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Rethinking Resynch: Exploring Mechanisms of Cardiac Resynchroniztion Beyond Wall Motion Control

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Abstract

Cardiac resynchronization (CRT) is a widely used clinical treatment for heart failure patients with depressed function and discoordinate contraction due to conduction delay. It is unique among heart failure treatments as it both acutely and chronically enhances systolic function yet also prolongs survival. While improved chamber mechano-energetics has been considered a primary mechanism for CRT benefit, new animal model data are revealing novel and in many instances unique cellular and molecular modifications from the treatment. Examples of these changes are the reversal of marked regional heterogeneity of the transcriptome and stress kinase signaling, improved ion channel function involved with electrical repolarization, enhanced sarcomere function and calcium handling and upregulation of beta-adrenergic responses, and improved mitochondrial energetic efficiency associated with targeted changes in the mitochondrial proteome. Exploration of these mechanisms may reveal key insights into how CRT can indeed get the failing heart to contract more and perform more work, yet not worsen long-term failure. These changes may provide a more biological marker for both the appropriate patients for CRT as well as point the way for new therapeutic avenues for heart failure in general.

Introduction

Heart failure is the leading cause of morbidity and mortality in older adults in the United States, and its incidence world-wide continues to rise. Current drug treatment focuses on neurohumoral blockade and volume unloading, whereas agents to stimulate pump function have been historically less successful. Though many had concluded that enhancing inotropy was like “beating a sick horse”, and should be avoided, the development of a device-based treatment called cardiac resynchronization (CRT) suggests it can all depend on how it is achieved. CRT treats discoordinate contraction due to electrical conduction delay. Approximately 25-45% of dilated HF patients have dyssynchrony, which generates heterogeneous contractions with early (low stress) and late (high stress)-regions and consequent pump inefficiency. CRT employs bi-ventricular stimulation to restore synchrony and improve systolic function. Yet despite acutely and chronically enhancing cardiac work and systolic performance, CRT also lowers mortality. New data is now revealing that its

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mechanisms are more complex than previously thought, and these insights may pave the road for new heart failure treatment more generally.

CRT and Chamber Mechano-Energetics

Until recently, the prevailing view of CRT efficacy is that it reduces mechanical inefficiency from discoordinate contraction, allowing more blood to be ejected at less energy cost. This can be documented rapidly (mechanics within a single beat). While QRS duration was first used to identify patients, dyssynchronous wall motion often detected by tissue Doppler evolved rapidly as a more direct approach. However, studies have not found that markers of basal dyssynchrony or even how much dyssynchrony is reduced by CRT provides a reliable predictor of outcome. This has raised the question of whether wall motion is all there is to it, or if effective CRT might involve more complex chronic changes in the heart unique to the therapy.

Hints at changes in cellular signaling pathways first came from human studies of responders versus “non-responders” where gene expression of calcium handling proteins, beta-receptors, and natriuretic peptides were improved in the former[1,2]. Patients with effective CRT display chronic enhancement of circulating apelin, a secreted hormone that can block adverse remodeling and has positive inotropic effects[3]. Circulating biomarkers of extracellular matrix remodeling also accompany successful CRT therapy, including decreases in tenascin-C, and metalloproteinases (2 and 9)[4]. Chronic CRT also has anti-inflammatory effects effect, reducing monocyte chemoattractant protein-1, interleukin-8, and interleukin-6[5]. These studies do not identify underlying mechanisms per se, but may ultimately suggest biomarkers for therapies that both enhance systole and survival in HF patients.

Development of a CRT animal model

To better elucidate cellular and molecular mechanisms by which dyssynchrony and CRT impacts the failing heart, we needed an animal model that could recapitulate chamber-level features of both conditions, but also provide tissues for more reductionist analysis. We developed one using the established canine rapid pacing model of dilated HF with minor modifications[6,7]. Dogs were subjected to left-bundle branch radiofrequency ablation, and either atrially paced for 6 weeks (dyssynchronous HF, DHF), or atrial for 3 weeks and bi-ventricular for the remaining 3 weeks (CRT). As in humans, the CRT model led to a modest improvement in chamber function from week 4-6 in CRT, declining slightly in DHF. Tissue Doppler parameters of dyssynchrony improved in CRT. Thus, we generated two conditions: both involving 6-wks of tachypacing (and thus HF), but one with the pattern of contraction altered midway. It is worth keeping this in mind, as the changes observed with the CRT model are quite remarkable given how little was manipulated.

CRT fixes regional molecular polarization

After 3-wks of dyssynchronous tachypacing, left ventricular protein expression of calcium handling proteins, connexin43, and mitogen-activated protein kinases display striking regional differences notably in the late activated wall[8]. This behavior, which we termed molecular polarization, was further evaluated in our DHF/CRT models, where we revealed greater p38 MAPK and calcium-calmodulin kinase II activation and increased TNF- α expression in the lateral wall, further showing CRT reversed these changes[7]. Disparities in calcium handling proteins were less marked than we had previously observed. This localized difference in stress kinase activation is consistent with disparities in regional workload in DHF and its equilization by CRT. More recently, we performed non-biased genome wide analysis, showing profound and broad based regional differences in mRNA expression

between early and late contracting myocardium in DHF, which were largely reversed by CRT[9]. Kegg pathway analysis showed reduction in energy/metabolism genes and increased matrix/remodeling genes in the anterior wall; changes reversed by CRT. This pattern indicates that DHF is not simply another form of failure, but one with consistent expression and enzyme activation heterogeneity. The specifics of what is most strikingly altered by CRT may point towards therapeutic targets itself.

Electrophysiologic influences of DHF and CRT

A major cause of death associated with HF is arrhythmia, and there are many known abnormalities involving ion channel function that occur with the disease. Intriguingly, CRT can reverse many of these changes. The Carnes laboratory first reported that prolongation of the action potential, reduction in L-type Ca^{2+} current, and reduced whole-cell Ca^{2+} transients observed in a model of DHF were all reversed by CRT[10]. We recently reported on these modifications in more detail, revealing DHF significantly reduced the inward rectifier, delayed rectifier, and transient outward potassium currents in both anterior and lateral myocytes. CRT partially restored DHF-induced reductions all but the last, with upregulation of gene and preprotein expression playing a partial role[11]. DHF also reduced peak inward Ca^{2+} current density and slowed the transient decay more in lateral than anterior myocytes, and CRT restored the current amplitude though did not impact decay rates. The changes in current were not easily explained by channel expression disparities. Similarly, while DHF reduced phospholamban, ryanodine receptor, and sarcoplasmic reticulum Ca^{2+} -ATPase and increased NCX mRNA and protein, there were few changes in these levels with CRT despite the functional improvements. This points to post-translational modifications of critical proteins involving SR Ca^{2+} uptake and release, and these are presently under study.

A more integrated reflector of electrophysiologic changes is the net action potential, which was significantly prolonged in DHF, especially in lateral cells. CRT abbreviated this duration. This correlated with an increase in the frequency of after depolarizations in DHF than CRT myocytes, which could reflect mechanisms by which CRT suppresses lethal arrhythmia[12]. An intriguing feature of these electrophysiologic changes is that many were more globally distributed. Thus, while we tend to focus on regional changes in stress and strain coupled with CRT, it is important to remember that with re-coordination of contraction timing comes global changes in chamber function, and neurohumoral activation as well.

CRT and Survival Signaling

Although mechanism by which CRT may confer benefit is by suppressing signaling cascades associated with reduced cell survival. As reported both in humans[13] and in our canine model[7], DHF hearts display an increase in myocellular apoptosis. In the canine model, this was supported by TUNEL staining, caspase-3 activity, and nuclear poly ADP-ribose polymerase cleavage. Importantly, this was suppressed by CRT. One of the most striking changes was a marked decline in Akt phosphorylation/activity with DHF that was also reversed by CRT. Akt is generally considered a pro-survival kinase, and its phosphorylation of the pro-apoptotic protein BAD results interaction of BAD with the chaperone 14-3-3, reducing apoptosis. In the canine model, we observed reduced BAD phosphorylation (and 14-3-3 interaction) with DHF, that was reversed with CRT. As with the electrophysiologic effects, the anti-apoptotic impact of CRT appears global in nature. There are many other factors that regulate cell survival signaling and these may also be modified by CRT. The mechanism by which the loss of dyssynchrony re-activates Akt to modify its downstream protein targets such as BAD remains unknown. It is unlikely due

improved global net function, which as mentioned was very modest in this model. However, activation of secreted factors coupled to the abnormal cell-mechanical loading may prove an important pathway, and this is currently being explored.

CRT and Mitochondria

Much of the initial mechanistic focus regarding CRT was on its improvement of chamber level mechano-energetics. However, Agnetti et al.[14] recently provided evidence of benefits of CRT on mitochondrial function and protein expression, showing that the sub-cellular energetic machinery is potently effected. Using an optimized 2D electrophoresis of the mitochondrial sub-proteome, the investigators resolved~ 1200 protein spots, and showed 31 quantitative protein changes between DHF and CRT, keeping a false discovery rate at 30%. Most changes were related to the respiratory chain, consistent with CRT modulating ATP production, including all of the complexes of oxidative phosphorylation (except complex IV). CRT also increased the metabolic pathways supplying the substrates (pyruvate carboxylase and pyruvate dehydrogenase, E1 and E2 subunits) and key enzymes (aldehyde dehydrogenase, α -keto acid dehydrogenase E2, and ferredoxin reductase) fueling the Krebs cycle. Importantly, mitochondrial oxidative efficiency (ADP/O_2) was depressed by DHF and enhanced by CRT. CRT was also shown to reduce oxidative stress, potentially by enhanced mitochondrial ROS scavenging proteins. These changes have not been specifically reported with other heart failure therapies, and may indeed be more selective to the CRT response.

Rest and Beta-adrenergic-stimulated myocyte function

The first hints of a positive impact of CRT on cardiac beta signaling were provided by clinical studies showing CRT enhanced neural norepinephrine reuptake and retention[15], and reduces muscle sympathetic nerve activity in patients with severe HF and asynchrony[16]. To more directly study myocyte β -adrenergic signaling, we isolated cells from early and late activated territories of control, DHF, and CRT hearts, and determined sarcomere shortening and calcium transients. DHF myocytes had highly blunted rest and isoproterenol-stimulated shortening and Ca^{2+} transients over controls, consistent with many models of heart failure. Yet, both were markedly improved with CRT, despite the fact that the hearts from which the cells came from still displayed an overall HF phenotype (i.e. dilated with reduced function). This improvement was accompanied by a decline in myocardial catecholamines, so CRT restored the balance of neurostimulation and myocyte responsiveness towards normal. The depression of β -signaling depression with DHF and improvement with CRT was global – not regional.

There are several intriguing mechanisms that likely underlie β -AR signaling in DHF and its amelioration by CRT. Both β 1- and β 2-receptor gene expression and number were depressed by DHF, and CRT enhanced β 1- but not β 2-receptor number, as it did in humans[17]. Functional analysis of adenylate cyclase activity revealed it to also be depressed by DHF, and CRT improved its cAMP production. Another change that appears specific to CRT was a modification of inhibitory G-protein coupled signaling. In DHF, we observed enhanced G_i signaling, evidenced by a marked augmentation of β -AR responsiveness in the presence of the G_i -inhibitor, pertussis toxin (PTX). However, in CRT, rest responses were greater and PTX had no further impact as if $\text{G}_i\alpha$ already was inhibited by CRT. $\text{G}_i\alpha$ was up-regulated similarly by DHF and CRT (as seen in human HF), so could not itself explain the change. However, we found selective up-regulation of proteins called regulators of G-protein signaling (RGS) proteins. RGS proteins negatively regulate G-coupled signaling by acting as GTPase accelerators, removing GTP from the activated α -subunit so that the trimeric G-protein complex re-formed to suppress coupled signaling. RGS3, a protein known to suppress G_i , was selectively up-regulated in CRT hearts, and this may be quite

important to improved functional reserve with this therapy. To our knowledge, this is the first demonstration of a therapy for heart failure resulting in upregulation of a specific RGS species coupled to improvement in catecholamine stimulated reserve. It is conceivable that this effect from CRT may be leveraged to assist other forms of heart failure more generally.

Conclusion

In a relatively short period, CRT has evolved from a clinical therapy where mechanistic understanding was largely relegated to imaging and biomarkers, to one where we are revealing far more complex cellular and molecular modifications (see summary Figure). Importantly, changes are now being identified that look to be characteristic features of DHF, and also those identifying specific targets through which CRT ameliorates the syndrome. As stated up front, a major lesson of CRT is that one can indeed treat the failing heart in a way that enhances both its mechanical work and global pump function and also long-term survival. We believe that unmasking the secrets of CRT will improve not only our ability to better target this particular therapy, but more importantly, point the way to new small molecule therapies that might well prove effective for heart failure more generally.

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Figure.

Mechanisms by which cardiac resynchronization (CRT) can ameliorate the dyssynchronous failing heart (DHF). Top center: two reconstructions of circumferential strain from magnetic resonance tagged images show early shortening of the septum (blue) with lateral wall stretch in DHF, versus early bi-ventricular shortening with no regional stretch with CRT.

Mechanisms of benefit – (moving counter clockwise): increased chamber function with left shift of the end-systolic pressure-volume points and increased PV loop width (stroke volume) and area (work; red DHF, blue CRT). Both basal and isoproterenol stimulated shortening a calcium transients are enhanced by CRT to levels seen in normal controls. Myocardial catecholamine levels rise in DHF, particularly in lateral wall, and decline with CRT. Action potential prolongation (shown at varying basic cycle lengths) with DHF over control is diminished by CRT. Inward rectifier K^+ tail current is depressed with DHF and partially restored by CRT. Mitochondrial proteins are altered by CRT, improving oxidative phosphorylation, potentially improving metabolism, reducing oxidant stress and increasing mitochondrial efficiency. Cell survival signaling coupled with Akt phosphorylation state is markedly depressed with DHF and restored by CRT. Last, genome wide regional heterogeneity of mRNA expression. First sets of columns are from DHF depicting genes that are differentially down or upregulated between septal and lateral walls. The pattern in normals (middle third of columns) is similar to CRT (rightward columns).