

## Summary

It is well established that a variety of pathological conditions induces structural and electrical remodeling of the heart which can lead to heart failure and cardiac arrhythmias. Clinically, structural remodeling is characterized by changes in the shape, size and function of the heart. These changes are based on diverse and complex cellular reactions to injury and involve both cardiomyocytes and non-cardiomyocytes. Histopathologically, remodeling typically involves cardiomyocyte hypertrophy, activation and proliferation of fibroblast, increased extracellular matrix deposition and cell death. Functionally, structural remodeling induces mechanical dysfunction and is accompanied by an increased likelihood of occurrence of life-threatening cardiac arrhythmias (Adamson et al., 2005). The electrical remodeling is usually defined as changes related to ion channels, transporters, passive electrical properties, redistribution of the gap junctions and it involves disturbances of the initiation, conduction, and coordination of the electrical stimulus in the heart. An increase in temporal variability of repolarization has been found in different pathologies to follow the electrical remodeling of the heart.

In the first part of my work I have studied heterocellular electrotonic interactions between myofibroblasts and cardiomyocytes, whereas in the second part I have focused on temporal variability of ventricular cardiomyocytes repolarization. Both mechanisms can be highly relevant in arrhythmogenesis. I briefly summarize here the scientific background which enlightens the physiopathological relevance of these mechanisms.

### **Role of (myo)fibroblasts-cardiomyocytes coupling in triggering arrhythmias.**

Mechanistically, arrhythmias in structurally remodeled hearts can be divided into (1) mechanisms related to the remodeling of the extracellular matrix (“fibrosis”) and (2) mechanisms related to altered cellular electrophysiological properties of the cardiomyocytes (“electrical remodeling”). Fibrosis, in turn, favors the initiation and perpetuation of arrhythmias by producing collagenous septa which separate bundles of cardiomyocytes over distances up to several millimeters, thus inducing structural discontinuities at the cellular level (LeGrice et al., 1995). Based on the tenet that fibrotic laminae act as electrical insulators which induce interstitial resistive

discontinuities, the spread of electrical activity under these conditions is impeded and results in non-uniform conduction or zig-zag activation of the myocardium (De Bakker et al., 1993). Structural remodeling of the myocardium during pressure overload and following infarction is typically accompanied by the appearance of interstitial myofibroblasts which contribute to cardiac fibrosis by excessive secretion of extracellular matrix proteins (Weber et al., 2004). The resulting collagenous septa contribute to arrhythmogenesis in structurally remodeled hearts by inducing discontinuous slow conduction (Spach et al., 1997). More recently, studies in vitro demonstrated that myofibroblasts can directly induce arrhythmogenic slow conduction following establishment of heterocellular gap junctional coupling with cardiomyocytes (Miragoli et al., 2006). This slowing of conduction is the result of a decrease in inward currents secondary to the partial depolarization of the cardiomyocytes by the less polarized myofibroblasts. Since partial depolarization of cardiac tissue has previously been shown to induce abnormal automaticity (Katzung et al., 1975), we investigated in the first chapter of this thesis (which resulted in the following publication: “Myofibroblasts induced ectopic activity in cardiac tissue”, *Circulation Research*, 101:755-758, 2007) whether heterocellular electrotonic interactions between myofibroblasts and cardiomyocytes might precipitate spontaneous ectopic activity. The effects of myofibroblasts on cardiac excitability were investigated in patterned growth strands of neonatal rat ventricular cardiomyocytes using optical recording of transmembrane voltage, immunocytochemistry and patch clamp recording techniques. These studies were performed in the Institute of Physiology, University of Bern (Switzerland), under the supervision of Prof. Stephan Rohr.

### **Dispersion of repolarization.**

Electrical remodeling affects a large number of ion channels, exchangers and calcium handling proteins and ultimately predisposes the heart to arrhythmias by, for example, inducing calcium overload related triggered activity or by causing dispersion of repolarization and thereby enabling reentrant electrical activity (Wasson et al., 2004). Temporal dispersion of ventricular repolarization is commonly documented at the organ level, where it is considered a relevant parameter of propensity to the development of arrhythmia (Thomsen et al. 2005). An intrinsic beat-to-beat variability of action potential duration (APD), which induces temporal

variability of contraction (Spitzer et al. 2006), is also reported in isolated ventricular myocytes, electrically paced at a fixed rate (Zaniboni et al. 2000). A constant pacing rate, though, is not a physiological condition, since sinus rhythm is known to be variable, with a beat-to-beat component which, for example in rat ECG, can reach up to 8% of the average R–R interval (Aubert et al. 1999). Changes in heart rate affect ventricular action potential (AP) profile, with an intrinsic mechanism which has classically been studied in mammalian cardiomyocytes in terms of steady-state differences at different pacing frequencies (the so-called ‘rate dependency’; Boyett & Jewell, 1978) and of sudden changes for variably delayed test stimuli following pacing trains at a constant rate (the so-called ‘electrical restitution’; e.g. Franz, 2003). In particular, electrical restitution is considered a potential target for anti-arrhythmic therapies (Koller et al. 2005) following the discovery that flattening the restitution curve can prevent the transition from tachycardia to ventricular fibrillation (VF; Weiss et al. 2005). Many sophisticated pacing protocols have been applied to whole-heart preparations in order to study adaptation of the recovery time to step or randomly changing pacing cycle length (CL) or diastolic interval (DI) (Wu & Patwardhan, 2004). In addition, ‘dynamic restitution’, defined as the relationship between each APD and the preceding DI during VF, has been studied (Watanabe & Koller, 2002). Nevertheless, an account of the dynamic restitution properties of single ventricular myocytes and its interplay with intrinsic temporal dispersion of APD is still lacking. The aim of the second chapter of this thesis (which resulted in the following publication: “Temporal variability of repolarization in rat ventricular myocytes paced with time-varying frequencies”, *Experimental Physiology*, 92: 859-869, 2007) was therefore to better define beat-to-beat APD variability at constant pacing rate and to study the cellular mechanism of APD adaptation to random and linear changes in pacing frequency, in the absence of autonomic and electrotonic effects, which have been shown to modulate these properties in the intact heart (Laurita et al., 1997). To this end single cells, enzymatically isolated from adult rat left ventricles, were studied using patch clamp recording techniques. It was first demonstrated how intrinsic beat-to-beat APD variability is dependent on both mean APD and pacing rate and how it limits the accuracy of classical estimates of repolarization properties. Then a random-CL pacing protocol was developed and

tested the ability of each APD to track changes in the preceding CL during stimulations that mimicked the frequency and variability of rat heart rhythm. It was finally showed how two ion channel blockers affected beat-to-beat APD variability during constant pacing and modulated the capability of the APD to track the preceding CL, during both randomly and linearly changing pacing trains. These studies were performed in the “Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma (Italia)”, under the supervision of dr. Massimiliano Zaniboni.

In summary, we found that 1) structural remodeling of the myocardium during pressure overload and following infarction is accompanied by the appearance of myofibroblasts which contribute to cardiac fibrosis by secretion of extracellular matrix proteins and to arrhythmogenesis by inducing discontinuous slow conduction secondary to establishment of heterocellular gap junctional coupling with cardiomyocytes which in turn induces depolarization of the cardiomyocytes; and 2) temporal dispersion of ventricular repolarization and, particularly, electrical restitution properties can be measured in dynamic conditions (randomly or linearly changing pacing rate) at the cellular level where they show pharmacological modulation properties comparable to those measured at the organ level. The two phenomena can be very relevant to each other in affecting cardiac propensity to arrhythmias. In fact, both local membrane currents and electrotonic current flow between neighboring cells govern transmembrane potential and changes of these currents have been demonstrated to affect the synchronization of repolarization between neighboring myocytes and hence the dispersion of repolarization.

Therefore the appearance of interstitial myofibroblasts could contribute to establish higher propensity to arrhythmias not only inducing depolarization of cardiomyocytes but also inducing alteration in the intercellular electrical coupling, and significantly affecting in turn cellular temporal dispersion of the repolarization and the electrical restitution properties of the cardiac tissue.

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