Heart Failure: The Final Frontier for Biophysics in Cardiovascular Medicine?

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Excitation Transcription Coupling, the New Frontier in HF Therapeutics

Alteration in how the cardiomyocyte manages intracellular calcium is not only important acutely in alteration of the cardiac action potential leading to potentially arrhythmogenic changes within the myocardium, but long-term, global changes in intracellular calcium have been linked to hypertrophy and heart failure [1–3]. Recent work implicates the NFATc3 transcription factor as a key player that translates these changes in intracellular calcium into changes in gene expression, ion current remodeling, and ultimately reshaping of the cardiac action potential via reduction in repolarizing Kv currents. As early as 48 h post MI, this reduction in repolarizing K+ currents leads to an increase in action potential duration (APD), QT interval prolongation, and thereby increases the probability for developing potentially life-threatening arrhythmias [4]. Under more chronic conditions, this reshaping of the cardiac action potential leads to a global increase in intracellular calcium via an increase in the open probability of the LTCC due to prolongation of phase 2 of the cardiac action potential and ultimately activation of genes leading to hypertrophy and HF. Data suggest that the initiating event for these changes in intracellular calcium is the increase in β-AR stimulation seen with the catecholamine surge during acute MI or decompensated HF [5, 6].

With the exception of acutely decompensated HF, it is clear that beta-blockers are important in improving clinical outcomes following infarction. Numerous randomized control trials have shown that the use of beta-blockers in HF not only leads to subjective improvement of NYHA class, but leads to an increase in survival and decreased hospitalizations [7–9]. Brophy et al. in a 2001 meta-analysis that included 22 trials involving more than 10,000 patients with left ventricular ejection fraction (LVEF) <35–45 % noted that beta-blockers significantly reduced 1-year

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mortality (OR 0.65; 95 % CI 0.53–80) and estimated that the use of beta-blockers saved 3.8 lives per 100 patients treated in 1 year. In the same study the use of beta-blockers significantly reduced hospitalizations for heart failure (odds ratio 0.64, 95 % CI 0.53–0.79) with an absolute benefit of four fewer hospitalizations in the first year per 100 patients treated. Based on these findings, the authors concluded "beta-blocker therapy is associated with clinically meaningful reductions in mortality and morbidity in patients with stable congestive heart failure and should be routinely offered to all patients similar to those included in the trials" [7]. Major society guidelines including ACC/AHA (2005 guidelines), Heart Failure Society of America (HFSA, 2006 guidelines), and the 2006 European Society of Cardiology (ESC, 2006 guidelines) recommend the use of beta-blockers in conjunction with ACE inhibitors regardless of NYHA class [10–14].

Despite this preponderance of evidence of the utility of beta-blockers to improve clinical outcomes, we are only recently beginning to understand this benefit at the molecular level. Increases in PKA activity, via activation of the βAR can illicit dramatic changes in calcium handling within the cardiomyocyte. These changes include an increase in I_{ca} via phosphorylation of the LTCC, increased SR calcium release as observed with increased calcium transients, and the quantal release of calcium from the SR, known as calcium sparks both as a result of phosphorylation of the RYR. Multiple studies have implicated the Ca^{2+}-activated phosphatase, calcineurin (Cn), as a pivotal player, in translating these changes in intracellular calcium to changes in gene expression via the activation of NFATc3 [2–4]. On activation, Cn dephosphorylates NFATc3, allowing for its translocation to the nucleus where it modulates the transcription of multiple genes within the cardiomyocyte including the voltage-gated potassium channels Kv 1.2, 2.1, 4.2, 4.3, as well as the Kv4 accessory protein, KChIP2 that acts as a molecular chaperone to increase surface expression of Kv 4 proteins. Together these channels and accessory proteins comprise the molecular entities that underlie the repolarizing Kv currents I_{to} (Kv 4.2, 4.3, and KChIP) and I_{sust} (Kv 1.5 and 2.1) that shape phase 2 and 3 of the cardiac action potential. A reduction in the expression of these channel proteins occurs as rapidly as 48 h after infarction, leading to elongation of the cardiac action potential, which in itself leads to an increased influx of intracellular calcium via increasing the activity of the LTCC and increasing SR calcium load [4, 15, 16]. Ultimately, these changes perpetuate the changes in intracellular calcium signaling establishing an intracellular environment that promotes sustained activation of Cn/NFAT, the genes responsible for hypertrophy and ultimately the HF phenotype. The use of beta-blockers, in a mouse model of infarction was shown to prevent these changes in intracellular calcium and thereby prevent activation of the Cn/NFAT signaling pathway [4]. Importantly, in the same study, the use of beta-blockers also prevented the down regulation of repolarizing Kv currents and at a molecular level, preventing the down regulation of Kv 1.5, 2.1, 4.2, and 4.3 mRNA and protein [4]. Adding more weight to his hypothesis, pharmacological inhibition of NFAT (with CsA or FK506) or the use of NFAT null animals in similar experiments prevented the down regulation of Kv transcript and protein, and thereby prevented pathological ion current remodeling [4]. Conversely, overexpression of a constitutively active form of

NFAT was shown induced reduction in Kv currents, channel proteins, and transcript similar to that seen after infarction. These findings provide a mechanism for the cardioprotective effects of beta-blockers, both acutely (ion current remodeling) and chronically (HF).

Refining of the Model

The cell sees changes in cytosolic [Ca] that varies from beat to beat with contraction of the cardiomyocyte; however, there are clearly some changes in intracellular calcium that lead to variations on genes expression. Numerous groups have worked to gain a better understanding of this signal that leads to the pathological changes repolarizing Kv currents and hypertrophy/HF. Studies have shown that in the mammalian heart there is a striking difference in the amplitude of I_{to} between cells isolated from the epicardium and the endocardium of the left ventricle [17, 18] with I_{to} being larger in epicardium [17, 19, 20]. This transmural gradient in I_{to} function is important for normal ventricular repolarization [19, 21, 22]. Calcium signaling also differs between the endocardium and epicardium with in the left ventricle. Dilly et al., described difference in both diastolic and systolic [Ca(2+)](i) which was higher in paced endocardial cells in comparison to their epicardial counterparts [15]. They noted that while differences in the action potential waveform could account for some of these differences in intracellular calcium, the amplitude of the $[Ca^{2+}]_i$ transient itself was larger. This study went on to describe spontaneous Ca^{2+} spark activity that was almost threefold higher in the endocardium and that expression of the ryanodine receptor type 2 (RyR2) was nearly twofold higher in endocardium in comparison to the epicardium. Additionally, they observed that efflux of calcium during the cardiac action potential via activity of the Na^+–Ca^{2+} exchanger was reduced in the endocardium. Interestingly, this study did not show a difference in the trigger of CICR, showing no difference between L-type calcium current between the endocardium and epicardium. The authors proposed that transmural differences in AP waveform, SR Ca^{2+} release, and Na^+–Ca^{2+} exchanger function together underlie differences in intracellular calcium across the left ventricular free wall.

Based on these observations and the recently established role of the Cn/NFATc3 signaling pathway in down regulation of repolarizing Kv currents after MI, Rossow et al. proposed that this pathway played a role in establishing the I_{to} gradient under normal physiological conditions, due to variations in calcium signaling across the left ventricle [16]. In support of this, this study found that both Cn and NFAT activity were higher in the endocardium when compared to the epicardium using a luciferase reporter mouse to compare NFAT activity between the two cell types. The study found that differential expression of I_{to} between the cells isolated from the epicardium and endocardium resulted from NFATc3-dependent regional differences in *only* Kv4.2 expression. Providing further evidence of the pivotal role of the Cn/NFAT pathway in establishing this gradient, NFAT null animals

showed no difference in I_{to} expression between the endocardium and epicardium. The study went on to describe a calcium-sensitive threshold for down regulation of other proteins contributing to AP repolarization via the outward Kv current, including Kv 4.3, Kv 1.2, Kv 1.5, and KChiP, all of which like Kv 4.2, contain multiple NFAT binding elements within their 5' UTR. While Kv 4.2 was the only gene that showed regional differences in expression under normal physiological conditions, Kv 4.3, Kv 1.2, Kv 1.5, and KChiP all showed down regulation after MI [4]. The mechanism underlying the differential expression of the RYR between the endocardium and epicardium is unknown, but it is reasonable to speculate that it too may be regulated via regional differences in gene expression regulated by this gradient in intracellular calcium similar to its Kv counterparts. In these studies evidence suggests that increased calcium leads to increased activity of Cn/NFAT which leads to down regulation of Kv 4.2 expression, this leads to reduction in the repolarizing Kv current and increased APD and further increases in $[Ca^{2+}]_i$, establishing the transmural gradient under normal physiological conditions. Further up-regulation of this pathway under pathological conditions such as post MI or decompensated HF, driven by a dramatic increase in catecholamines, leads to further increases in intracellular calcium and further down regulation of the molecular entities that compose these currents. These changes promote resetting of intracellular $[Ca^{2+}]_i$ to perpetuate these changes once established leading to HF and hypertrophy.

Despite the preponderance of evidence in support of this model, the source of calcium responsible for pathological hypertrophy has not been clearly defined. The role of the LTCC in CICR and contraction has been well described. These channels are predominantly localized in the T-tubules, in close apposition to the ryanodine receptor composing the fundamental signaling complex for CICR. However, recent evidence suggests that *this* calcium signal may not be the etiology of pathologic changes within the myocyte that lead to hypertrophy. In addition to the Cn-NFAT signaling pathway described above, other calcium signaling pathways including CaM Kinase and PKC have been implicated in pathological hypertrophy (ref). Mounting evidence suggests that in the intact heart simple increases in diastolic Ca^{2+} alone may not account for activation of these signaling pathways, as such the source of the calcium signal that is responsible for the initiation of this cascade of events has yet to be definitively established. Potential sources include the LTCC [23, 24], T-type calcium channel [25, 26], and TRP channels [27].

Makarewich et al. in a recent publication have proposed an intriguing model that describes two disparate populations of L-type calcium channels responsible for what they have dubbed as "contractile calcium" and small population of LTCCs within caveolae responsible for "hypertrophic calcium" [28]. This subpopulation of LTCC was previously described by Nichols et al. [29], which are stabilized by scaffolding protein cavelolin-3, the predominant caveolin in heart, forming a microdomain for localized calcium signaling. In this study the authors used a novel LTCC inhibitor, REM, which they targeted to cav-3 containing membranes, selectively targeting the LTCC with in the caveolae microdomains. The authors were able to selectively inhibit this subpopulation of LTCCs without altering whole

L-type calcium currents or affecting contractility. Interestingly, the selective inhibition of this population of calcium channels prevented the Ca^{2+}-induced translocation of NFAT, presumably by disrupting the calcium signaling complex required for activating of this transcription factor.

Further underscoring the importance of localized calcium signaling within discrete microdomains within the cardiomyocyte, in the study by Nichols et al. describing this subpopulation of LTCCs associated with the scaffolding protein AKAP 150 (also called AKAP150/79), this scaffolding protein was shown to also target adenylyl cyclase, PKA, and calcineurin to caveolin-3 enriched membranes within the t-tubule forming a microdomain for calcium signaling. In this study the authors used cardiomyocytes isolated from wild type and AKAP null mice to investigate the role of this signaling complex in β-adrenergic signaling. The study found that β-adrenergic augmentation of calcium transients and the phosphorylation of substrates involved in calcium handling were disrupted in AKAP5 knockout cardiomyocytes. The authors found that under normal conditions, only the caveolin 3-associated Cav1.2 channels are phosphorylated by PKA in response to sympathetic stimulation in wild-type heart. However, with loss of this signaling complex in AKAP5 null hearts, adenylyl cyclase 5/6 no longer associated with caveolin 3 in the T-tubules, and noncaveolin 3-associated calcium channels become phosphorylated after β-adrenergic stimulation. Functionally, the disruption of this complex leads to the loss of increase in the calcium transient normally seen in wild-type animals in response to β-adrenergic signaling. The authors went on to show that this signaling microdomain orchestrated by AKAP5 was also essential for the PKA-dependent phosphorylation of ryanodine receptors and phospholamban. These findings demonstrate that AKAP5 anchoring protein is essential in organizing a signaling complex that is associated with caveolin-3 within the t-tubules and is essential for sympathetic modulation of the calcium transient in the heart. These observations of spatially localized calcium signaling microdomains acting on functionally disparate populations of calcium channels raises the exciting possibility of a novel therapeutic targets for treatment if HF and hypertrophy that does not effect contractility, a tool that would be useful during decompensated HF when traditionally the use of beta-blockers has been contraindicated due to the negative inotropic effects.

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