New and Notable

Cardiac Electrophysiology Delivered a "Grand Slam" by Angiotensin II: The Third Explanation of Transmural Cardiac Electrical Activity Gradients

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Evidence has accumulated for decades that the mechanosensitive release of angiotensin II (ATII) by cardiac myocytes and fibroblasts can importantly regulate myocyte excitation-contraction coupling, electrical activity, and growth. In this issue of the *Biophysical Journal,* Gao et al. (1) present evidence for a "grand slam" hypothesis that the release of ATII by cardiac myocytes depends on a gradient of mechanical stress from endocardium to epicardium. As a result, ATII release is constitutively more active in the endocardium. That cardiac myocytes release more ATII with an increase of either afterload or preload is now well documented to underlie the slowly developing, load-dependent increases of cardiac Ca transients and contraction via the Anrep effect (2).

The authors hypothesize further that the resulting transmural gradient of ATII receptor activation, proposed to occur within T-tubules, promotes the generation of ion channel and transporter activity gradients via posttranslational ATII signaling.

In support of their hypotheses, the ATII-dependencies of three important cardiac currents are analyzed across the canine left ventricle: transient outward K current, Na/K pump current, and L-type Ca current. The former two become upregulated in endocardial myocytes when ATII signaling is blocked by ATII antagonists. Conversely, the larger epicardial currents are downregulated by ATII so that current densities become nearly equal across the myocardium. Ca channels are initially downregulated by ATII, but their activity rebounds beyond baseline activity. These ATII-dependent current changes are in the right direction, and they are large enough in magnitude, to substantively modulate both contractile and electrical activity of the heart.

In baseball, a "grand slam" is a home run hit with the bases loaded, and certainly, many recent developments in cardiac signaling related to electrical remodeling have primed the field for a potentially major breakthrough. Of course, you can still lose the ball game after hitting a grand slam. In this case, at least two opposing runners have been around the bases. The first competing hypothesis is that the transcription factor *Irx5* establishes the cardiac ventricular repolarization gradient by suppressing expression of the potassium channel subunit, Kv4.2, and thereby transient outward current, in endocardial myocardium (3). This developmental mechanism follows the model of the cardiac conduction system, whereby differentiation is driven by multiple transcriptional programs that are turned on or off in the targeted myocytes. The second competing hypothesis, presently at play in the ball park, is that the Ca-dependent calcineurin/NFAT pathway regulates expression of Kv4 channel subunits; NFATc3-null mice show complete loss of the transmural gradient of transient outward current and Kv4 itself (4). Here, there is some room for a compromise.

One could speculate that ATII signaling establishes an excitation-contraction coupling gradient (i.e., higher Ca transients in the endocardium) that, in turn, drives calcineurin/NFAT signaling. However, that is not what Gao et al. (1) propose. Indeed, the changes of electrical activities are occurring on a timescale of one to a few hours, too fast for most cardiac translational programs to be involved. As a result, our field is now graced with three very different mechanisms to account for the transmural gradient of transient outward current in the heart. It may be, therefore, that transient outward current is regulated by multiple redundant mechanisms. It may also be that different regulatory mechanisms are active in different species. With certainty, a major controversy in cardiac electrophysiology has now erupted with increased potential fall out.

For cardiac physiology and pathology, the importance of resolving this controversy can hardly be overestimated. Transmural differences in the electrophysiology and excitation-contraction coupling of cardiac myocytes appear to be universal. In zebra fish, frogs, and mammals from mice to men, action potentials in the ventricular endocardium are longer than those occurring in the epicardium, thereby giving rise to a repolarization wave that travels from epicardium to endocardium. Importantly, the T-wave of the ECG is caused by the transient electrical gradient occurring during this repolarization wave. In addition, it appears to be the rule that Ca transients, developed pressure, and the rate of pressure development are larger in the endocardium versus the epicardium (5). Almost certainly, these transmural differences in myocyte function optimize ejection of blood by the heart and are therefore required for effective cardiac function across species. As many readers of the *Biophysical Journal* will know, other prominent features of cardiac excitation-contraction are remarkably different between species. These include ventricular action potential shapes, the specific K channels involved, the role of Ca influx in activating contraction, and

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the contraction staircases that occur upon changes of heart rate.

The idea that a gradient of ventricular wall stress (and/or strain) promotes a gradient of ATII release is attractive because it would explain how a simple biophysical principle controls a complex cellular regulatory pathway. Not surprisingly, the subject of ventricular wall stress has a long and complex history. Even in a hollow rubber ball with a thick wall, wall stress is a fairly difficult physical problem (6). Certainly, more mechanical stress will occur close to the inside of a thick rubber ball than close to the outer surface when pressure is applied to the inside of the ball. However, the principles that determine wall stress in intact cardiac ventricles are much more complex. To begin, it is essential to analyze fiber orientation and curvature through the myocardium, and then reconstruct wall tension in three dimensions. To make matters even more complex, the measurement of wall stress in the intact heart is technically challenging. Best estimates of myocardial strain suggest that with a left ventricular pressure of 8 mm Hg, both circumferential and longitudinal strains increase by a factor of 2–3 from epicardium to endocardium (7). The obvious consequence of transmural stress and work gradients will be a gradient of metabolic rate. In this connection, it seems firmly established that transmural differences in cardiac energy metabolism for intermediate-sized, normal hearts are $<20\%$ (8). Nevertheless, much larger inhomogeneities develop rapidly upon stopping blood flow, and probably explain why the endocardium is relatively more vulnerable to ischemic damage (9).

One persuasive study relevant to this article concerns the release of adenosine by cardiac myocytes (10). The adenosine concentration of the cardiac interstitium is known to reflect cardiac metabolic rate and to increase in the presence of metabolic stress. In a well-perfused heart, the extracellular adenosine concentration is entirely homogeneous across the left ventricular wall. However, adenosine quickly increases to 12-times-higher concentrations in the endocardial interstitium versus the epicardial interstitium when coronary flow is modestly reduced. The large magnitude of the transmural adenosine gradient suggests that other paracrine and autocrine hormones may well also develop transmural gradients during metabolic stress. Clearly, a similar approach with an appropriate dialysis method, such as the porous nylon sampling disks used by Zhu et al. (10), is now essential to test the ATII hypothesis. In short, it should be very feasible to determine whether transmural ATII gradients exit normally and/or whether ATII gradients might be induced by changes of passive load, work load, or coronary flow.

Gao et al. (1) demonstrate that most of the electrophysiological changes mediated by ATII occur in T-tubules, and they speculate that endocytic mechanisms may be involved. In support of the plausibility of this mechanism, recent work suggests that cardiac myocytes can indeed internalize large fractions of their sarcolemma (11), as would be required to downregulate a transport mechanism with a high sarcolemmal density (i.e., Na/K pumps). Previous studies of ATII signaling have examined both short- and long-term effects on many conductances. Although the modulation of cardiac Ca current by ATII has been a controversial subject, studies with the perforated patch-clamp technique have convincingly demonstrated a facilitating effect of ATII mediated by protein kinase $C(s)$ (12).

Downregulation of transient outward K currents by ATII has also been reported variously over both short and long timescales. Using a myocyte culture model that allows application of mechanical stretch, Saygili et al. (13) demonstrated that chronic stretch promotes the expression of many cardiac K channels; however, expression of the transient outward current Kv 4.2 channel subunit was decreased, and the decrease was blocked by an ATII antagonist. In other myocyte culture models, it has been demonstrated that ATII downregulates K channel subunits involved in transient outward current by destabilizing their mRNA, and the specific mechanisms that regulate Kv4.3 mRNA stability have been at least partially elucidated (14). Together with the results of the article under discussion, it seems unambiguous that ATII downregulates transient outward currents in the heart by multiple mechanisms. However, the suggested involvement of endocytosis remains to be established.

Finally, the downregulation of Na/K pumps by ATII requires consideration. Liu et al. (15) have described extensively that ATII downregulates cardiac Na/K pump activity in rabbit myocytes by a mechanism that involves reactive oxygen species' generation and glutathionylation of β-subunits of the pump. The functional consequence of glutathionylation is that the apparent affinity of the pump for cytoplasmic Na is decreased. In other words, ATII downregulates Na/K pump activity rather potently by a mechanism that does not involve removal of Na/K pumps from the surface membrane. Importantly, Gao et al. (1) have employed a very high cytoplasmic Na concentration (70 mM) in their experiments in the absence of cytoplasmic K. In this condition, the mechanism described by Liu et al. (15) will not be operational. Therefore, the results of Gao et al. (1) are inconsistent with previous studies of ATII effects on cardiac Na/K pumps. It will have to be established by future studies how such results using canine myocytes can be related to those of Liu et al. (15), using rabbit myocytes, and whether endocytic mechanisms are involved.

In summary, the cardiac field is presently confronted with three different hypotheses concerning the generation and maintenance of transmural gradients of cardiac electrical activity, in particular the gradient of

transient outward current that keeps the epicardial action potential relatively short. The proposal that post-translational mechanisms initiated by mechanosensitive ATII release shape or even cause the transmural gradient of electrical activity is attractive for several reasons. It is very familiar territory. The initial steps of this pathway increase cardiac Ca transients during short-term ATII signaling in response to changes of pre- and afterload (2), and over long times the pathway drives cardiac growth via transcriptional mechanisms in response to hypertension.

At the end of the day, the hypothesis of Gao et al. (1) is attractive simply because it makes many important predictions that can be readily tested. Two obvious ones stand out:

1. It should be possible to demonstrate the existence of an extracellular ATII gradient across the left ventricular wall.
2. High concentrations of ATII antagonists, as well as a constant infusion of ATII, should cause significant changes of the T-wave of the cardiac ECG in living animals within one to a few hours.

Experimental progress in these areas could quickly move the working hypothesis from the plausible to the likely category. It seems certain that we will see decisive experimental evidence for and/or against the provocative hypothesis outlined in the article by Gao et al. (1) in a relatively short time.

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REFERENCES

1. Gao, J., X. Sun, …, J. H. Kim. 2014. Autocrine A2 in the T-system of ventricular myocytes creates transmural gradients in ion transport: a mechanism to match contraction with load? *Biophys. J.* 106:2364–2374.
2. Cingolani, H. E., N. G. Pérez, …, I. L. Ennis. 2013. The Anrep effect: 100 years later. *Am. J. Physiol. Heart Circ. Physiol.* 304:H175–H182.
3. Costantini, D. L., E. P. Arruda, …, B. G. Bruneau. 2005. The homeodomain transcription factor *Irx5* establishes the mouse cardiac ventricular repolarization gradient. *Cell.* 123:347–358.
4. Rossow, C. F., K. W. Dilly, …, L. F. Santana. 2009. NFATc3-dependent loss of I_{to} gradient across the left ventricular wall during chronic β-adrenergic stimulation. *J. Mol. Cell. Cardiol.* 46:249–256.
5. Cordeiro, J. M., L. Greene, …, C. Antzelevitch. 2004. Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. *Am. J. Physiol. Heart Circ. Physiol.* 286:H1471–H1479.
6. Moriarty, T. F. 1980. The law of Laplace. Its limitations as a relation for diastolic pressure, volume, or wall stress of the left ventricle. *Circ. Res.* 46:321–331.
7. Omens, J. H., K. D. May, and A. D. McCulloch. 1991. Transmural distribution of three-dimensional strain in the isolated arrested canine left ventricle. *Am. J. Physiol.* 261:H918–H928.
8. van der Vusse, G. J., T. Arts, …, R. S. Reneman. 1990. Transmural differences in energy metabolism of the left ventricular myocardium: fact or fiction. *J. Mol. Cell. Cardiol.* 22:23–37.
9. Kuwada, Y., and K. Takenaka. 2000. Transmural heterogeneity of the left ventricular wall: subendocardial layer and subepicardial layer. *J. Cardiol.* 35:205–218.
10. Zhu, Q. Y., J. P. Headrick, and R. M. Berne. 1991. Transmural distribution of extracellular purines in isolated guinea pig heart. *Proc. Natl. Acad. Sci. USA.* 88:657–660.
11. Lin, M. J., M. Fine, …, D. W. Hilgemann. 2013. Massive palmitoylation-dependent endocytosis during reoxygenation of anoxic cardiac muscle. *eLife.* 2:e01295.
12. Aiello, E. A., and H. E. Cingolani. 2001. Angiotensin II stimulates cardiac L-type Ca^{2+} current by a Ca^{2+}- and protein kinase C-dependent mechanism. *Am. J. Physiol. Heart Circ. Physiol.* 280:H1528–H1536.
13. Saygili, E., O. R. Rana, …, C. Zobel. 2007. Losartan prevents stretch-induced electrical remodeling in cultured atrial neonatal myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 292:H2898–H2905.
14. Zhou, C., C. Z. Vignere, and E. S. Levitan. 2008. AUF1 is upregulated by angiotensin II to destabilize cardiac Kv4.3 channel mRNA. *J. Mol. Cell. Cardiol.* 45:832–838.
15. Liu, C. C., A. Garcia, …, H. H. Rasmussen. 2012. Susceptibility of β1 Na^+-K^+ pump subunit to glutathionylation and oxidative inhibition depends on conformational state of pump. *J. Biol. Chem.* 287:12353–12364.