Mechanoelectric coupling and arrhythmogenesis in cardiomyocytes contracting under mechanical afterload in a 3D viscoelastic hydrogel

Bence Hegyi,a Rafael Shimkunas,b,b Zhong Jian,b,b Leighton T. Izu,b Donald M. Bers,a and Ye Chen-Izu,b,b,c,1

*Department of Pharmacology, University of California, Davis, CA 95616; †Department of Biomedical Engineering, University of California, Davis, CA 95616; and ‡Division of Cardiovascular Medicine, Department of Internal Medicine, University of California, Davis, CA 95616

Edited by Richard W. Aldrich, The University of Texas at Austin, Austin, TX, and approved June 29, 2021 (received for review May 5, 2021)

The heart pumps blood against the mechanical afterload from arterial resistance, and increased afterload may alter cardiac electrophysiology and contribute to life-threatening arrhythmias. However, the cellular and molecular mechanisms underlying mechanoelectric coupling in cardiomyocytes remain unclear. We developed an innovative patch-clamp-in-gel technology to embed cardiomyocytes in a three-dimensional (3D) viscoelastic hydrogel that imposes an afterload during regular myocyte contraction. Here, we investigated how afterload affects action potentials, ionic currents, intracellular Ca2+ transients, and cell contraction of adult rabbit ventricular cardiomyocytes. We found that afterload prolonged action potential duration (APD), increased transient outward K+ current, decreased inward rectifier K+ current, and increased L-type Ca2+ current. Increased Ca2+ entry caused enhanced Ca2+-transients and contractility. Moreover, elevated afterload led to discordant alternans in APD and Ca2+ transient. Ca2+ alternans persisted under action potential clamp, indicating that the alternans was Ca2+-dependent. Furthermore, all these afterload effects were significantly attenuated by inhibiting nitric oxide synthase 1 (NOS1). Taken together, our data reveal a mechano-chemo-electrotansduction (MCET) mechanism that acutely transduces afterload through NOS1-nitric oxide signaling to modulate the action potential, Ca2+-transient, and contractility. The MCET pathway provides a feedback loop in excitation–Ca2+-signaling–contraction coupling, enabling autoregulation of contractility in cardiomyocytes in response to afterload. This MCET mechanism is integral to the individual cardiomyocyte (and thus the heart) to intrinsically enhance its contractility in response to the load against which it has to do work. While this MCET is largely compensatory for physiological load changes, it may also increase susceptibility to arrhythmias under excessive pathological loading.

mechano-chemo-transduction | cardiac arrhythmia | action potential | mechanosensitive ion channels | intracellular calcium cycling

Biological cells are capable of sensing and responding to mechanical forces. In every heartbeat, cardiomyocytes experience large dynamic changes in mechanical forces during diastolic filling (preload, which exerts mechanical strain or stretch on the cell) and systolic contraction against resistance (afterload, which exerts mechanical stress on the cell). In addition to neurohormonal regulation, the heart has intrinsic abilities to regulate the contractile force in response to preload [via the Frank–Starling mechanism (1–3)] or afterload [via the Anrep effect (4, 5)] in order to maintain cardiac output. Changes in mechanical load also regulate electrical activity, referred to as mechano-electric coupling [MEC (6, 7)], which may contribute to cardiac arrhythmias (8, 9). Several ion channels have been found directly mechano-gated or modulated by the mechanical load–induced signaling pathways (10). However, more than 100 years after Bainbridge’s discovery of MEC (11), the exact ionic mechanisms by which mechanical load modulates the electrical activity of the cardiomyocyte remain elusive.

We recently developed a patch-clamp-in-gel technique (12) based on our cell-in-gel system in which isolated cardiomyocytes are embedded in a three-dimensional (3D) viscoelastic hydrogel polymer matrix to apply afterload during cell contraction and investigate the cellular and molecular consequences of increased afterload (13, 14). This approach to apply afterload (mechanical stress) on the cardiomyocyte is profoundly different from previous studies using osmotic swelling, inflation, or uniaxial stretch to apply preload (mechanical strain) on the cardiomyocyte (15). Moreover, the cell-in-gel system imposes afterload during cyclic cardiomyocyte contraction as opposed to a static preload stretch used in most previous studies, with a few exceptions (16). To our knowledge, no data has been available on the ionic mechanisms of MEC under any kind of cyclic mechanical load during cardiomyocyte contraction.

Hence, we aimed to study the afterload effects on regulating ion channels and action potential (AP) in cardiomyocytes during contraction in the beat-by-beat cardiac cycle.

Our previous study showed that mouse ventricular cardiomyocytes contracting in a 3D viscoelastic hydrogel exhibited increased intracellular Ca2+ transients and increased diastolic Ca2+ sparks, with both dependent on localized nitric oxide synthase 1 (NOS1) and Ca2+/calmodulin-dependent protein kinase II (CaMKII) signaling (13). In addition to mechanical loading, pathological changes in Ca2+ handling and nitric oxide (NO) signaling have also been linked to cardiac arrhythmias (17, 18). Therefore, we investigated the ionic mechanisms of afterload-induced MEC in rabbit (rather

Author contributions: B.H., L.T.I., D.M.B., and Y.C.-I. designed research; B.H., R.S., and Z.J. performed research; B.H. and R.S. analyzed data; and B.H., L.T.I., D.M.B., and Y.C.-I. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1To whom correspondence may be addressed. Email: ychenizu@ucdavis.edu.

This article contains supporting information online at https://www.pnas.orglookup/suppl/doi:10.1073/pnas.2108484118/-/DCSupplemental.

Published July 29, 2021.
than mouse) ventricular myocytes in which the electrophysiology and Ca$^{2+}$ handling properties resemble that of the human heart. We tested the hypothesis that mechanical afterload affects ion channels and Ca$^{2+}$-handling molecules to regulate the AP, Ca$^{2+}$ transient, and contractility in cardiomyocytes. Here, we used the patch-clamp-in-gel methodology to perform current-clamp, voltage-clamp, and AP-clamp experiments while the cardiomyocyte is contracting under afterload in a 3D viscoelastic hydrogel. We found that the afterload on a contracting cardiomyocyte regulated multiple ionic currents, including L-type Ca$^{2+}$ current ($I_{Ca,L}$), transient outward K$^+$ current ($I_{to}$), inward rectifier K$^+$ current ($I_{K1}$), and a mechanosensitive current inhibited by GsMTx-4 (19), leading to prolonged AP duration (APD) and enhanced Ca$^{2+}$ transient and contractility. However, this afterload also promoted discordant alternans in APD and Ca$^{2+}$ transient, increasing the susceptibility to cardiac arrhythmias. Furthermore, we found that the afterload effects were critically mediated by the localized NOS1–NO signaling.

**Results**
**Mechanical Afterload–Induced Changes in AP.** First, we examined how mechanical afterload acutely alters the AP morphology in single rabbit ventricular myocytes contracting in a 3D viscoelastic hydrogel. Fig. 1 shows a schematic of the patch-clamp-in-gel technique and a representative experiment. The patch-clamp experiment was performed in cardiomyocytes embedded in Tyrode’s solution containing polyvinyl alcohol (PVA; Fig. 1A, Left). After achieving the whole-cell current-clamp configuration, steady-state APs were recorded at 0.5 Hz pacing frequency at room temperature (referred to as “load-free” control; Fig. 1B). Next, adding cross-linker tetraboronate-polyethylene glycol (4B-PEG, 7.5 wt%) to PVA (10 wt%) caused formation of the 3D viscoelastic hydrogel polymer matrix (for details, see Materials and Methods) surrounding the myocyte–electrode assembly (referred to as “in-gel” condition; Fig. 1A, Right). As the gel stiffens over 2 to 3 min, there is an initially relatively stable prolongation of the APD (Fig. 1B and C) followed by development of APD alternans (characterized by alternating beats that exhibit long and short APD).

To investigate whether cell contraction by itself influences AP morphology, we measured the AP under load-free conditions in the presence of the myofilament contraction uncoupler blebbistatin (myosin II ATPase inhibitor, 10 μmol/L). As expected, blebbistatin stopped the contraction and relaxed the cardiomyocyte (Fig. 2A and C), but preventing contraction in the absence of afterload (load-free) had no effect on AP shape or APD. This also agrees with a previous study showing that blebbistatin did not alter APs in Langendorff-perfused rabbit hearts (20). Therefore, the myocyte shortening per se, in the absence of afterload, had no significant acute effect on the electrical activity. This further

Fig. 1. Experimental approach to study mechanoelectric coupling. (A) Whole-cell patch-clamp experiments were performed in isolated rabbit ventricular cardiomyocytes embedded in PVA and perfused with Tyrode’s solution (load-free control). Then, 4B-PEG cross-linker was added to form a 3D viscoelastic hydrogel around the cell, which applies mechanical load during contraction. (B) Representative APs before gel formation (control, black) and after formation of the gel (in-gel, red) showing APD prolongation and changes in AP morphology under mechanical load. Later, a significant APD alternans (two subsequent APs are shown, blue) developed within 3 min of steady-state pacing (0.5 Hz, room temperature) in gel. (C) Time course of changes in APD$_{90}$ before and after application of the cross-linker, which forms the gel within seconds.
supports the notion that the electrophysiological changes in Figs. 1 and 2 are the consequences of afterload during cell contraction rather than by sarcomere length changes.

Fig. 2 D–I shows APs and contractions of cardiomyocytes under afterload during contraction in the hydrogel (1 min after adding the cross-linker). The AP morphology showed characteristic changes under afterload (Fig. 2D): the AP duration measured at 90% repolarization (APD90) increased from the load-free 517 ± 20 ms to 606 ± 23 ms in gel (Fig. 2D, E, and G). The AP in gel also exhibited a slightly depolarized resting membrane potential and a more negative early plateau potential (Plateau20) but more positive late plateau potential (Plateau75; Fig. 2G and H). This corresponds to faster phase-1 and slower phase-3 repolarization of the AP, indicating altered kinetics of the underlying ionic currents. The plateau potential at which the in-gel AP crossed the membrane potential of the load-free AP was 28.9 ± 1.6 mV. As expected, the sarcomere shortening was slightly smaller under mechanical load with fractional shortening decreased from 21.3 ± 0.9% in load-free to 19.2 ± 0.8% in-gel (Fig. 2 E and F). The maximal velocity of sarcomere shortening was slightly decreased in cardiomyocytes contracting under mechanical load, and the relaxation velocity was significantly reduced (τ of relaxation increased from 84.4 ± 6.8 ms load-free to 143.5 ± 11.4 ms in-gel; Fig. 2f). All mechanical load effects on AP were fully reversible upon blebbistatin application (SI Appendix, Fig. S1).

NOS1 Signaling Mediates Mechanoelectric Coupling. We previously found that nitric oxide synthase (NOS) mediates the mechano-chemo-transduction effect on Ca2+ handling under afterload (13). Here, we further tested whether the NOS pathway is also involved in the electrophysiological response of the myocyte to mechanical afterload. Under the load-free condition, pretreating the cells with the NOS inhibitor L-NAME (1 mmol/L) had no significant effect on any AP parameters (APD90 was 521 ± 28 ms in L-NAME–pretreated cells versus 517 ± 20 ms in control; Fig. 3 A and D, white bars). Similarly, both fractional shortening and relaxation kinetics during load-free contraction were unchanged in L-NAME (21.5 ± 0.8% versus 21.3 ± 0.9% in L-NAME treatment and control, respectively; Fig. 3 B, E, and F, white bars).

In sharp contrast to the lack of L-NAME effects on load-free cardiomyocyte results, L-NAME pretreatment nearly abolished the afterload-induced changes in APD and AP morphology (Fig. 3A and D). A slight APD90 prolongation was still observed (by 18.1 ± 4.7 ms; Fig. 3 C and D). In L-NAME–pretreated ventricular myocytes, the afterload produced by the gel caused substantially less fractional shortening than in control (Fig. 3 B and E), and the mechanical load–induced increase in the relaxation decay was prevented (Fig. 3F).

Fig. 3 G–J compares L-NAME with isoform-selective NOS inhibitor effects in which the afterload-induced changes in APD90, shortening, and relaxation velocity are compared to the control without NOS inhibition. The selective NOS1 inhibitor Nω-propyl-L-arginine (L-NPA, 5 µmol/L) prevented the afterload-induced APD prolongation, further depressed shortening amplitude, and prevented the slowing of relaxation, like the L-NAME effects. In contrast, the NOS3 inhibitor L-ω-(1-iminoethyl)-L-ornithine (L-NIO, 10 µmol/L) had no effect on any of these parameters when compared to control (Fig. 3 G–J). These results indicate that MEC is mediated predominantly by NOS1 signaling in rabbit ventricular myocytes contracting in a 3D viscoelastic hydrogel.

Mechanical Load–Induced Changes in Ito and AP Phase 1. Next, we investigated which ion channels might mediate the afterload-induced increased early AP repolarization (phase 1). Cardiomyocytes were pretreated with selective ion channel inhibitors prior to subjecting them to mechanical load (Fig. 4 A–C). Ito was inhibited using 4-aminopyridine (4-AP, 3 mmol/L), which markedly reduced early repolarization and slightly prolonged load-free APD90 (Fig. 4 A–C, white bars). Similarly, both fractional shortening and relaxation kinetics during load-free contraction were unchanged in L-NAME (21.5 ± 0.8% versus 21.3 ± 0.9% in L-NAME treatment and control, respectively; Fig. 3 B, E, and F, white bars).

In sharp contrast to the lack of L-NAME effects on load-free cardiomyocyte results, L-NAME pretreatment nearly abolished the afterload-induced changes in APD and AP morphology (Fig. 3A and D). A slight APD90 prolongation was still observed (by 18.1 ± 4.7 ms; Fig. 3 C and D). In L-NAME–pretreated ventricular myocytes, the afterload produced by the gel caused substantially less fractional shortening than in control (Fig. 3 B and E), and the mechanical load–induced increase in the relaxation decay was prevented (Fig. 3F).

Fig. 3 G–J compares L-NAME with isoform-selective NOS inhibitor effects in which the afterload-induced changes in APD90, shortening, and relaxation velocity are compared to the control without NOS inhibition. The selective NOS1 inhibitor Nω-propyl-L-arginine (L-NPA, 5 µmol/L) prevented the afterload-induced APD prolongation, further depressed shortening amplitude, and prevented the slowing of relaxation, like the L-NAME effects. In contrast, the NOS3 inhibitor L-ω-(1-iminoethyl)-L-ornithine (L-NIO, 10 µmol/L) had no effect on any of these parameters when compared to control (Fig. 3 G–J). These results indicate that MEC is mediated predominantly by NOS1 signaling in rabbit ventricular myocytes contracting in a 3D viscoelastic hydrogel.

Mechanical Load–Induced Changes in Ito and AP Phase 1. Next, we investigated which ion channels might mediate the afterload-induced increased early AP repolarization (phase 1). Cardiomyocytes were pretreated with selective ion channel inhibitors prior to subjecting them to mechanical load (Fig. 4 A–C). Ito was inhibited using 4-aminopyridine (4-AP, 3 mmol/L), which markedly reduced early repolarization and slightly prolonged load-free APD90 (Fig. 4 A–C, white bars). Similarly, both fractional shortening and relaxation kinetics during load-free contraction were unchanged in L-NAME (21.5 ± 0.8% versus 21.3 ± 0.9% in L-NAME treatment and control, respectively; Fig. 3 B, E, and F, white bars).

In sharp contrast to the lack of L-NAME effects on load-free cardiomyocyte results, L-NAME pretreatment nearly abolished the afterload-induced changes in APD and AP morphology (Fig. 3A and D). A slight APD90 prolongation was still observed (by 18.1 ± 4.7 ms; Fig. 3 C and D). In L-NAME–pretreated ventricular myocytes, the afterload produced by the gel caused substantially less fractional shortening than in control (Fig. 3 B and E), and the mechanical load–induced increase in the relaxation decay was prevented (Fig. 3F).

Fig. 3 G–J compares L-NAME with isoform-selective NOS inhibitor effects in which the afterload-induced changes in APD90, shortening, and relaxation velocity are compared to the control without NOS inhibition. The selective NOS1 inhibitor Nω-propyl-L-arginine (L-NPA, 5 µmol/L) prevented the afterload-induced APD prolongation, further depressed shortening amplitude, and prevented the slowing of relaxation, like the L-NAME effects. In contrast, the NOS3 inhibitor L-ω-(1-iminoethyl)-L-ornithine (L-NIO, 10 µmol/L) had no effect on any of these parameters when compared to control (Fig. 3 G–J). These results indicate that MEC is mediated predominantly by NOS1 signaling in rabbit ventricular myocytes contracting in a 3D viscoelastic hydrogel.

Mechanical Load–Induced Changes in Ito and AP Phase 1. Next, we investigated which ion channels might mediate the afterload-induced increased early AP repolarization (phase 1). Cardiomyocytes were pretreated with selective ion channel inhibitors prior to subjecting them to mechanical load (Fig. 4 A–C). Ito was inhibited using 4-aminopyridine (4-AP, 3 mmol/L), which markedly reduced early repolarization and slightly prolonged load-free APD90 (Fig. 4 A–C, white bars). Similarly, both fractional shortening and relaxation kinetics during load-free contraction were unchanged in L-NAME (21.5 ± 0.8% versus 21.3 ± 0.9% in L-NAME treatment and control, respectively; Fig. 3 B, E, and F, white bars).

In sharp contrast to the lack of L-NAME effects on load-free cardiomyocyte results, L-NAME pretreatment nearly abolished the afterload-induced changes in APD and AP morphology (Fig. 3A and D). A slight APD90 prolongation was still observed (by 18.1 ± 4.7 ms; Fig. 3 C and D). In L-NAME–pretreated ventricular myocytes, the afterload produced by the gel caused substantially less fractional shortening than in control (Fig. 3 B and E), and the mechanical load–induced increase in the relaxation decay was prevented (Fig. 3F).

Fig. 3 G–J compares L-NAME with isoform-selective NOS inhibitor effects in which the afterload-induced changes in APD90, shortening, and relaxation velocity are compared to the control without NOS inhibition. The selective NOS1 inhibitor Nω-propyl-L-arginine (L-NPA, 5 µmol/L) prevented the afterload-induced APD prolongation, further depressed shortening amplitude, and prevented the slowing of relaxation, like the L-NAME effects. In contrast, the NOS3 inhibitor L-ω-(1-iminoethyl)-L-ornithine (L-NIO, 10 µmol/L) had no effect on any of these parameters when compared to control (Fig. 3 G–J). These results indicate that MEC is mediated predominantly by NOS1 signaling in rabbit ventricular myocytes contracting in a 3D viscoelastic hydrogel.
and D). Importantly, under mechanical load, the enhanced phase-1 repolarization was abolished in 4-AP–pretreated cells (Fig. 4A).

Moreover, the mechanical load–induced APD90 prolongation and alternans were enhanced in 4-AP–pretreated cells (Fig. 4E and F).

We conclude that an afterload-induced increase in Ito is likely a main contributor to the afterload-induced enhancement of early AP repolarization, an effect that may limit larger afterload-induced increases in APD90 and alternans.

Ca2+-activated Cl− current (ICl, Ca), which has also been implicated in early repolarization (21), was inhibited using CaCCinh-A01 (30 μmol/L). CaCCinh-A01 slightly prolonged load-free APD90 but failed to alter the afterload-induced effects on early repolarization.

Fig. 3. Mechanoelectric coupling is predominantly mediated by NOS1 signaling. (A and B) Representative AP and sarcomere shortening traces in cardiomyocyte pretreated with the nonsubtype selective NOS inhibitor L-NAME (1 mmol/L) in load-free (LF) and in-gel (Gel) conditions. (C) Time course of APD change under mechanical load. (D–F) L-NAME pretreatment significantly attenuated APD prolongation (n = 8 cells from four animals), reduced fractional shortening, and attenuated the slowing of relaxation (n = 7 cells from four animals) in gel compared to control (n = 25 cells from seven animals and n = 8 cells from four animals for APD and shortening, respectively). (G–I) The mechanical load–induced APD prolongation was markedly attenuated by the selective NOS1 inhibitor L-NPA (5 μmol/L, n = 6 cells from three animals), whereas the NOS3 inhibitor L-NIO (10 μmol/L, n = 5 cells from three animals) had no effect. The sarcomere shortening under mechanical load was markedly reduced in L-NPA (n = 5 cells from three animals) but not in L-NIO (n = 5 cells from three animals). ANOVA with Dunnett’s post hoc test.

Fig. 4. Transient outward K+ current and a mechanosensitive current contribute to AP changes under mechanical load. (A–C) Representative APs following inhibition of Ito by 4-AP (3 mmol/L), ICl, Ca by CaCCinh-A01 (30 μmol/L), and IMSC by GsMTx-4 (5 μmol/L). (D) Load-free APD in control (Ctrl; n = 25 cells from seven animals) and following pretreatment with 4-AP (n = 10 cells from five animals), CaCCinh-A01 (CaCC; n = 6 cells from three animals), and GsMTx-4 (GsMTx; n = 6 cells from three animals). (E) Mechanical load–induced APD prolongation was more pronounced following 4-AP treatment. (F) Magnitude of mechanical load–induced APD alternans (difference between long and short APDs) was increased by 4-AP (n = 10 cells from five animals), unaltered by CaCCinh-A01 (n = 6 cells from three animals), and reduced by GsMTx-4 (n = 6 cells from three animals) compared to control (n = 19 cells from seven animals). ANOVA with Dunnett’s post hoc test.
or ΔAPD90 versus control (Fig. 4 B, D, and E). We also tested the potential role of mechanosensitive nonspecific cation current (IMSC) that can be inhibited using GsMTx-4 (5 μmol/L). GsMTx-4 had no effect on APD90 in load-free cells in solution. In the cells in gel, GsMTx-4 did not change the afterload effects on the early repolarization and APD90, although it did reduce the magnitude of APD alternans (Fig. 4 C–F). Hence, neither ICl,Ca nor IMSC plays a major role in the afterload-induced early repolarization or APD90 prolongation, but IMSC plays a role in APD alternans.

Profile of Mechanical Load–Induced Membrane Current under AP Clamp. Next, we investigated the changes in ionic currents that underlie the APD prolongation under afterload. A challenge in measuring individual ionic currents in our cell-in-gel system arises from our emphasis on maintaining physiological intracellular Ca2+ cycling during the excitation-contraction coupling under afterload. That precludes the conventional voltage-clamp experiments that typically use exogenous Ca2+ buffers to eliminate intracellular Ca2+ transient and cell contraction. Therefore, we used our physiological self-AP-clamp technique (22) to preserve Ca2+ transient and contraction and recorded the total membrane current induced by afterload. First, we recorded the cell’s own steady-state AP under the load-free condition and then used it as the voltage command in self-AP clamp (Fig. 5A). The load-free APD90 in self-AP-clamp experiments was similar to the APD90 measured in conventional current-clamp experiments (Fig. 5A, Inset). Without self-AP clamp, afterload induced a ∼10% decrease in sarcomere shortening (as in

![Figure 5](https://doi.org/10.1073/pnas.2108484118)
Mechanical Load Increases ICa,L and Decreases IK1 under Voltage Clamp. Prompted by the AP-clamp data suggesting mechanosensitive ICa,L, we directly tested for afterload-induced change of ICa,L. We measured ICa,L using conventional voltage-clamp protocol while preserving the Ca2+ transients and cell contractions by titrating the Ba2+ buffer in the pipette solution (see Materials and Methods).

The ICa,L was measured during depolarizing pulses to +5 mV from a holding potential of −40 mV (to inactivate Na+ channels) and calculated by subtracting the nifedipine-sensitive background current. Mechanical load increased the peak ICa,L density from load-free −4.8 ± 0.3 A/F to −6.3 ± 0.4 A/F in gel (Fig. 6 A and B), confirming that afterload induced a significant increase in the ICa,L current density.

Next, because IK1 is known to be important in stabilizing the negative resting Vm in ventricular myocytes, we measured IK1 using conventional voltage-clamp protocol with a hyperpolarizing pulse to −140 mV. IK1 was slightly decreased under mechanical load (−23.7 ± 1.4 A/F in gel versus −29.3 ± 1.5 A/F load free; Fig. 6 C and D). These data indicate that an afterload-induced reduction in IK1 may underly the small depolarization in resting Vm. This reduction in IK1 is expected to make the diastolic Vm less stable during events like delayed afterdepolarizations (24), which increases the risk of arrhythmias.

Mechanical Afterload Induces Alternans in APD, [Ca2+]i, [Ca2+]SR, and Contraction. We have observed that some rabbit ventricular myocytes (19 out of 25 cells) not only exhibited APD prolongation but also progressively developed APD alternans after 3 min into gel formation (Fig. 7 A–C). The observed alternans was always discordant; that is, the longer APD occurred during the smaller contraction, while the shorter APD occurred with the larger contraction (Fig. 7 D). This load-induced alternans dissipated by either dissolving the gel (SI Appendix, Fig. S2A) or using contraction uncoupler blebbistatin (SI Appendix, Fig. S2B) to relieve the afterload on cardiomyocytes. In comparison, similar discordant alternans occurred in load-free cells following high-dose (1 μmol/L) angiotensin II treatment and higher pacing rate (SI Appendix, Fig. 5 E and F). These data show that mechanical afterload affects multiple ion channels at different phases of the AP and that the dominant inward currents are regulated by intracellular Ca2+ (based on alteration in AP shape at different phases of the AP and that the dominant inward ICa,L, as observed. Conversely, the larger Ca2+ transient would drive a larger inward Na+/Ca2+ exchange current that is most apparent in late repolarization and at diastole, as observed (Fig. 5 E and F).

The mechanical afterload increases ICa,L and reduces IK1 under voltage clamp. (A) Representative ICa,L traces during a depolarization pulse to +5 mV in load-free (LF) and in-gel (Gel) conditions. The step current was inhibited by 10 μmol/L nifedipine (Nife). (B) Mechanical load increased ICa,L peak density (n = 9 cells from five animals). (C) Representative IK1 traces during a hyperpolarizing pulse to −140 mV. The current was inhibited by 300 μmol/L Ba2+. (D) IK1 was significantly decreased under mechanical load (n = 9 cells from five animals). ANOVA with Dunnett’s post hoc test.
S3) or following treatment with the L-type Ca²⁺ channel activator Bay K8644 (100 nmol/L) (SI Appendix, Fig. S4), suggesting a Ca²⁺-driven mechanism for discordant alternans.

Importantly, afterload-induced APD alternans was not observed in any of the L-NAME–pretreated cells contracting in gel (Fig. 7E). In contrast, afterload-induced contraction alternans was robust even under self-AP clamp (constant AP waveform; Fig. 7F), in which the mechanoelectrical feedback is absent but mechanopCa²⁺ feedback is present (although the contraction alternans were smaller under self-AP clamp). These data indicate that the afterload-induced Ca²⁺ handling changes are responsible for alternans, and the associated APD prolongation may further amplify contraction alternans. Moreover, simultaneous recording of sarcomere shortening and intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ, using Fura-2 fluorescence ratio) showed alternans in systolic [Ca²⁺]ᵢ. There were no beat-to-beat changes in diastolic [Ca²⁺]ᵢ or myofilament Ca²⁺ sensitivity, as evidenced by the overlapping [Ca²⁺]ᵢ versus sarcomere length curves during relaxation (Fig. 7G–I).

To examine whether the alternans arises from SR Ca²⁺ overload, we directly measured intra-SR [Ca²⁺] ([Ca²⁺]SR) using Fluo-5N loaded into the SR lumen (25). We found afterload-induced alternating small-large SR Ca²⁺ releases that were paralleled by fluctuations of diastolic [Ca²⁺]SR levels (Fig. 7 J–L). Notably, tachypacing-induced alternans is usually concordant and starts without variation in diastolic [Ca²⁺]SR but can progress to diastolic [Ca²⁺]SR alternans (26, 27), which is consistent with lowered diastolic [Ca²⁺]SR after the large Ca²⁺ transient in the previous beat drives more Ca²⁺ efflux via Na⁺/Ca²⁺ exchange and less Ca²⁺ influx due to stronger Ca²⁺-dependent inactivation of ICa,L.

**Discussion**

**Patch-Clamp-in-Gel Method to Study Afterload Effects on Cardiac Electrophysiology.** This is our first study on how the mechanical afterload on cardiomyocyte during beat-to-beat contraction regulates the AP and ionic currents. We explored the newly developed patch-clamp-in-gel methodology to perform various patch-clamp experiments (i.e., current clamp, voltage clamp, and AP clamp) while the cell was embedded in a 3D viscoelastic hydrogel and contracting under afterload. Here, we discuss our findings in the context of the previous literature but also keep in mind that the...
patch-clamp-in-gel setting applies 3D mechanical stress on the cell, which differs significantly from most previous studies of MEC by stretching the cell membrane to apply mechanical strain (i.e., uniaxial stretch, osmotic swelling, pipette suction, inflation, etc.). Given that different stress and strain fields on the rod-shaped cell architecture may activate different mechanosensors, the data from different experimental settings may not be directly comparable. Our intention here is to piece together information from relevant studies to gain more comprehensive understanding of the mechano-transduction mechanisms in cardiomyocytes.

**Afterload Effects Are Mediated by NOS1–NO Signaling.** Our previous studies using the cell-in-gel system show that the mechano-chemo-transduction is mediated by localized NOS1 signaling that increases the systolic Ca$^{2+}$ transient and contractility in compensatory response to increased afterload (13), but the diastolic spontaneous Ca$^{2+}$ spark rate was also significantly increased under afterload, which could increase the susceptibility to arrhythmias (28). However, little was known about how afterload affects APs and ion channels in cardiomyocytes and how the mechanoelectric coupling relates to Ca$^{2+}$ handling and cell contraction.

Furthermore, the near abolition of afterload-induced AP changes by inhibition of NOS1 indicates that this signaling pathway is critical to the electrophysiological effects of afterload, just as we have reported for the afterload-induced effects on myocyte Ca$^{2+}$ handling (13). Thus, afterload-induced NOS1–NO signaling affects multiple ion channels and Ca$^{2+}$ handling molecules. Future studies are needed to elucidate whether afterload-induced NO signaling directly alters the gating of these channels or indirectly by activating other signaling pathways, such as CaMKII and protein kinase G (PKG), among others. CaMKII has been shown to be activated by S-nitrosylation at serine 290 (29) and modulate sarcosomal ion channel activities and intracellular Ca$^{2+}$ handling in a very similar way, as observed here (30, 31). In contrast, PKG tends to inhibit I_{Ca,L} by phosphorylating serine 496 in the β2a subunit of the L-type Ca$^{2+}$ channel (32); however, details are still debated. Interestingly, NOS1 inhibition (without mechanical load) prolonged APD and increased I_{Ca,L} in guinea-pig cardiomyocytes (33). Thus, the precise molecular mechanisms by which mechanical afterload and NOS1–NO signaling affect each ion conductance require further investigation. Importantly, this afterload–inhibitor system (NOS1–NO signaling pathway differs from the stretch–inhibitor system) allows pre- and post-transduction effects on cardiomyocyte Ca$^{2+}$ handling reported by the Prosser and Lederger groups (16, 34), which involved stretch-induced rapid mecano-transduction mediated by microtubular deformation and NADPH oxidase 2 activation. Cell structural elements that sense and transduce mechanical afterload may include the costameres formed by the dystrophin–glycoprotein complex and the vinculin–talin–integrin system and require further investigation (15). These preload and afterload induced mechano-transduction pathways should combine in the working heart where both afterload and preload are present.

Our data using specific inhibitors of NOS1 (Fig. 3) indicates that NOS1 but not NOS3 is critical—a almost entirely—for the main afterload effects on AP. However, the GsMTx-4–sensitive I_{Msc} (Fig. 4) also contributes to the APD alternans. Afterload also affects the major cardiac voltage-gated ion channels I_{to}, I_{Ca,L}, and I_{K1}, which contribute to remodeling the AP at various phases (Figures 4–6). These ion channels have been reported to be mechanosensitive (7, 10) and are known to be regulated by NOS1 signaling (17). These data support the notion that afterload-induced NOS1–NO signaling modulates all these ion channels. The differential effect of NOS1 versus NOS3 indicates localized NOS1–NO signaling to these ion channels.

**Afterload Effects on Regulating Ion Channels and AP.** Remodeling of AP morphology under mechanical stretch was demonstrated previously in both single-cell (10) and whole-heart (8) levels. Stretching the cardiomyocytes (preload) caused accelerated early AP repolarization and slowed terminal repolarization, which are similar to our finding of the afterload effects (Fig. 2). However, the reversal potential (−30 mV) of the afterload-induced current under ap-AP clamp (Fig. 5C) is far more positive than that reported for stretch-activated currents (between 0 and −20 mV) (35). Moreover, in contrast to the stretch-induced currents with a typical linear current–voltage relationship (35), the afterload-induced membrane current under ap-AP clamp clearly showed a composite current that changed dynamically during the AP time course (Fig. 5C). Several factors may account for the differences between the afterload effects here and the preload effects in previous studies: the different types of mechanical stimuli applied on cardiomyocytes (mechanical stress under afterload versus mechanical strain under preload), different dimensionality of the forces (3D-resistive afterload versus one-dimensional uniaxial prestretch), cyclic beat-by-beat afterload versus static prestretch, AP clamp versus rectangular-pulse voltage clamp, and preserved Ca$^{2+}$ transient and contraction versus buffered [Ca$^{2+}$]_i and no contraction.

Our finding of the slight depolarization of resting V_m under afterload can be readily explained by the decrease of I_{to} observed in voltage-clamp experiments here (Fig. 6C) and in a previous report using stylus pressure on a mouse myocyte (36). The accelerated phase-1 early repolarization (Fig. 2) is in line with the early outward current enhancement observed in the ap-AP clamp (Fig. 5C), and it was mediated by the I_{to} inhibitor 4-AP (Fig. 4B). The inward current was also increased because of the increased Ca$^{2+}$ transients under afterload (37). However, a role for I_{Ca,L} seems unlikely because the afterload-induced early repolarization was not affected by selective I_{Ca,L} inhibition (Fig. 4B) and the early outward current in ap-AP clamp did not change during Ca$^{2+}$ alternans (Fig. 5E). It merits mention here that the I_{to}-dependent enhanced early repolarization also enhances the early Ca$^{2+}$ entry through I_{Ca,L} and the efficacy in triggering SR Ca$^{2+}$ release (38) and therefore may contribute to the afterload-induced contraction enhancement. Hence, physiological changes in blood pressure during daily activities is expected to alter the afterload encountered by the heart, which is expected to finetune the AP and Ca$^{2+}$ signaling to autoregulate contractility in compensatory response to load changes (39).

The APD prolongation and elevation of plateau potentials under afterload are likely to be a consequence of increased I_{Ca,L}, as tested directly using the conventional voltage-clamp protocol under afterload (Fig. 6D) and in line with previous reports on mechanosensitive I_{Ca,L} (40, 41). The current–voltage relationship of the afterload-induced inward plateau current (Fig. 5C, Inset) also resembled the profile of I_{Ca,L} under AP clamp (42, 43). The alternans data in ap-AP clamp (Figs. 5E and 7F) showed that a larger contraction and larger [Ca$^{2+}$]_i coincided with a decreased inward plateau current and shortened APD in line with the enhanced Ca$^{2+}$-dependent in-activation of I_{Ca,L} during larger Ca$^{2+}$ release from the SR (44). The late inward current was increased during the larger contraction and larger Ca$^{2+}$ transient, and the current showed a slow decay even after terminal AP repolarization (Fig. 5E), a feature most consistent with an inward Na$^+$/Ca$^{2+}$ exchange current elicited by the larger Ca$^{2+}$ transient.

**Afterload-Induced Arrhythmogenic Alternans.** The afterload-induced changes in ionic currents led to significant APD prolongation (Fig. 2) and, later, the development of APD alternans (Fig. 7), which increases susceptibility to arrhythmias (45). The discordant alternans was predominantly Ca$^{2+}$ driven (Fig. 7F) and critically dependent on SR Ca$^{2+}$ load (Fig. 7K), as previously demonstrated (26, 27, 46). In line with our data on alternans and its attenuation with GsMTx-4 (Fig. 4), a computer modeling study also predicted that an increase in I_{Msc} current promotes electromechanically discordant alternans when the alternans is Ca$^{2+}$ driven with negative-voltage Ca$^{2+}$ coupling (47). Ca$^{2+}$-dependent ionic currents and alternans in [Ca$^{2+}$]_i transient are known to significantly

**Mechanoelectric coupling and arrhythmogenesis in cardiomyocytes contracting under mechanical afterload in a 3D viscoelastic hydrogel**

Hegyi et al.
impact the beat-to-beat APD variability (48–50). Hence, the prior knowledge on cardiac alternans is consistent with our findings that the afterload on the cardiomyocyte during contraction against resistance causes activation of NOS1–NO signaling, which regulates multiple voltage-gated Ca2+ and K+ channels leading to enhanced phase-1 AP repolarization, APD prolongation, and enhanced intracellular Ca2+ transient and contractility. However, excessive afterload causes SR Ca2+ overload and fluctuation leading to discordant [Ca2+]i and APD alternans. APD prolongation and discordant APD alternans are known to provide vulnerable substrates for arrhythmias in the intact heart (45), suggesting that high mechanical load can markedly increase arrhythmia susceptibility in vivo. In line with this, abrupt high mechanical impact on the heart (“commotic cordis”) is strongly associated with cardiac arrhythmias and sudden cardiac death (51). Chronic conditions with increased mechanical load include hypertension (increased afterload), dilated cardiomyopathy (increased wall stress), and myocardial infarction ( stiffening of the myocardium and nonuniform mechanical stresses), all of which are associated with increased risk of arrhythmias and heart failure (7). QT interval prolongation on the electrocardiogram and alternans (either T wave or pulsus alternans) can be found in a significant fraction of these patients and are associated with poor prognoses (52). At the cell level, remodeling of electrophysiology (APD prolongation, alternans, and increased Ca2+ influx) and Ca2+ homeodynamics (Ca2+ overload and elevated diastolic Ca2+) have been shown in cardiomyocytes in many studies of cardiomyopathies (reviewed in ref. 53). Our present study provides insights on how mechanical load also causes electrophysiology and Ca2+ remodeling that contribute to arrhythmogenic activities.

Limitation and Perspective. The patch-clamp-in-gel methodology provides an informative experimental tool for studying the afterload effects on ion channels and APs while the cardiomyocyte is contracting in a 3D viscoelastic hydrogel. The cardiomyocyte is adhered by the hydrogel via binding of the cell-surface glycans to boronated-PEG cross-linked PVA polymer matrix. This experimental setting resembles the binding of cell-surface glycosylated molecules to the extracellular matrix in the 3D viscoelastic myocardium. However, in relating single-cell electrophysiology to arrhythmogenesis in the heart, one must also consider other important factors including the electrical coupling between cells, mechanosensitive properties of noncardiomyocytes, nonuniform anatomical distribution of the mechanical field, and the presence of both preload and afterload in the beating heart. The cell-in-gel methodology is well suited for studying the afterload effect in separation from preload (without prestretch), which is complementary to the various stretching methods for studying the preload effects. Hence, our study of the afterload effects on ion channels and action potentials complements the studies of the preload effects. It is necessary to combine the knowledge obtained from using all these methods to gain a comprehensive understanding of the heart’s intrinsic responses to mechanical loading.

1. S. W. Patterson, E. H. Starling, On the mechanical factors which determine the output of the ventricles. J. Physiol. 48, 357–379 (1914).
2. A. M. Katz, Ernest Henry Starling, his predecessors, and the “Law of the Heart”. Circulation 106, 2986–2992 (2002).
3. P. P. de Tombe et al., Myofilament length dependent activation. J. Mol. Cell. Cardiol. 48, 851–858 (2010).
4. G. von Anrep, On the part played by the suprarealns in the normal vascular reactions of the body. J. Physiol. 45, 307–317 (1912).
5. H. E. Gingolani, N. G. Pérez, O. H. Gingolani, I. L. Ennis, The Anrep effect: 100 years later. Am. J. Physiol. Heart Circ. Physiol. 304, H175–H182 (2013).
6. P. Kohl, F. Sachs, M. R. Franz, Cardiac Mecho-Electric Coupling and Arrhythmias. (Oxford University Press, 2 ed., 2011).
7. L. T. Izu et al., Mechano-electric and mecho-chemo-transduction in cardiomyocytes. Physiol Rev. 98, 1285–1305 (2020).
8. M. R. Franz, D. Burkhoff, D. Y. Yue, K. Sagawa, Mechanically induced action potential changes and arrhythmia in isolated and in situ canine hearts. Cardiovasc. Res. 23, 213–223 (1989).
9. D. E. Hansen, C. S. Craig, L. M. Hondogether, Stretch-induced arrhythmias in the isolated canine ventricle. Evidence for the importance of mechanoelectrical feedback. Circulation 81, 1094–1105 (1990).
10. P. Peyronnet, J. M. Nerbonne, P. Kohl, Cardiac mehano-gated ion channels and arrhythmias. Circ. Res. 118, 311–329 (2016).
11. F. A. Bainbridge, The influence of venous filling upon the rate of the heart. J. Physiol. 50, 65–84 (1915).
12. R. Shimkunas et al., Mechanical load regulates excitation-Ca2+ signaling-contraction in cardiomyocyte. Circ. Res. 128, 772–774 (2021).
13. Z. Jiao et al., Mechanocathemotransduction during cardiomyocyte contraction is mediated by localized nitric oxide signaling. Sci. Signal. 7, ra27 (2014).
14. J. Shav, L. Izu, Y. Chen-Izu, Mechanical analysis of single myocyte contraction in a 3-d elastic matrix. PLoS One 8, e75492 (2013).
15. Y. Chen-Izu, L. T. Izu, Mechano-chemo-transduction in cardiac myocytes. J. Physiol. 595, 3949–3958 (2017).

Materials and Methods
Detailed methods can be found in SI Appendix.

Cell-in-Gel System. The 3D viscoelastic gel matrix was made of a hydrogel system composed of undervisualized PVA (10 wt%, 89 to 90 kDa, hyrolyzed, Sigma-Aldrich) and a 4B-PEG cross-linker (7.5 wt%) as previously described (13). The tetravalent boronate group cross-links the PVA hydrogel, embedding the cell in a 3D gel matrix. Based on rheology measurements, mechanical analysis using a viscoelastic model estimated the elastic shear modulus of the hydrogel to be 4.7 kPa, which is comparable to the elasticity in infarcted hearts (54); for further details, see ref. 55. Importantly, the boronate group of 4B-PEG also cross-links the cis-diols of the cell surface glycans to PVA, thereby tethering the cell surface to the gel (13).

Animal Model and Cell Isolation. Ventricular cardiomyocytes were isolated from 20 young adult (3- to 4-mo-old, male, 2.5 to 3 kg) New Zealand White rabbits (Charles River Laboratories) using a standard enzymatic technique as previously described (56). All animal handling and laboratory procedures were in accordance with the approved protocols of the local Institutional Animal Care and Use Committee at University of California Davis, conforming to the Guide for the Care and Use of Laboratory Animals published by the US NIH (57).

Patch-Clamp-in-Gel Technique. Freshly isolated cardiomyocytes were first suspended in PVA and then perfused with Tyrode's solution. APs and ionic currents were recorded using whole-cell patch clamp with physiological solutions (for ionic composition, see SI Appendix: Materials and Methods). The cardiomyocytes were continuously stimulated in current-clamp experiments with suprathreshold depolarizing pulses delivered via the patch pipette at 0.5 Hz frequency. To measure the net membrane current induced by mechanical load under physiological conditions, intra-AP-clamp experiments were conducted as previously described (42). Formation of 3D viscoelastic hydrogel around the contracting cardiomyocyte was then achieved by applying the 4B-PEG cross-linker. All experiments were conducted at room temperature (22 ± 1 °C).

Ca2+ Concentration and Cardiomyocyte Contraction. Parallel cardiomyocyte contraction and intracellular (cytosolic) Ca2+ concentration, [Ca2+]i, or intra-SR Ca2+ concentration, [Ca2+]SR, were assessed by sarcomere length measurements, Fura-2 fluorescence ratio (F340/F380), and Fluo-30F fluorescence in field-stimulated cardiomyocytes (at 22 °C and 0.5 Hz pacing) using an IonOptix system as previously described (13).

Statistical Analysis. Data are expressed as the mean ± SEM. The statistical significance of differences was evaluated using a two-tailed Student’s t test (paired or unpaired) to compare two groups and ANOVA to compare multiple groups with Dunnett’s or Tukey’s post hoc tests. Origin 2016 and GraphPad Prism 9 software were used for data plotting and analysis. P < 0.05 was considered statistically significant.

Data Availability. All study data are included in the article and/or SI Appendix.

ACKNOWLEDGMENTS
We thank Dr. Tamás Bányász for scientific and technical discussions. We thank Mark Jude, Benjamin W. Van, Maura Ferrero, and Dr. Julie Bossuyt for their help in animal care, cell isolation, and laboratory tasks. This work was supported by NIH Grant Nos. R01-HL123526 (to Y.C.-I.), R01-HL141460 (to Y.C.-I.), R01-HL149431 (to Y.C-I. and L.T.I.), R01-HL030077 (to D.M.B.), R01-HL142282 (to D.M.B.), and F31-HL129746 (to R.S.) and the American Heart Association Grant No. 14GRNT20510041 (to Y.C.-I.).
16. B. L. Prosser, C. W. Ward, W. J. Lederer, X-ROS signalling is enhanced and graded by cyclic cardiomyocyte stretch. Cardiovasc. Res. 98, 307–314 (2013).

17. J. Tamargo, R. Caballero, R. Gómez, E. Delpón, Cardiac electrophysiological effects of nitric oxide. Cardiovasc. Res. 87, 593–600 (2010).

18. B. M. Bers, Calcium cycling and signaling in cardiac myocytes. Annu. Rev. Physiol. 70, 23–49 (2008).

19. T. M. Suchyna et al., Identification of a peptide toxin from Grammmostola spatulata spider venom that blocks cation-selective stretch-activated channels. J. Gen. Physiol. 115, 583–598 (2000).

20. V. V. Fedorov et al., Application of blebbistatin as an excitation-contraction uncoiler for electrophysiological study of rat and rabbit hearts. Heart Rhythm 4, 619–626 (2007).

21. B. Hegyi et al., Ca2+-activated Cl− current is antiarrhythmic by reducing both spatial and temporal heterogeneity of cardiac repolarization. J. Mol. Cell. Cardiol. 109, 27–37 (2017).

22. Y. Chen-Izu, L. T. Izu, B. Hegyi, T. Bányaỳsz, “Recording of ionic currents under physiological conditions: Action potential-clamp and ‘onion-peeling’ techniques” in Modern Tools of Biophysics, T. Jue, Ed. (Springer, New York, NY, 2017), pp. 31–48.

23. D. A. Eisner, J. L. Caldwell, K. Kistamás, A. W. Trafford, Calcium and excitation-contraction coupling in the heart. Circ. Res. 123, 181–195 (2017).

24. S. M. Pogwizd, K. Schlotthauer, L. W. Yuan, D. M. Bers, Arrhythmogenesis and contractile dysfunction in heart failure: Roles of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. Circ. Res. 88, 1159–1167 (2001).

25. T. R. Shannon, T. Guo, D. M. Bers, Ca2+ sparc: Local depletions of free [Ca2+] in cardiac sarcomplasmic reticulum during contractions leave substantial Ca2++ reserve. Circ. Res. 93, 40–45 (2003).

26. E. Pichl, J. DeSantiago, L. A. Blatter, D. M. Bers, Cardiac alternans do not rely on diastolic sarcomplasmic reticulum calcium content fluctuations. Circ. Res. 95, 740–748 (2000).

27. L. Wang et al., Optical mapping of sarcomplasmic reticulum Ca2+ in the intact heart: Rymyodine receptor refractoriness during alternans and fibrillation. Circ. Res. 114, 1410–1421 (2014).

28. D. M. Bers, Cardiac sarcomplasmic reticulum calcium leak: Basis and roles in cardiac dysfunction. Annu. Rev. Physiol. 76, 107–124 (2014).

29. J. R. Erickson et al., S-nitrosylation induces both autonomous activation and inhibition of calcium/calmodulin-dependent protein kinase II α. J. Biol. Chem. 290, 25646–25656 (2015).

30. B. Hegyi, D. M. Bers, J. Bossuyt, CaMKII signaling in heart diseases: Emerging role in heart failure: Roles of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. Circ. Res. 88, 1159–1167 (2001).

31. B. Hegyi et al., Hyperglycemia regulates cardiac K+ channels via O-GlcNAc-CaMKII and NOX2-ROS-PKC pathways. Basic Res. Cardiol. 115, 71 (2020).

32. L. Yang et al., Protein kinase G phosphorylates Cav1.2 alpha1c and beta2 subunits. Circ. Res. 103, 1304–1306 (2008).

33. C. Ronchi.

34. B. L. Prosser, C. W. Ward, W. J. Lederer, X-ROS signaling: Rapid mechano-chemo et al.

35. V. Dyachenko, B. Husse, U. Rueckschloss, G. Isenberg, Mechanical deformation of ventricular myocytes modulates both TRPC6 and KCa3.3 channels. Cell Calcium 45, 38–54 (2009).

36. B. Hegyi et al., Sarcollemmal Ca2+ entry through L-type Ca2+ channels controls the profile of Ca2+-activated Cl− current in canine ventricular myocytes. J. Mol. Cell. Cardiol. 97, 125–139 (2016).

37. R. Sah et al., Regulation of cardiac excitation-contraction coupling by action potential repolarization: Role of the transient outward potassium current (Ito). J. Physiol. 546, 5–18 (2003).

38. L. Izu et al., Emergence of mechano-sensitive contraction autoregulation in cardiomyocytes. Life (Basel) 11, 503 (2021).

39. Z. Pedrozo et al., Polyeyttn-1 is a cardiomyocyte mechanosensor that governs L-type Ca2+ channel protein stability. Circulation 131, 2131–2142 (2015).

40. G. L. Lyford et al., alpha1C(CaV1.2) L-type calcium channel mediates mechano-sensitive calcium regulation. Am. J. Physiol. Cell Physiol. 283, C1001–C1008 (2002).

41. B. Hegyi et al., Complex electrophysiological remodeling in postinfarction ischemic heart failure. Proc. Natl. Acad. Sci. U.S.A. 115, E3036–E3044 (2018).

42. B. Hegyi et al., Enhanced depolarization drive in failing rabbit ventricular myocytes: Calcium-dependent and β-adrenergic effects on late sodium, L-type calcium, and sodium-calcium exchange currents. Circ. Arrhythm. Electrophysiol. 12, e007061 (2019).

43. B. Hegyi et al., Changes in intracellular calcium concentration influence beat-to-beat variability of action potential duration in canine ventricular myocytes. Pflugers Arch. 467, 1431–1443 (2015).

44. K. Kistamás et al., Changes in intracellular calcium concentration influence beat-to-beat variability of action potential duration in canine ventricular myocytes. J. Physiol. Pharmacol. 66, 73–81 (2015).

45. D. M. Johnson et al., Diastolic spontaneous calcium release from the sarcoplasmic reticulum increases beat-to-beat variability of repolarization in canine ventricular myocytes after β-adrenergic stimulation. Circ. Res. 112, 246–256 (2013).

46. C. Másias, B. J. Maron, J. Weinstock, N. A. Estes III, M. S. Link, Commotio cordis: Sudden cardiac death with chest wall impact. J. Cardiovasc. Electrophysiol. 18, 115–122 (2007).

47. S. Galice, D. M. Bers, D. Sato, Stretch-activated current can promote or suppress cardiac alternans depending on voltage-calcium interaction. Biophys. J. 110, 2671–2677 (2016).

48. N. Zsontandrásy et al., Contribution of ion currents to beat-to-beat variability of action potential duration in canine ventricular myocytes. PLoS Arch. 467, 1431–1443 (2015).

49. K. Kistamás et al., Changes in intracellular calcium concentration influence beat-to-beat variability of action potential duration in canine ventricular myocytes. J. Physiol. Pharmacol. 66, 73–81 (2015).

50. D. M. Johnson et al., Diastolic spontaneous calcium release from the sarcoplasmic reticulum increases beat-to-beat variability of repolarization in canine ventricular myocytes after β-adrenergic stimulation. Circ. Res. 112, 246–256 (2013).

51. C. Medias, B. J. Maron, J. Weinstock, N. A. Estes III, M. S. Link, Commotio cordis: Sudden cardiac death with chest wall impact. J. Cardiovasc. Electrophysiol. 18, 115–122 (2007).

52. D. S. Rosenbaum et al., Electrical alternans and vulnerability to ventricular arrhythmias. N. Engl. J. Med. 330, 235–241 (1994).

53. S. Nattel, A. Maguy, S. Le Bouter, Y. H. Yeh, Arrhythmogenic ion-channel remodeling in the heart: Failure, myocardial infarction, and atrial fibrillation. Physiol. Rev. 87, 425–456 (2007).

54. C. Pislaru, M. W. Urban, S. V. Pislaru, R. R. Kinnick, J. F. Greenleaf, Viscoelastic properties of normal and infarcted myocardium measured by a multifrequency shear wave method: Comparison with pressure-segment length method. Ultrasound Med. Biol. 40, 1785–1795 (2014).

55. M. A. Kazemi-Lari et al., A viscoelastic Eshelby inclusion model and analysis of the Cell-in-Gel system. Int. J. Eng. Sci. 165, 103489 (2021).

56. B. Hegyi, T. Bányaỳsz, T. R. Shannon, Y. Chen-Izu, L. T. Izu, Electrophysiological determination of submembrane Na(+) concentration in cardiac myocytes. Biophys. J. 111, 1304–1315 (2016).

57. National Research Council, Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, ed. 8, 2011).