. and J.M.R. conducted the experiments, H.Z. and J.M.R. analyzed the results, H.Z. and J.M.R. wrote the manuscript. All authors reviewed the manuscript.

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