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Original research

The zebrafish as a novel animal model to study the molecular mechanisms of mechano-electrical feedback in the heart

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ABSTRACT

Altered mechanical loading of the heart leads to hypertrophy, decompensated heart failure and fatal arrhythmias. However, the molecular mechanisms that link mechanical and electrical dysfunction remain poorly understood. Growing evidence suggest that ventricular electrical remodeling (VER) is a process that can be induced by altered mechanical stress, creating persistent electrophysiological changes that predispose the heart to life-threatening arrhythmias. While VER is clearly a physiological property of the human heart, as evidenced by "T wave memory", it is also thought to occur in a variety of pathological states associated with altered ventricular activation such as bundle branch block, myocardial infarction, and cardiac pacing. Animal models that are currently being used for investigating stretch-induced VER have significant limitations. The zebrafish has recently emerged as an attractive animal model for studying cardiovascular disease and could overcome some of these limitations. Owing to its extensively sequenced genome, high conservation of gene function, and the comprehensive genetic resources that are available in this model, the zebrafish may provide new insights into the molecular mechanisms that drive detrimental electrical remodeling in response to stretch. Here, we have established a zebrafish model to study mechano-electrical feedback in the heart, which combines efficient genetic manipulation with high-precision stretch and high-resolution electrophysiology. In this model, only 90 min of ventricular stretch caused VER and recapitulated key features of VER found previously in the mammalian heart. Our data suggest that the zebrafish model is a powerful platform for investigating the molecular mechanisms underlying mechano-electrical feedback and VER in the heart.

1. Introduction

1.1. Stretch-induced ventricular electrical remodeling in the heart

Ventricular electrical remodeling (VER) is a persistent change in the electrophysiological properties of myocardium in response to a change in heart rate or activation sequence (Jeyaraj et al., 2010). Abnormal ventricular activation is commonly associated with a variety of cardiac pathologies including conduction system disease, myocardial infarction, hypertrophy and heart failure and is induced by ventricular pacing. The alteration of ventricular activation by pacing causes an inversion of the T wave of the electrocardiogram which was first described by Chatterjee et al. (1969). This T wave abnormality was later termed "T-wave memory" by Rosenbaum et al. (1982), because it persists long after the cessation of pacing and dissipates at a rate that depends on the history of prior pacing (Wecke et al., 2005). Importantly, T-wave memory exhibits accumulation, i.e. the duration and the degree of the T wave abnormality observed is dependent on the duration and degree of abnormal activation (del Balzo and Rosen, 1992). Because electrical excitation and mechanical contraction are tightly coupled (Bers, 2002), any alteration of electrical activation changes the temporal and spatial properties of contraction, leading to mechanical dyssynchrony (Bank et al., 2011; Gjesdal et al., 2011). Therefore, it was hypothesized that an alteration of myocardial mechanics is the underlying cause of cardiac memory. Sosunov et al. (2008) examined this possibility in a Langendorff-perfused rabbit heart. They reported that stretching just a single point on the epicardium induced a T-wave alteration which was similar to that observed in cardiac memory. This result clearly supports the

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hypothesis that altered mechanical activity, rather than the direction of current flow *per se*, is a key determinant of cardiac memory. Recently, using an *in vivo* dog model of pacing-induced VER, we found that periods of mechanical stretch are in fact a mechanism for triggering VER and T wave memory (Jeyaraj et al., 2007). Importantly, action potential durations were markedly increased in high-strain regions and unchanged in low-strain regions, accounting for the inversion of the T wave.

Taken together, these reports suggest that tissue stretch is a prerequisite for T-wave memory and that long-term alteration of ventricular activation causes persistent electrical remodeling via a mechano-electrical feedback mechanism (Marrus and Nerbonne, 2008). It is important to point out that in this context, VER produces persistent electrophysiological changes, such as action potential prolongation, which outlast the period of applied stretch. This should be distinguished from transient mechano-electrical effects which have been studied extensively and are only present as long as cardiac muscle is actively stretched (Iribe et al., 2009; Kohl et al., 2006; Nishimura et al., 2006, 2008).

1.2. Clinical consequences of ventricular electrical remodeling

Results from both randomized clinical trials and smaller mechanistic studies have suggested that long-term ventricular pacing has significant adverse effects, ranging from impaired mechanical function (Tantengco et al., 2001) to increased risk of hospitalization, new or worsened heart failure, and death (Sweeney et al., 2003; Thambo et al., 2004). The DAVID trial showed that patients who received continuous dual-chamber pacing had significantly worse outcomes than patients with back-up, i.e. less frequent, ventricular pacing, presented by worsening of heart failure and increased mortality (Wilkoff et al., 2002). This result was confirmed by the MADIT II trial, which demonstrated that ventricular pacing was linked to worsening of heart failure (Moss et al., 2002). Some reports suggested that there is a direct relationship between ventricular pacing, cardiac memory, mechanical dysfunction and increased susceptibility to arrhythmias (Alessandrini et al., 1997; Medina-Ravell et al., 2003), thus highlighting the need to further investigate the effects of ventricular pacing. This is particularly paramount for pacing therapy in children with congenital or acquired atrioventricular (AV) block because in these patients, pacing has been shown to be highly beneficial (van Geldorp et al., 2011).

When the normal pattern of activation is disturbed by ventricular pacing, impulse propagation occurs between ventricular muscle cells, rather than through the fast His-Purkinje network, causing slowing of conduction (Scher and Young, 1955; Scher et al., 1953; Vassallo et al., 1984) and mechanical dyssynchrony (Fang et al., 2010; Rosenbush et al., 1982). In a study of 93 patients with sinus node dysfunction who had been paced for at least 6 months, Fang et al. (2010) found that half had developed significant mechanical dyssynchrony. Those patients who developed dyssynchrony had significantly larger ventricles and lower ejection fraction than those who did not develop dyssynchrony. Furthermore, in another report, Zhang et al. (2008a) showed that, of 79 patients who were paced from the right ventricle more than 90% of the time, 26% developed systolic heart failure within the 8-year follow-up period. Right-ventricular pacing created left-ventricular mechanical dysfunction and accelerated the progression of heart failure (Tse and Lau, 1997; Zhang et al., 2008a). Conversely, clinical studies aimed at synchronizing electrical activation and mechanical contraction by bi-ventricular pacing improved survival (Abraham et al., 2002; Bristow et al., 2004; Tu et al., 2011). In a recent review, Bank et al. (2011) have summarized many of these clinical observations.

1.3. Molecular mechanisms of ventricular electrical remodeling

The aforementioned studies established clear correlations between pacing-induced mechanical dyssynchrony and electrical dysfunction. Therefore, a number of investigations have been aimed at understanding the molecular mechanisms that link abnormal mechanical stretch with adverse electrical remodeling in the heart. Recent reports have been focused on the G protein-coupled mechanoreceptor pathway as a possible mechanism (Patel et al., 2010; Sharif-Naeini et al., 2010; Storch et al., 2012). This pathway is an ideal candidate to explain stretch-induced VER because it couples a pressure sensor (for example, the angiotensin II type I receptor, AT1R) to a depolarizing, calcium-permeable ion channel such as the transient receptor potential canonical, TRPC, channel (Rowell et al., 2010) via a Gq protein-coupled signaling cascade. There is substantial evidence that Gq protein-mediated signaling is involved in the maladaptive hypertrophic response of the heart to pressure overload (Akhter et al., 1998; Wettschureck et al., 2001) that causes cellular Ca^{2+} influx and hypertrophy via activation of the NFAT-calcineurin pathway (Bush et al., 2006; Kuwahara et al., 2006; Nakayama et al., 2006; Onohara et al., 2006; Seth et al., 2009). Moreover, expression of the protein 'regulator of G-protein signaling 2' (RGS2), an endogenous inhibitor of Gqα function, was reduced in ventricular myocardium from hypertensive patients (Semplicini et al., 2006), from end-stage heart failure patients with left-ventricular assist devices (Takeishi et al., 2000), and in a dog model of dyssynchronous heart failure (Chakir et al., 2009). Consistent with this, mice lacking RGS2 responded more rapidly to pressure overload than their wild-type counterparts because of Gq hyper-activation, leading to hypertrophy, heart failure and early death (Takimoto et al., 2009). Activation of AT1R (Bkaily et al., 2003; Gusev et al., 2009), down-regulation of RGS2 (Calo et al., 2008; Klaiber et al., 2010; Semplicini et al., 2006) and membrane stretch (Malhotra et al., 1999; Onohara et al., 2006) have been shown to independently activate Gq signaling and Ca^{2+} influx through TRPC channels.

Other signaling pathways are known to transduce mechanical signals into electrophysiological and structural remodeling events, and these pathways include: protein kinase A, protein kinase C, protein kinase G, calmodulin-activated kinase, calcineurin, mitogen-activated protein kinase, integrin and tyrosine kinase (Heineke and Molkentin, 2006; Romer et al., 2006; Sadoshima and Izumo, 1997). They activate a number of key transcription factors (for example, MEF2, GATA4, etc.) that regulate myocyte growth and function. Some mechanosensitive ion channels (MSC) that are present in the sarcolemmal membrane and in the extracellular matrix/cytoskeleton have been shown to be directly gated by stretch, thereby affecting excitation-contraction coupling on a beat-to-beat basis (Iribe et al., 2009; Kohl et al., 2006). In the beating heart, it is likely that the final common pathway linking mechanical signals to electrophysiological and structural remodeling is not represented by a single signaling pathway, but rather a combination of these mechanisms.

1.4. Limitations of current animal models for studying mechano-electrical feedback mechanisms in the heart

Studies in intact hearts from large animals (for example dog, sheep or lamb) with physiologies that are close to human have provided some important insights into the VER phenotypes which are clinically relevant and would have otherwise been difficult to obtain from patients (Dixon and Spinale, 2009). Although these large-animal experiments at the intact-organ level are informative because of their high level of complexity, they allow only limited control of tissue parameters. For example, perfusion of whole-organ