

## Regional differences in arrhythmogenesis

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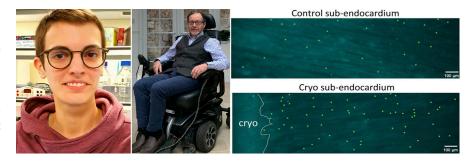
JGP study shows that the subendocardium is more susceptible to spontaneous Ca<sup>2+</sup> release events that can initiate arrhythmias, and this may be reduced by local CaMKII inhibition.

Calcium release and uptake must be carefully controlled in cardiomyocytes to ensure that the heart maintains a regular beat, and spontaneous Ca<sup>2+</sup> release (SCR) from the sarcoplasmic reticulum—due to leaky ryanodine receptors, for example—can trigger lethal ventricular arrhythmias. In this issue of *JGP*, Dries et al. demonstrate that the subendocardial layer of the ventricular wall is particularly susceptible to arrhythmogenic SCR, and that this could potentially be treated by local inhibition of calcium/calmodulin-dependent kinase II (CaMKII; 1).

SCRs have been extensively studied in isolated cardiomyocytes, but arrhythmias are multicellular events (2) in which the behavior of individual cells is influenced by their interactions with neighboring cells and the extracellular matrix. "In addition, myocardial electrophysiology changes at different depths of the ventricular wall, and the vast majority of studies do not account for this transmurality," explains Cesare Terracciano, a professor at the National Heart and Lung Institute, Imperial College London.

Terracciano's group has pioneered the use of living myocardial slices prepared from different layers of the ventricular wall to study regional differences in the electrical and mechanical properties of healthy hearts (3,4). However, it is unclear how these differences are impacted by injury or disease and whether this leaves some layers of the heart wall more susceptible to SCRs and arrhythmogenesis.

Terracciano and colleagues, including first author Eef Dries, therefore prepared



Using living myocardial slices, Eef Dries (left), Cesare Terracciano (center), and colleagues show that, following injury, the subendocardial layer of the rat ventricular wall is more susceptible than the subepicardial layer to arrhythmogenic SCR events. High-resolution  $Ca^{2+}$  imaging of the subendocardium shows the increased number of SCRs (green dots) in the region bordering the injured tissue. The frequency of SCRs and ectopic contractions can be reduced by CaMKII inhibition.

myocardial slices from different layers of the rat ventricular wall and subjected them to cryoinjury (1). Structural remodeling—in the form of reduced T-tubule density—was similar in both subendocardial and subepicardial slices after injury, but only subendocardial slices showed an increase in spontaneous, arrhythmic contractions.

Dries et al. used a fluorescent Ca<sup>2+</sup> indicator and high-resolution imaging to examine Ca<sup>2+</sup> signaling in the "border zone" surrounding the cryoinjury, as this region has been implicated in triggering arrhythmias following myocardial infarction. "Intriguingly, and only in subendocardial slices after injury, we observed a reduction in the amplitude of calcium transients that also became slower to decline, changes that are hallmarks of heart failure," Terracciano says. "SCR events were more frequent and more closely distributed when we cryoinjured the slices but, again, only in the subendocardium."

The clustering of multiple SCRs in both space and time makes them more likely to trigger an ectopic contraction. One possibility is that the open probability of ryanodine receptors is increased in subendocardial slices. This could be caused by enhanced CaMKII-mediated phosphorylation of ryanodine receptors and, indeed, Dries et al. found that, after cryoinjury, receptor phosphorylation is increased in subendocardial, but not subepicardial, slices (1).

Accordingly, Terracciano and colleagues found that the CaMKII inhibitor AIP reduced the frequency of SCRs and spontaneous contractions in cryoinjured subendocardial slices. In contrast, AIP had no effect on injured subepicardial slices or on normal, healthy cardiac tissue. CaMKII inhibitors have been proposed as potential therapies for cardiac arrhythmias, but their use has so far been limited by off-target effects. Dries et al.'s results suggest that

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targeting CaMKII inhibitors to specific regions of the ventricular wall (using localized gene therapy, for example) could greatly improve their efficacy.

"A picture is emerging that subendocardial slices are more susceptible to arrhythmogenic stimuli, and this can be important for understanding and treating arrhythmias," Terracciano says. He now plans to study injured myocardial slices over longer time periods and investigate the molecular changes underlying the enhanced arrhythmogenic susceptibility of the subendocardium, as well as testing localized gene therapy approaches in animal models of disease.

## References

- 1. Dries, E., et al. 2021. J. Gen. Physiol. https://doi .org/10.1085/jgp.202012737
- Houser, S.R. 2000. Circ. Res. https://doi.org/10 .1161/01.RES.87.9.725
- 3. Pitoulis, F.G., et al. 2020. *J. Mol. Cell. Cardiol.* https://doi.org/10.1016/j.yjmcc.2020.03.007
- 4. Pitoulis, F.G., et al. 2020. *Cardiovasc. Res.* https://doi.org/10.1093/cvr/cvz341