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Mechano-Chemo Transduction Tunes the Heartstrings

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Abstract

In the beating heart, mechanical stretch triggers the production of reactive oxygen or nitrogen species that target Ca²⁺-signaling proteins. Termed mechano-chemo transduction, this pathway “tunes” the calcium release machinery in the healthy heart; when dysregulated, it contributes to disease. In this issue of *Science Signaling*, Jian *et al.* used a “cell-in-gel” method to show that contractions in healthy heart cells elicit a steep, viscosity-dependent increase in mechano-chemo transduction in which nitric oxide synthase (NOS), NADPH oxidase 2 (Nox2), and Ca²⁺/calmodulin-dependent kinase II (CaMKII) contribute. These authors provide evidence for a role of neuronal NOS (nNOS) over endothelial NOS; they supported their findings with super-resolution microscopy, which localized nNOS nearest to the Ca²⁺ release sites. In a disease model, signaling through nNOS and CaMKII rather than through Nox2 was enhanced, supporting the independent mechano-activation of these enzymes. The coupling of these quantitative approaches will provide a new understanding of mechano-chemo transduction.

More than 60 years ago, Sandow coined the term excitation-contraction (EC) coupling and proposed cytosolic calcium as the critical activator of muscle contraction (1); it has now become clear that contraction itself provides important feedback in shaping this calcium signal. In this issue of *Science Signaling*, Jian *et al.* extend the concept of mechano-chemo transduction, a term that encompasses how mechanical stress dynamically modulates calcium handling through chemical effectors (2). Jian *et al.* showed the utility of a tool in which they embedded cardiomyocytes in a variable-viscosity gel that interacts with the cell membrane. The “cell-in-gel” contracts under viscoelastic load with stress transmitted in three dimensions (3). Using this technique, they identified neuronal nitric oxide synthase (nNOS), NADPH oxidase 2 (Nox2), and Ca²⁺/calmodulin-dependent kinase II (CaMKII) as key enzymes involved in the mechanical modulation of EC coupling and raise intriguing questions about the mechanism involved. Here, we offer a mechanistic discussion and

testable hypotheses for future work aimed at defining the interplay of cell mechanics and calcium signaling in healthy and diseased myocardium.

Key players in cardiomyocyte mechano-chemo transduction are located in the dyad (Fig. 1), the intersection between a junctional sarcoplasmic reticulum (jSR) and transverse tubule. The type 2 ryanodine receptors (RyR2s) cluster in the jSR membrane, directly opposed to L-type calcium channels in the t-tubule, and are activated by calcium-induced calcium release. The importance of RyR2 regulation is underscored not simply by its role in releasing Ca^{2+} stores to drive contraction, but also because abnormal RyR2 activity contributes to Ca^{2+} -dependent arrhythmias, hypertrophy, and heart failure. At the dyad, previously established regulators of RyR2 function include nNOS and endothelial NOS (eNOS), both of which produce NO; Nox2, which produces superoxide that is rapidly dismutated to hydrogen peroxide, resulting in ROS in the dyadic space; and CaMKII.

The principal finding of Jian *et al.* is that compared to myocytes contracting under little or no load, cell-in-gel contractions increased global calcium release and led to a high frequency of Ca^{2+} sparks (4) without detectably changing SR calcium content. Although this finding is consistent with enhanced activation of RyR2, simple pump-leak balance (5) would suggest that SR Ca^{2+} content should decrease, thereby implicating additional factors. The authors also propose that the enhanced RyR2 activation contributes to the Anrep effect, the slow augmentation of calcium release and contractility that occurs with an increase in cell stress (6).

Furthermore, the authors identified NOS as a key regulator of the augmented response. Although this result extends the work of the Solott group and others (7), the use of pharmacologic agents and genetic ablation revealed that nNOS rather than eNOS was the dominant chemoeffector, and super-resolution imaging showed colocalization of nNOS near the RyR2s. This combination of live-cell and super-resolution imaging sets a new standard for examining the micro-domain signaling of these molecules.

Nox2 or CaMKII inhibition were equally effective to nNOS inhibition in blocking mechanically induced activation of RyR2 during cell-in-gel contractions. This confirms reports from our group and others showing that Nox2-generated ROS (X-ROS) is a potent mechano-chemoeffector of RyR2, but also raises the question of apparently redundant signaling.

One possibility is that Nox2 and NOS are triggered by separate mechanical stimuli that are each activated during cell-in-gel contractions. X-ROS signaling is activated by longitudinal and axial stresses through the microtubule cytoskeleton (2, 8, 9), whereas NOS is modulated by membrane shear stress through yet-unidentified elements. As modeled (10), a cell-in-gel contraction results in both longitudinal and axial stress as well as three-dimensional shear stress at the membrane. This provides a parsimonious explanation for dual activation of NOS and Nox2 in the cell-in-gel experiments, yet incompletely explains their redundant roles.

Another nuance of the cell-in-gel system that may contribute to augmented signaling is the steep, nonlinear dependence of RyR2 activation on gel density. In fact, healthy cells

contracting in the denser gel exhibited very high Ca^{2+} spark frequencies consistent with destabilized RyR2s more commonly seen in disease models. Although we speculate that the stiffer gel may more closely approximate supraphysiologic stress, the signaling generated in these experiments has resulted in important new findings. A critical next step is to define the viscoelastic environment of the healthy and diseased heart in situ; with these parameters, the gel can be adjusted to evaluate cardiomyocytes under pathophysiologic and physiologic stresses. An additional advancement would be the ability to apply diastolic stretch to the cell-in-gel system to separate the effects of preload and afterload stress, as each may have different effects on downstream signaling.

Jian *et al.*'s investigation into an arrhythmia model arising from a troponin T (TnT) mutation reveals that similar stresses may elicit different signaling depending on the pathophysiological background. In contrast to healthy cells, the cell-in-gel enhancement of RyR2 activation in TnT mutant myocytes was dependent on NOS and CaMKII but independent of Nox2—a finding that supports independent activation pathways. However, in a dystrophin-deficient cardiomyopathy model, the microtubule cytoskeleton is stiffened and Nox2 abundance is increased; consistently, microtubule-dependent Nox2 hyperactivation underlies the Ca^{2+} instability induced by preload stress (2). Results in disease states will undoubtedly correlate with the abundance and regulation of the key mechanosensitive enzymes and transducers; without this information, it is difficult to place the TnT mutant results in context.

Regardless of the activation stress, signaling interactions among nNOS, Nox2, and CaMKII likely contribute to their apparently equal enhancement of RyR2 activation (Fig. 1B). Nox2-generated superoxide, which becomes H_2O_2 , can directly oxidize and activate RyR2, as can NO. However, superoxide can also interact with NO to form peroxynitrite, which also modifies target proteins such as RyR2. CaMKII is a likely player in this pathway because it can be activated by oxidation by Nox2-ROS (11) and NO (12). Synergism among Nox2, NOS, and CaMKII has become increasingly evident. We find that with frequent passive stretches, X-ROS production dominates RyR2 modulation initially (within seconds), but NOS and CaMKII signaling arise after a delay (tens of seconds) (13). Consistent with this finding, Dries *et al.* reported that Nox2-dependent activation of CaMKII likely underlies RyR2 modulation during high-frequency mechanical stress (14).

An additional target of this synergistic interplay not addressed by Jian *et al.* is the mechanosensitive channel (MSC) (Fig. 1). MSCs are modulated by both ROS and NO and are implicated in the Anrep effect. X-ROS activation of MSCs contributes to increased cytosolic calcium during mechanical stress (9) and may contribute to the RyR2 activation reported here. The role of NO is more complex: Kass and co-workers have demonstrated that activation of protein kinase G, which is driven by NO, suppresses MSC activity to blunt the Anrep effect (15). Initially, this appears in opposition to the results presented by Jian *et al.*, which suggest that NO augments the Anrep effect through modulation of RyR2; how can these seemingly disparate results be reconciled?

First, bear in mind that mechano-chemo transduction depends on the nature of the mechanical stress and abundance of key players. Studies using various methods of stress in

complex disease models require both independent and unified interpretation. Although mechanotransduction in the cell-in-gel system is currently uncalibrated and complex, it is a methodological approach that makes measuring mechanotransduction accessible to the scientific community. However, there are still two issues to reconcile: (i) the conflicting role of NO as a modulator of the Anrep effect, and (ii) the seemingly redundant effects of Nox2, CaMKII, and nNOS on EC coupling. We thus propose a conceptual hypothesis to unify both unresolved issues (Fig. 1).

In the presence of superoxide, nNOS becomes “uncoupled” and switches from NO to superoxide production (16). We hypothesize that healthy cells contracting in a stiff viscous gel will generate substantially more Nox2-ROS, which can uncouple nearby nNOS, leading to an additional source of superoxide. Both superoxide sources may then directly modify RyR2 and synergistically effect EC coupling through CaMKII (Fig. 1). This would explain the redundant effects seen by Jian *et al.*, while also separating the influence of NO from uncoupled nNOS (and thus ROS) on the Anrep effect. New work with the cell-in-gel and complementary approaches will enable direct testing of this and alternative hypotheses in healthy and diseased heart.

Regardless of the nature or magnitude of stress, the study of Jian *et al.* highlights the importance of conducting experiments under mechanical load. Mechanical stress clearly influences calcium signaling, and studies in unloaded myocytes present a valuable but incomplete story. The new tools and questions stemming from the work of Jian *et al.* show that exciting discoveries await those who study mechanochemo transduction.

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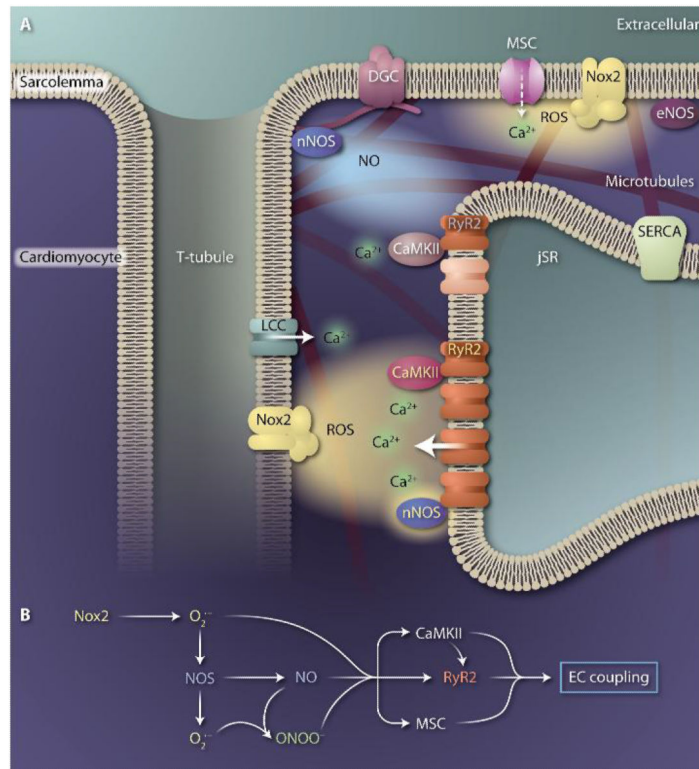


Fig. 1. Mechano-chemo transduction in the heart

(A) Simplified model of key players at the calcium release unit of a cardiomyocyte. Upon mechanical stress, mechanosensitive enzymes generate local signals that target calcium handling proteins. These include Nox2, which produces ROS in the sarcolemmal and T-tubule membrane, and NOS, which produces NO and is localized to the SR and sarcolemma membranes as part of the dystrophin-dystroglycan complex (DGC). ROS and NO can directly regulate RyR2 activity through oxidation and nitrosylation, respectively, or can activate CaMKII, which modulates RyR2 through phosphorylation. Superoxide in the subspace can uncouple nNOS, resulting in superoxide rather than NO production. Superoxide and NO can also interact to form peroxynitrite. Additional targets of ROS and NO include the mechanosensitive channel (MSC), the L-type Ca^{2+} channel (LCC), and the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA). ROS and NO preferentially modify target proteins in their immediate vicinity, as depicted by the sensitized RyR2 and CaMKII (in darker colors). Although mechano-chemo coupling tunes EC coupling in the healthy heart, altered localization or expression of key players underscores the destabilized calcium signaling seen with increased mechanical stress and in disease. (B) Potential linear reaction schemes involved in mechano-chemo transduction.