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Aalborg, March 15th, 2017

Lars Hvilsted Rasmussen

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Contents

This thesis is partly based on the following publications (referred to as studies I to XI in the text)

I. Osadchii, O.E, Larsen, A.P. & Olesen, S.P. 2010. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. *Am J Physiol Heart Circ Physiol* 298, H210-H220.

II. Osadchii, O.E. 2012a. Electrophysiological determinants of arrhythmic susceptibility upon endocardial and epicardial pacing in guinea-pig heart. *Acta Physiol (Oxf.)* 205, 494-506.

III. Osadchii, O.E. 2012b. Effects of ventricular pacing protocol on electrical restitution assessments in guinea-pig heart. *Exp Physiol* 97, 807-821.

IV. Osadchii, O.E. 2012c. Impact of Na^+ channel blockers on transmural dispersion of refractoriness and arrhythmic susceptibility in guinea-pig left ventricle. *Eur J Pharmacol* 691, 173-181.

V. Osadchii, O.E., 2012d. Dofetilide promotes repolarization abnormalities in perfused guinea-pig heart. *Cardiovasc Drugs Ther* 26, 489-500.

VI. Osadchii, O.E., 2012e. Flecainide-induced proarrhythmia is attributed to abnormal changes in repolarization and refractoriness in perfused guinea-pig heart. *J Cardiovasc Pharmacol* 60, 456-466.

VII. Osadchii, O.E., 2013. Quinidine elicits proarrhythmic changes in ventricular repolarization and refractoriness in guinea-pig. *Can J Physiol Pharmacol* 91, 306-315.

VIII. Osadchii, O.E., 2014a. Procainamide and lidocaine produce dissimilar changes in ventricular repolarization and arrhythmogenicity in guinea-pig. *Fundam Clin Pharmacol* 28, 382-393.

IX. Osadchii, O.E., 2014b. Effects of Na^+ channel blockers on extrasystolic stimulation-

evoked changes in ventricular conduction and repolarization. *J Cardiovasc Pharmacol* 63, 240-251.

X. Osadchii, O.E., 2014c. Impaired epicardial activation-repolarization coupling contributes to the proarrhythmic effects of hypokalemia and dofetilide in guinea-pig ventricles. *Acta Physiol (Oxf.)* 211, 48-60.

XI. Osadchii, O.E., 2014d. Impact of hypokalemia on electromechanical window, excitation wavelength and repolarization gradients in guinea-pig and rabbit hearts. *PLoS* One 9, e105599.

Preface

The present thesis is based on my studies performed at the Danish Cardiac Arrhythmia Research Center (DARC), Department of Biomedical Sciences, Panum Institute, University of Copenhagen (from 2007 to 2012), with subsequent data analysis and interpretation continued in Department of Health Science and Technology, Aalborg University, wherein I have been employed thereafter. I am grateful to Søren Peter Olesen, Director of DARC, for providing access to excellent research facilities, and for supporting these studies both intellectually and financially. All people from his group are acknowledged for creating a friendly, professional and stimulating environment I enjoyed being part of. I am also sincerely thankful to Olga Sosnovtseva for sharing her office with me and being a good friend.

Looking back to my academic career over the last decade, I have a pleasure to thank Gavin Norton and Angela Woodiwiss for introducing me into the field of cardiac pathophysiology, and for providing professional training and excellent supervision during my postdoctoral studies in South Africa. I also greatly appreciate the inspiration and insightful inputs from Eric Dubuis and Alexander Gourine, with whom I was glad to maintain nice social and professional contacts while working in England.

Finally, I am indebted to all my family for continuous support and encouragement I always felt during the many years of working in different countries, and for persuading me that learning new things and disseminating knowledge to students are the most enjoyable life endeavors.

List of abbreviations

APD, action potential duration

APD_{90}, action potential duration at 90% repolarization

ATP, adenosine triphosphate

AV, atrioventricular

BAPTA, bis-aminophenoxyethane-tetraacetic acid

CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II

DAD, delayed afterdepolarization

EAD, early afterdepolarization

ECG, electrocardiogram

EGTA, ethylene glycol tetraacetic acid

ERP, effective refractory period

LV, left ventricular

mRNA, messenger ribonucleic acid

RV, right ventricular

S_1, regular pacing stimulus

S_2, premature (extrasystolic) stimulus

SERCA, sarcoplasmic reticulum Ca^{2+} ATP-ase

SR, sarcoplasmic reticulum

TRIaD: triangulation, reverse use dependence, instability and dispersion

V_{max}, the maximum velocity of the action potential upstroke

VT, ventricular tachyarrhythmia

VF, ventricular fibrillation

Abstract

In cardiac patients, life-threatening tachyarrhythmia is often precipitated by abnormal changes in ventricular repolarization and refractoriness. Repolarization abnormalities typically evolve as a consequence of impaired function of outward K^+ currents in cardiac myocytes, which may be caused by genetic defects or result from various acquired pathophysiological conditions, including electrical remodeling in cardiac disease, ion channel modulation by clinically-used pharmacological agents, and systemic electrolyte disorders seen in heart failure, such as hypokalemia. Cardiac electrical instability attributed to abnormal repolarization relies on the complex interplay between a provocative arrhythmic trigger and vulnerable arrhythmic substrate, with a central role played by the excessive prolongation of ventricular action potential duration, impaired intracellular Ca^{2+} handling, and slowed impulse conduction. This review outlines the electrical activity of ventricular myocytes in normal conditions and cardiac disease, describes classical electrophysiological mechanisms of cardiac arrhythmia, and provides an update on repolarization-related surrogates currently used to assess arrhythmic propensity, including spatial dispersion of repolarization, activation-repolarization coupling, electrical restitution, TRIaD (triangulation, reverse use dependence, instability, and dispersion), and the electromechanical window. This is followed by a discussion of the mechanisms that account for the dependence of arrhythmic vulnerability on the location of the ventricular pacing site. Finally, the review clarifies the electrophysiological basis for cardiac arrhythmia produced by hypokalemia, and gives insight into the clinical importance and pathophysiology of drug-induced arrhythmia, with particular focus on class Ia (quinidine, procainamide) and Ic (flecainide) Na^+ channel blockers, and class III antiarrhythmic agents that block the delayed rectifier K^+ channel (dofetilide).

1. Introduction

Cardiovascular disease is the leading cause of mortality in developed countries. In cardiac patients, about 50% of deaths are sudden, and typically caused by fatal tachyarrhythmia such as ventricular fibrillation (VF). The rapid and highly disorganized electrical activation in VF contributes to inefficient cardiac systole, which provokes acute circulatory failure and leads to death within minutes, unless the arrhythmia is immediately terminated by electrical cardioversion. The risk stratification parameters for sudden arrhythmic death largely remain empirical, and the existing preventive therapies are often not effective. Consequently, malignant ventricular tachyarrhythmia (VT) represents a major health problem that requires the development of novel adequate and patient-specific therapies based on in-depth knowledge of the contributing electrophysiological mechanisms.

The focus of the present thesis is on the detailed description of abnormal cardiac repolarization mechanisms that drive electrical derangements in VT, with particular emphasis on arrhythmias provoked by hypokalemia and antiarrhythmic drugs. Initially, the ionic basis for the electrical activity of ventricular myocytes (chapter 2) and the classical electrophysiological mechanisms for cardiac arrhythmia, such as abnormal automaticity, early and delayed afterdepolarizations, and re-entry (chapter 3) will be outlined. The next few chapters will provide information on repolarization-related arrhythmic surrogates, including spatial repolarization gradients (chapter 4), activation-repolarization coupling (chapter 5), electrical restitution (chapter 6), TRIaD (triangulation, reverse use dependence, instability, and dispersion) (chapter 7), and electromechanical window (chapter 8). Chapter 9 highlights the importance of the location of ventricular pacing site for inducing arrhythmia, when electrical stimulation protocol is applied at the endocardium vs. the epicardium, or in the LV vs. the RV chamber. Chapter 10 overviews the clinical impact and the electrophysiological

mechanisms of arrhythmia caused by hypokalemia, a side effect of diuretic therapy in cardiac patients. Chapter 11 provides insight into the clinical aspects and electrophysiological mechanisms of arrhythmia provoked by antiarrhythmic drugs, with major focus on Na^+ channel blockers (quinidine, procainamide, and flecainide) and class III agents (dofetilide). Finally, the summary of research findings and conclusions are given in chapter 12.

2. Electrical activity of ventricular cells

2.1 Ventricular action potential and the ionic basis for repolarization

Ventricular contraction is promoted by a synchronous excitation of cardiac myocytes, which is accomplished via generation and fast myocardial spread of the electrical signal called *action potential*. From biophysical point of view, action potential is a transient alteration in transmembrane voltage in cardiac cell that is produced by the orchestrated activity of multiple voltage-gated ion channels embedded in the sarcolemma (for review see Amin *et al*. 2010; Schmitt *et al*. 2014; Bartos *et al.* 2015). The human cardiac action potential includes five phases (indicated by the numbers 0 through 4), and its configuration is determined by the precise balance between inward (depolarizing) and outward (repolarizing) ionic currents, as schematically outlined in Figure 1. Electrical activation starts with *fast depolarization* (phase 0) of the cardiac myocyte up to the voltage level of +30 to +40 mV, which is initiated by Na^+ influx contributing to the action potential upstroke. The inward Na^+ current (I_{Na}) is very rapid and transient, as the most Na^+ channels inactivate within one millisecond, thus terminating phase 0. Subsequent activation of the transient outward K^+ current (I_{to}) contributes to a brief *early repolarization* (phase 1). The I_{to} inscribes a notch on the action potential waveshape, and sets the level of take-off potential for subsequent activation of ion channels involved into the plateau phase. The *plateau* (phase 2), or slow repolarization, is characterized by a balance between an inward current generated by the influx of Ca^{2+} ions via the L-type Ca^{2+} channels

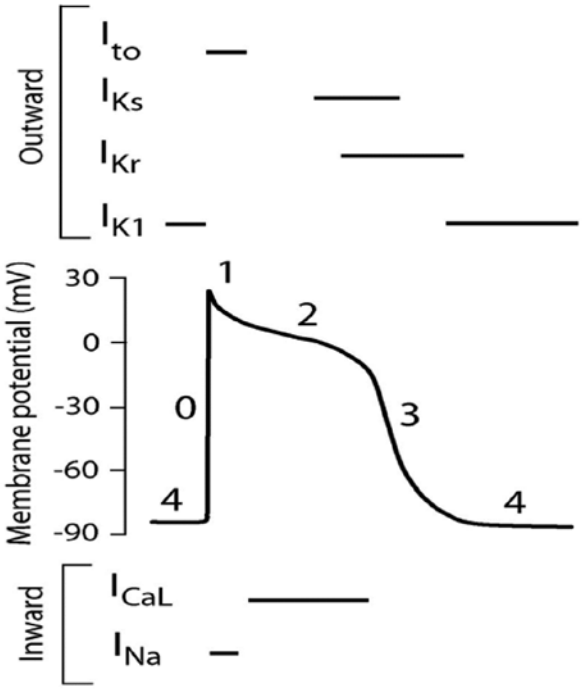

Figure 1. Schematic representation of the human ventricular action potential and contributing inward and outward ionic currents

The numbers above the action potential shape refer to the five phases of the action potential, as outlined in the text. Adapted from Amin, A.S. *et al.* 2010. Cardiac ion channels in health and disease. *Heart Rhythm* 7, 117-126, with permission from publisher.

(I_{Ca}), and outward K^+ currents, such as the rapid and slow components of the delayed rectifier (I_{Kr} and I_{Ks}, respectively). Other contributors to the plateau phase are the late Na^+ current and the Na^+/Ca^{2+} exchanger. The late Na^+ current (I_{NaL}) is maintained by a small fraction of Na^+ channels (0.1-1%) that remain open after completing phase 0. The sarcolemmal Na^+/Ca^{2+} exchanger is an electrogenic transporter that can function either in *the forward mode* (Ca^{2+} efflux, Na^+ influx) or *in the reverse mode* (Na^+ efflux, Ca^{2+} influx), with a stoichiometry of 3 Na^+/1 Ca^{2+} (for review see Bers 2002). The reversed mode is favored by positive membrane potentials and increased intracellular Na^+ levels, whereas the forward mode is favored by negative membrane potentials and increased intracellular Ca^{2+} levels.

Increased Ca^{2+} entry via L-type Ca^{2+} channels subsequently promotes additional Ca^{2+} release from the sarcoplasmic reticulum (SR) via stimulation of the ryanodine receptor, an effect called *Ca^{2+}-induced Ca^{2+} release*. This, in turn, provokes Ca^{2+}-dependent inactivation of I_{Ca}, thus limiting further Ca^{2+} influx and terminating the plateau phase. The membrane voltage then proceeds to change in the negative direction owing to the ongoing activation of outward K^+ currents such as I_{Kr} and I_{Ks}, which accounts for the *late repolarization* (phase 3). In its final portion, the phase 3 is also maintained by I_{K1}, the inward rectifier K^+ current, until the membrane potential is stabilized at the level of -80 to -90 mV. This *resting membrane potential* (phase 4) persists in ventricular cells until the next electrical activation is initiated by cardiac pacemaker (sinoatrial node).

The mechanism of electrical activation outlined above presumes that ventricular repolarization is controlled by several outward K^+ currents, including I_{to}, I_{Kr}, I_{Ks}, and I_{K1}. This redundancy of repolarizing currents has been termed the *repolarization reserve* (Roden 1998; for review see Varro & Baczko 2011), meaning that a blockade of one of these currents can be partly compensated by recruiting others, which would ensure timely ordered repolarization. In this context, the relative importance of each individual repolarizing current can be related to the functional state of other K^+ channels. For example, in normal conditions, I_{Ks} is thought to play only little role in ventricular repolarization in humans and most animal species (one exception is guinea-pig: see Sanguinetti & Jurkiewicz 1990; Zeng *et al.* 1995; Bosch *et al.* 1998; O'Hara & Rudy 2012). Pharmacological blockade of I_{Ks} has been found to produce only small or no lengthening of action potential duration (APD) in rabbit (Lengyel *et al.* 2001; Lu Z. *et al.* 2001; Guerard *et al.* 2008; So *et al.* 2006, 2008), canine (Varro *et al.* 2000; Stengl *et al.* 2003; Jost *et al.* 2013) and undiseased human ventricular myocytes (Jost *et al.* 2005, 2013). However, I_{Ks} becomes much more important when APD is prolonged by blocking

other repolarizing K^+ currents. It was shown that preserved I_{Ks} current may prevent an excessive APD prolongation in circumstances wherein I_{Kr} is significantly reduced (Biliczki *et al.* 2002; Volders *et al.* 2003; Guerard *et al.* 2008). Once APD is prolonged upon I_{Kr} blockade, the I_{Ks} has more time to activate at positive voltages during the plateau phase, thereby limiting further APD lengthening. Accordingly, I_{Ks} is thought to serve as a negative feedback safety mechanism that reduces the risk for arrhythmogenic responses associated with interventions that delay ventricular repolarization (Varro *et al.* 2000).

Apart from conditions that reduce repolarization reserve, there are at least two other physiological regulations that increase the contribution of I_{Ks} to ventricular repolarization. First, I_{Ks} is increased upon β-adrenergic activation, thus causing APD to shorten (Sanguinetti *et al.* 1991; Volders *et al.* 2003; Overholser *et al.* 2009; O'Hara & Rudy 2012). When I_{Ks} is blocked with chromanol 293B, the adrenergic agonist-induced reduction in APD is completely abolished, or even reversed to APD lengthening, which is likely mediated via increased L-type Ca^{2+} current (Schreieck *et al.* 1997). This change facilitates early and delayed afterdepolarizations (to be discussed in sections 3.3. and 3.4). Based on this principle, the combination of an I_{Ks} blocker and β-adrenergic agonist has been used to develop the canine model of arrhythmia associated with the long-QT1 syndrome (Shimizu & Antzelevitch 1998; Gallacher *et al.* 2007). Second, I_{Ks} can importantly contribute to APD shortening at rapid cardiac beating rates. Because of slow deactivation kinetics, the I_{Ks} can accumulate at short pacing intervals, which accounts for APD reduction (Jurkiewicz & Sanguinetti 1993; Lu Z. *et al.* 2001; Overholser *et al.* 2009; O'Hara & Rudy 2012).

Various outward K^+ currents play dissimilar role in maintaining ventricular repolarization in different mammalian species. In mouse and rat, the major repolarizing K^+ current is I_{to}, and the ventricular action potential has a triangular shape, with short

repolarization and no high plateau phase (Watanabe *et al*. 1983; Varro *et al*. 1993; Knollmann *et al*. 2001). A reduction in I_{to} is associated with prolonged APD in these animal species. In contrast, in larger mammalian species, including humans, ventricular repolarization is primarily governed by I_{Kr} and I_{Ks}, although their relative contribution is variable (O'Hara & Rudy 2012). In human and canine ventricular cells, repolarization is controlled to a greater extent by I_{Kr} than I_{Ks} (the I_{Kr} to I_{Ks} ratio is about 3-5 to 1; human: Li *et al*. 1996; Jost *et al*. 2005; canine: Gintant 1996; Varro *et al*. 2000), whereas it almost entirely depends on I_{Kr} in rabbit (the I_{Kr} to I_{Ks} ratio is about 13:1; Lengyel *et al*. 2001). I_{Kr} is also the predominant repolarizing current in feline ventricular myocytes (Follmer & Colatsky 1990). On the other hand, in guinea-pig, ventricular myocytes do not express I_{to} (Varro *et al*. 1993; Zicha *et al*. 2003), and the repolarization is mostly controlled by I_{Ks} (the I_{Ks} to I_{Kr} ratio is about 8-11 to 1; Sanguinetti & Jurkiewicz 1990; Zeng *et al*. 1995; Bosch *et al*. 1998). These findings imply important interspecies differences in pharmacological responsiveness to I_{Kr} blockers, with the largest APD lengthening, and hence a greater tendency to develop arrhythmogenic early afterdepolarizations, to be expected to occur in rabbit and cat.

Based on similarities in I_{Kr} density and kinetics, canine cardiac tissue is commonly considered an optimal model to reproduce drug-induced repolarization abnormalities observed clinically in human patients. Nevertheless, this opinion has been challenged in recent studies that revealed much lower repolarization reserve in human vs. canine ventricular cells. The simulation studies suggest that I_{Kr} block results in APD increase by 80% in human myocytes vs. only 30% increase in canine cells (O'Hara & Rudy 2012). In experimental works, APD lengthening produced by a given concentration of I_{Kr} blocker was found to be about three-fold greater in human vs. canine myocytes (Jost *et al*. 2013). This difference has been attributed to a greater expression of two other repolarizing K^+ channels, I_{K1} and I_{Ks}, in dogs than humans,

which may significantly resist APD prolongation upon I_{Kr} blockade in the canine heart. In a practical context, these findings indicate that the clinical impact of drug-induced repolarization delay may be underestimated when solely based on assessments in canine ventricular tissue. Recent studies have raised the point that the clinically relevant pattern of arrhythmic susceptibility in the setting of reduced repolarization reserve could be more adequately reproduced when using the rabbit rather than canine models (Husti *et al*. 2015). The interspecies differences, therefore, should be carefully considered when extrapolating repolarization assessments from animal experiments to humans.

2.2 Ventricular effective refractory period

Immediately upon completing phase 0, Na^+ channels enter the inactivated state, and hence could not be recruited in order to produce a new depolarization. As a result, ventricular muscle fails to respond to electrical stimulations applied during the entire plateau phase and throughout the most of phase 3. This lack of excitability is called *refractoriness*. It could be quantified by measuring the effective refractory period (ERP), which refers to the portion of action potential over which the ventricular muscle is rendered non-excitable.

The recovery of Na^+ channels from inactivation is voltage-dependent, meaning that once the myocyte membrane repolarizes back to the level of resting potential, increasingly more Na^+ channels become available for activation. It is presumed that at least 25% of Na^+ channels must recover from inactivation, before the cardiac cell can be re-excited (Roden 1996). Under normal conditions, myocardial excitability typically recurs by the end of phase 3 (i.e., at membrane potentials of -65 to -80 mV), and an electrical stimulation applied at this time point can evoke a premature action potential. Based on this principle, the ERP could be measured by programmed electrical stimulation, wherein a train of regular (S_1) pulses is followed by a premature (S_2) stimulus applied at progressively reducing coupling intervals.

The longest S_1S_2 interval producing no extrasystolic beat is then taken as the refractory period.

A prolongation of ERP could be potentially achieved in two ways (Roden 1996). The ERP would be increased in the setting of reduced I_{Na} availability (e.g., upon Na^+ channel blocker administration), as the recovery of excitability in this case will occur at more negative membrane potentials. The ERP is also increased upon prolongation of APD (e.g., upon I_{Kr} blocker administration), as this would indicate that longer time is required in order to reach the level of repolarization at which a critical number of Na^+ channels has recovered from inactivation. Therefore, the variations in the steady-state APD value, for example, upon changing the cardiac beating frequency, are always accompanied by parallel and proportional changes in ERP (Guss *et al.* 1976; Morgan *et al.* 1990; Lee *et al.* 1992).

In a normal heart, a recovery of ventricular excitability typically occurs at a membrane voltage that corresponds to 80-90% of total repolarization, meaning that the ERP-to-APD ratio is 0.8-0.9 (Franz & Costard 1988; Koller *et al.* 1995; Schreieck *et al.* 1997; Knollmann *et al.* 2001; Fabritz *et al.* 2003). An increase in ERP by I_{Na} blockers may result in *post-repolarization refractoriness*, which is defined as a condition wherein ERP is greater than APD measured at 90% repolarization (APD_{90}) (Franz & Costard 1988; Kirchhof *et al.* 1998; Franz *et al.* 2014). The post-repolarization refractoriness is considered an antiarrhythmic change, because it protects the heart from premature activation in early diastole, which would otherwise trigger VT. Therefore, antiarrhythmic agents that produce a greater increase in ERP compared to an increase in APD_{90}, can be an effective therapy for preventing VT (Kirchhof *et al.* 2003; Frommeyer *et al.* 2012, 2014; Milberg *et al.* 2012a; Franz *et al.* 2014).

The opposite change, i.e. ERP reduction relative to APD_{90}, indicating a decreased ERP-to-APD ratio, could be observed in hypokalemia (Sabir *et al.* 2007; Osadchii & Olesen 2009),

or could be induced by the application of several closely-coupled premature pulses during programmed ventricular stimulation (Koller *et al.* 1995). This change is considered to be a state of "facilitated excitability" that allows ventricular capture to be produced at an earlier repolarization time point (*the "encroachment" effect*), resulting in increased inducibility of tachyarrhythmia (Koller *et al.* 1995; Franz *et al.* 2014).

2.3 Propagation of ventricular action potential

Once the electrical signal is generated by the cardiac cell, it should be rapidly conducted to other myocytes in order to promote a synchronous ventricular contraction. This process is governed by the *source-sink relationships* (for review see Kleber & Rudy 2004; Spector 2013; Dhein *et al.* 2014; Veeraraghavan *et al.* 2014). The *source* refers to the depolarizing current available for activation (i.e., generated by the proximal excited cell), and the *sink* is the current needed to activate the distal myocytes along the propagation path. The source-to-sink ratio is called the *safety factor*. The electrical impulse could successfully propagate from the site of its origin only when the source is larger than sink, meaning that safety factor should always be greater than 1.

The *source* is a local circuit current that originates from a voltage difference between the excited myocyte and the adjacent quiescent cell. This current is flowing via *gap junctions*, specialized low-resistance intercellular connections. Once the adjacent cell is activated, it will in turn generate a depolarizing current to excite the next, more distal myocyte. In this way, the excitation propagates across myocardial syncytium in a wave-like manner. The amplitude of the depolarizing current is largely determined by the maximal velocity of the action potential upstroke (V_{max}), which is directly proportional to the inward Na^+ flux during phase 0. Therefore, when Na^+ channel availability is reduced, the conduction velocity is slowed.

Apart from the *active properties* of the myocyte sarcolemma determined by Na^+ channel density, the impulse conduction depends on the *passive electrical* properties of myocardial tissue (for review see King *et al.* 2013; Dhein *et al.* 2014). That is, a certain fraction of the depolarizing current has to be used in order to discharge the *membrane capacitance* in the distal quiescent cell. Likewise, the impulse propagation is partly opposed by the *resistance* of extracellular and intracellular spaces. Hence, in real conditions, a certain critical number of cells, rather than just one single myocyte, must be excited in order to generate a depolarizing current that is sufficiently strong for initiating a propagating response. These depolarized cells form the original excitation focus, or *liminal area*, which normally has a radius of at least 200-250 μm (Lindemans & van der Gon 1978).

When considering the impulse conduction in a three-dimensional excitable media, the *source* is partly determined by the geometric characteristics of the depolarization wave, such as the *wave curvature* (Fast & Kleber 1997). When the wavefront is convexed, i.e. curved outward, the excitatory current it supplies is distributed over a larger downstream myocardial area. This dispersion of electrical current then contributes to a slower conduction velocity compared to that determined with a flat excitation wavefront. These relationships are particularly important during re-entrant arrhythmia (to be discussed in section 3.5), which is driven by a spiral depolarization wave with a curved leading edge.

The *sink* depends on the excitability of cardiac cells located ahead of the propagating action potential. This is determined by the difference between the resting membrane potential and the membrane voltage for activation of the fast inward Na^+ current. When this difference, called the *excitation threshold,* is increased (e.g. upon increased I_{K1} conductance which causes the resting membrane potential to become more negative), a greater excitatory current is required to depolarize the myocyte. The sink is also increased in conditions associated with a

reduced density of gap junctional channels, which contributes to increased axial resistance to the excitatory circuit current flow.

3. Electrophysiological mechanisms of ventricular tachyarrhythmia

3.1 Cardiac arrhythmia: definition and overview of contributing mechanisms

In a broad sense, *arrhythmia* refers to any irregularities in cardiac beating, or to abnormal heart rates, or both. As such, cardiac arrhythmia embraces various types of electrical disturbances, ranging from clinically asymptomatic single extrasystolic beats, to malignant, life-threatening tachyarrhythmias such as torsade de pointes and ventricular fibrillation. Classically, the mechanism of tachyarrhythmia relies on the dynamic interplay of a provocative *trigger*, the vulnerable tissue *substrate*, and the modulating factors, such as autonomic balance, plasma electrolytes, and pharmacological treatments, which influence both (Keefe *et al.* 1987; Surawicz 1989; Yan *et al.* 2001b; Fabritz *et al.* 2003; Gallacher *et al.* 2007; Merchant & Armoundas 2012; Qu & Weiss 2015).

Holter ECG recordings in victims of sudden cardiac arrest demonstrate that VT is typically triggered by a closely-coupled premature cardiac contraction or a series of rapid ectopic beats (Lewis *et al.* 1983; Panidis & Morganroth 1983; Kempf & Josephson 1984; Milner *et al.* 1985; Locati *et al.* 1995). The ectopic beat that serves as the initiating event for VT could either originate in myocardial regions with *abnormal automaticity*, or develop from *early* and *delayed afterdepolarizations* provoked by an excessive APD lengthening and/or intracellular Ca^{2+} overload.

The vulnerable *substrate* for arrhythmia is typically created by regional structural and functional heterogeneities in ventricular tissue (for review see Wolk *et al.* 1999a; Antzelevitch 2007a, b), either intrinsic or induced by cardiac disease. Most commonly, the favorable milieu for VT is formed by ventricular scarring following acute myocardial infarction, wherein

surviving myocardial fibers are interspersed in areas of patchy fibrosis. These changes contribute to the non-uniform propagation of the triggered action potential, with electrical conduction being preserved in some directions, but significantly slowed or even blocked in others. Under certain circumstances, the electrical impulse may start to circulate within a closed conducting pathway, thus perpetuating tachyarrhythmia via a mechanism called *re-entry* (to be discussed in section 3.5).

With functional tissue heterogeneities, the substrate for arrhythmia can be created upon non-uniform lengthening of APD, and hence effective refractory period, at distinct myocardial regions (Kuo *et al.* 1983, 1985; Laurita & Rosenbaum 2000; Akar & Rosenbaum 2003). This may be a consequence of electrical remodelling in cardiac disease, or produced by I_{Kr} blocker infusion, or caused by the inherited loss-of-function mutations of repolarizing ion channels. A premature depolarization generated by ectopic pacemaker then successfully propagates across myocardial regions with short ERP, whereas conduction in the direction of cells with prolonged ERP will be slowed and eventually die out, which would set a stage for re-entry.

Based on the underlying electrophysiological mechanisms, arrhythmias are traditionally categorized into those attributed to abnormal impulse *formation* and those resulting from abnormal impulse *conduction*. Classification of ventricular arrhythmias, nevertheless, is continuously evolving upon accumulation of novel findings from both experimental and clinical studies. For example, very recently, Weiss *et al.* (2015) proposed a dynamics-based classification, which subdivides cardiac arrhythmias into three primary categories related to unstable Ca^{2+} cycling, reduced repolarization, and excess repolarization, respectively. The following sections provide a more detailed overview of classic arrhythmogenic mechanisms.

3.2 Abnormal automaticity

Abnormal automaticity refers to the initiation of spontaneous firing in myocardial sites (*ectopic pacemakers*) that do not exhibit pacemaker activity in normal conditions (for review see Qu & Weiss 2015). This might originate from the downstream parts of the cardiac conduction system, such as Purkinje fibers, which normally exhibit only very slow spontaneous diastolic depolarization rate and are therefore suppressed by rapid firing from the sinoatrial node. Alternatively, abnormal automaticity may be acquired by atrial or ventricular cells that are quiescent in physiological conditions. The abnormal ventricular activations that occur in this setting are categorized as *escaped ectopic rhythms* that develop as a consequence of the sinus node dysfunction, and *accelerated ectopic rhythms* attributed to enhanced automaticity in the latent pacemaker. In both cases, the ectopic pacemaker is capable to generate premature depolarization that interferes with regular activation rate set by the sinoatrial node.

The enhanced automaticity in ectopic pacemakers is often a consequence of the partial depolarization of the myocyte sarcolemma, which brings the resting membrane potential closer to the threshold voltage for activation of the inward Na^+ current; this change facilitates premature action potential. The same is observed when the threshold voltage becomes more negative, or upon an increase in slope of the spontaneous diastolic depolarization in pacemaker cells. Antiarrhythmic drugs therefore may suppress abnormal automaticity by either reducing the slope of phase 4 depolarization, or increasing the maximum diastolic potential, or shifting the threshold potential to less negative values (Roden 2006).

The common pathophysiological factors that facilitate spontaneous firing in ectopic pacemakers include ischemia, acidosis, hypokalemia, increased adrenergic tone, and myocardial stretch. In these conditions, premature depolarization is produced either by

increasing the inward electrical current, such as I_f (a mixed Na^+-K^+ current contributing to spontaneous diastolic depolarization), or by reducing the outward K^+ current via I_{K1} channels.

3.3 Early afterdepolarizations

Early afterdepolarizations (EADs) are the positive voltage deflections that interrupt the smooth course of repolarization during phase 2 or phase 3 of the action potential (for review see El-Sherif *et al.* 1990; Antzelevitch & Sicouri 1994; Volders *et al.* 2000; Weiss *et al.* 2010; Qu *et al.* 2013; Qu & Weiss 2015). When EAD increases in amplitude, it may reach threshold voltage level for generating a propagating action potential, called a *triggered beat* (Figure 2), which is manifested on ECG as the extrasystolic complex. It is noteworthy, however, that in a well-coupled cardiac tissue, EAD generated in a single myocyte is unlikely to give a rise to the propagating response, because the depolarizing EAD current is dissipated in a large volume of surrounding myocardial tissue, thus causing the source-sink mismatch. The generation of triggered beat, therefore, requires the EAD to develop in a large number (hundreds to thousands) of ventricular cells localised in the same anatomical region (for review see Weiss *et al.* 2010).

EADs are typically precipitated by an excessive APD lengthening, which increases the time spent over the voltage window (-40 to 0 mV) for recovery from inactivation of the L-type Ca^{2+} current. Accordingly, shaping biophysical properties of I_{Ca} has been proposed as a powerful therapeutic strategy to suppress EADs-dependent arrhythmia (Madhvani *et al.* 2011). Other inward currents that contribute to EAD are the late I_{Na} (Horvath *et al.* 2013; Edwards *et al.* 2014; Pezhouman *et al.* 2015) and the Na^+-Ca^{2+} exchange current, $I_{Na/Ca}$ (Szabo *et al.* 1994; Spencer & Sham 2003). EADs most commonly originate in Purkinje fibers and midmyocardial cells (Burashnikov & Antzelevitch 1998; Yan *et al.* 2001b), which is attributed to reduced repolarization reserve in these types of cells compared to epicardial and

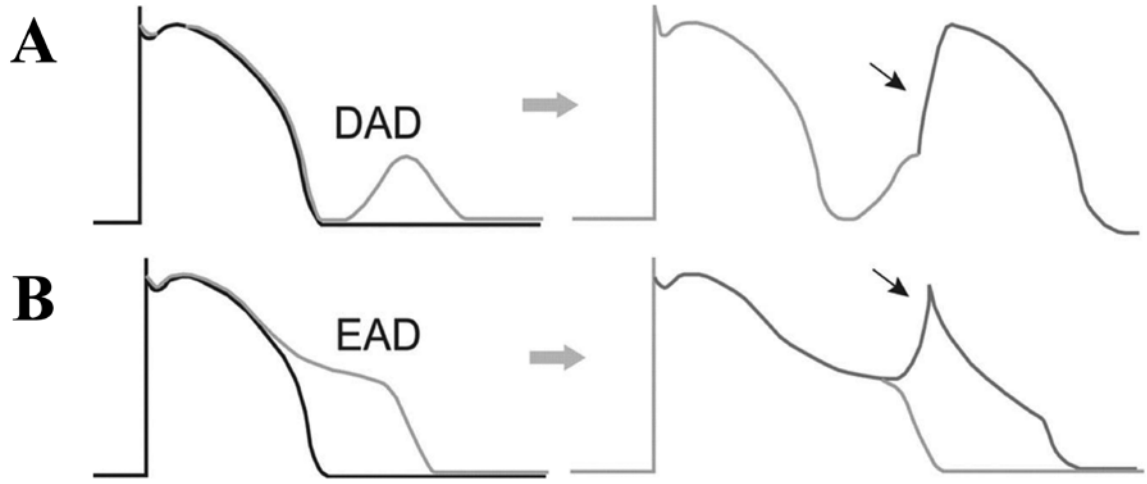

Figure 2. Afterdepolarizations and triggered beats in ventricular myocytes
In panels A and B, the left fragment shows ventricular action potentials recorded in basal conditions (solid line) and after inducing delayed (A) and early (B) afterdepolarizations (DAD and EAD, respectively). The right fragment in both panels shows that DAD and EAD may give a rise to the propagating premature action potential (a triggered beat; highlighted by arrow). Adapted from Khan, R. 2004. Identifying and understanding the role of pulmonary vein activity in atrial fibrillation. *Cardiovasc Res* 64, 387-394, with permission from publisher.

endocardial myocytes (to be discussed in section 4.3).

The EADs play a pivotal role in arrhythmogenesis in patients with long QT syndrome, a clinical condition characterized by congenital or acquired defects in repolarizing ion channels (for review see Keating & Sanguinetti 2001). Because cardiac cells enrol multiple ionic currents to ensure timely ordered repolarization, a single electrical defect would unlikely to result in a clinical long QT phenotype. An excessive APD lengthening that increases propensity for EAD is thought be the result of *multiple hits* on repolarization, including genetic predisposition, drug-induced blockade of repolarizing ion currents, low resting heart rates, and electrolyte abnormalities that cause APD to prolong, such as hypokalemia and hypomagnesiemia. Female patients are more susceptible to these electrical derangements compared to males owing to the gender-related differences in cardiac repolarization reserve.

A critical degree of APD prolongation that evokes EADs could be attained by decreasing outward currents, or by increasing inward currents, or via combinations of the two. The ionic mechanisms contributing to EADs are species dependent; in simulation studies, 90% I_{Kr} block has been shown to promote EADs in canine but not human or guinea-pig myocytes, whereas 90% I_{Ks} block promoted EADs in guinea-pig but not in humans or dog (O'Hara & Rudy 2012). Apart from I_{Kr}/I_{Ks} blockers, the agents that promote EADs include those that increase Ca^{2+} influx, or enhance the late Na^{+} current by delaying its inactivation (for review see El-Sherif *et al.* 1990). The lengthening and flattening of the action potential plateau provoked by these agents represents the *conditioning EAD phase* (January & Riddle 1989; Zeng & Rudy 1995). This provides more time at plateau potentials for I_{Ca} to recover from inactivation, which contributes to the transmembrane depolarizing current. Because I_{Ca} reactivation is voltage-dependent and regenerative, it develops as a positive feedback process, whereby a small initial Ca^{2+} entry via L-type channels causes a further increase in I_{Ca}. This initiates the EAD upstroke (*the depolarizing EAD phase*). Taken together, these changes constitute the sarcolemma-dependent mechanism for EAD generation. In addition, there is convincing evidence that at least some types of EADs could be promoted by spontaneous SR Ca^{2+} release, with subsequent stimulation of the forward mode of the Na^{+}/Ca^{2+} exchange (Priori & Corr 1990; Volders *et al.* 1997; Zhao *et al.* 2012). The *sarcolemma-dependent* and *sarcoplasmic reticulum-dependent* mechanisms are not mutually exclusive, and may act synergistically in inducing EADs (Weiss *et al.* 2010).

3.4 Delayed afterdepolarizations

Delayed afterdepolarizations (DADs) are oscillations of the membrane potential that arise after a full repolarization has been attained in a preceding beat. As with EADs, the amplitude of DADs may become sufficiently large in order to trigger a premature ventricular

activation (Figure 2, panel A), and precipitate tachyarrhythmia. While suprathreshold DADs provide an arrhythmic trigger, the subthreshold DADs may importantly contribute to the vulnerable tissue substrate by elevating the diastolic membrane potential and inactivating Na^+ channels, changes that facilitate conduction slowing (Liu *et al.* 2015).

The mechanism of DADs is related to Ca^{2+} overload in cardiac cells, which promotes depolarization of sarcolemma via an increase in the forward mode Na^+/Ca^{2+} exchange current, with a smaller contribution provided by the Ca^{2+}-activated chloride current, $I_{Cl(Ca)}$, and the non-selective cation channels (Marban *et al.* 1986; Zygmunt *et al.* 1998; Verkerk 2001). DADs are often provoked by interventions that either increase systolic Ca^{2+} fluxes, or reduce Ca^{2+} elimination in diastole. The former is typically observed at rapid cardiac activation rates, especially in the presence of Ca^{2+}-mobilizing agents, such as cardiac glycosides, or catecholamines, and the latter can be a consequence of downregulation of the SR Ca^{2+} ATPase in heart failure. Elevated cytosolic Ca^{2+} levels, in turn, contribute to increased SR Ca^{2+} load, which facilitates spontaneous oscillatory Ca^{2+} release in diastole. The probability of uncontrolled SR Ca^{2+} release is markedly increased in patients with mutant (i.e., "leaky") cardiac ryanodine receptors that exhibit increased sensitivity to luminal Ca^{2+}. This genetic defect is often associated with catecholaminergic polymorphic VT phenotype (Paavola *et al.* 2007). The arrhythmia is typically provoked by sympathetic overactivation during exercise or emotional stress, which promotes spontaneous Ca^{2+} waves and DADs via β-adrenoreceptor-cAMP-dependent mechanism.

It should be emphasized that a separation of electrophysiological mechanisms underlying DADs vs. EADs is quite conventional, as there are a variety of pathophysiological factors capable of inducing both types of afterdepolarizations, including hypokalemia, catecholamines, ischemia and reperfusion, etc. In an experimental setting, both EADs and

DADs can often be induced in the same cardiac preparation (Priori & Corr 1990; Volders *et al.* 1997; Pott *et al.* 2012; Zhao *et al.* 2012).

3.5 Re-entry

Re-entry, or circus movement, refers to the circulation of electrical impulse within a closed pathway (*the re-entrant circuit*), which allows the impulse to repetitively excite the site of its origin (for review see El-Sherif 1988; Rudy 1995; Kleber & Rudy 2004; Comtois *et al.* 2005; Roden 2006; Qu & Weiss 2015). This type of abnormal activation is based on several prerequisites. First, there must be an obstacle, which interrupts normal propagation of the action potential, thus causing the impulse to move around. The obstacle is the non-conductive medium that could be either anatomic (e.g. post-infarction scar) or functional (e.g. ischemic tissue with depressed excitability). Second, re-entry requires the transient unidirectional conduction block to be present at the proximal part of the circuit. The cells in this area typically have a prolonged refractory period, and therefore are not able to support conduction following upstream stimulation. The electrical impulse therefore starts to move around the obstacle in the opposite direction. While making a circle, the impulse can retrogradely approach the proximal area that imposed conduction block, and excite it, providing that myocytes in this area have already recovered from refractoriness. Therefore, the third requirement for re-entry to occur is that the impulse conduction time within the circuit should be longer than the effective refractory period. In this case, the impulse can return to the site of its origin, and start another circle. Depending on the number of round trips the impulse will make before dying out, the arrhythmic episode may be manifested either as a short series of several ectopic beats or as sustained tachycardia.

In order to support re-entry, the anatomical pathway available for impulse conduction must be long enough to ensure that each site within the circuit has regained excitability before

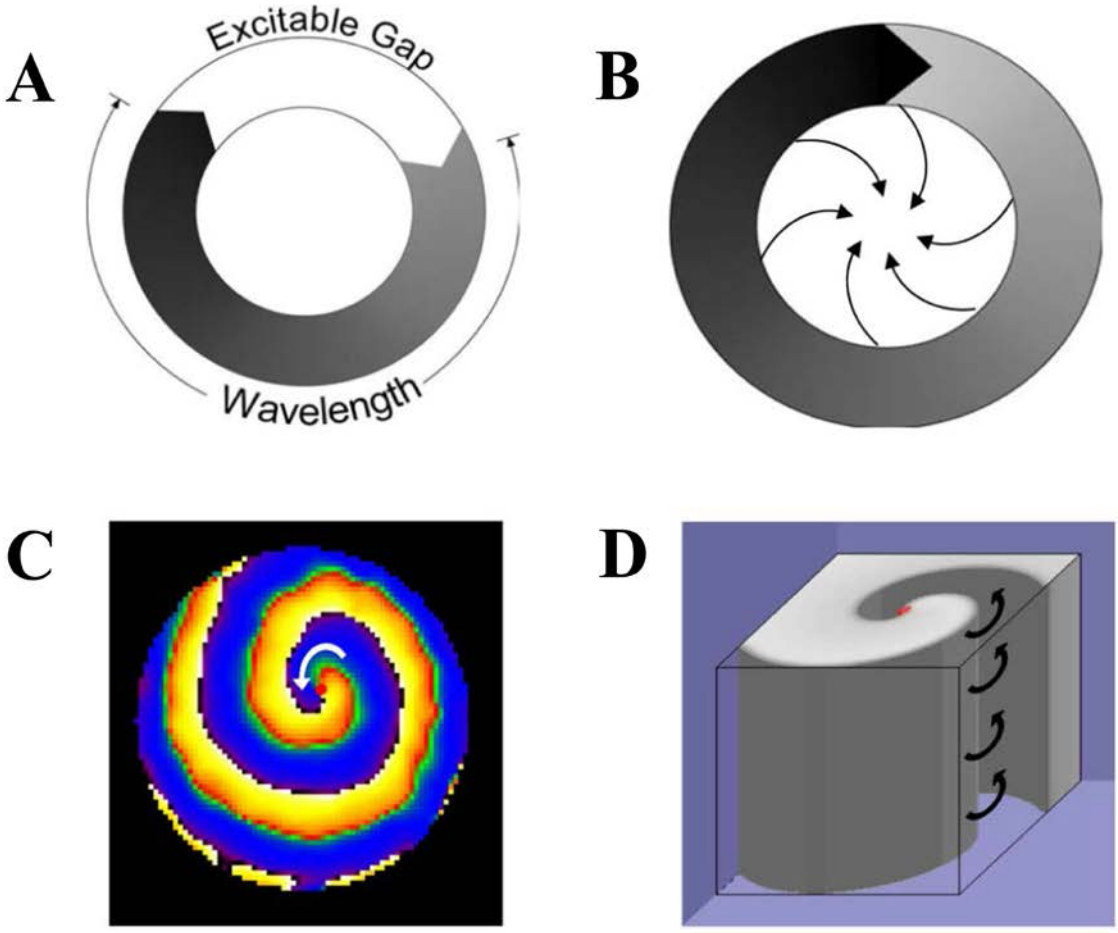

Figure 3. Different models of re-entry in cardiac tissue

Panel A: circus movement around an anatomical obstacle (central white circle). The area of depolarized cells (wavelength) is less than the size of the anatomical pathway available for impulse conduction, which creates a fully excitable gap separating the head of the depolarization wave (black part of the arrow shape) and its refractory tail (grey part of the arrow shape). Panel B: leading circle re-entry around a refractory central core, whereby depressed excitability is maintained by centripetal wavelets (curved arrows). The head of depolarization wave encroaches its partially refractory tail, indicating a lack of the fully excitable gap. Panel C: spiral depolarization wave (colored in yellow) rotates around the central core (red dot) which is excitable, but not excited, because the high wave front curvature at the tip of the spiral wave creates a source-sink mismatch that stops further propagation of depolarization. Panel D: a three-dimensional scroll wave rotating counterclockwise. Adapted from Jalife, J. 2009. Inward rectifier potassium channels control rotor frequency in ventricular fibrillation. *Heart Rhythm* 6, S44-S48, with permission from publisher.

the return of the circulating impulse. This creates a *spatial excitable gap* which separates the head of the depolarization wave and its refractory tail (Figure 3, panel A). Historically, this principle stems from the classical work by Garrey (1914) who postulated that VF could be maintained only when the amount of available cardiac tissue exceeds a certain critical size (*the critical mass hypothesis*). In human RV, VF is not inducible if the tissue mass is reduced below 20% of the cardiac weight (Wu *et al.* 1999). More specifically, the size of anatomical pathway must be greater than the *excitation wavelength*, which is the area of depolarized cells formed as the electrical impulse travels forward. The wavelength could be found as a product of conduction velocity and ERP. The interventions that reduce the excitation wavelength, either by slowing conduction or by decreasing ERP, would allow the re-entry to sustain in a smaller mass of cardiac tissue, indicating a *proarrhythmic effect*. In contrast, a prolongation of ERP may increase the excitation wavelength to such an extent that it no longer fits the size of the available anatomic pathway, indicating *antiarrhythmic effect*.

Traditionally, re-entry is categorized as *anatomical* and *functional*. The major difference is that the anatomical re-entry relies on the underlying tissue structure, meaning that the re-entrant pathway has a fixed location and length (typically determined by a perimeter of the anatomic obstacle), whereas with functional re-entry, the circuits may vary in size and appear at random locations. The prototypical model of anatomical re-entry was proposed more than a century ago by Mayer (1906) who performed experiments on the ring-like fragments from the muscular tissue of jellyfish. Upon single point electrical stimulation, he successfully induced a wave of contraction that propagated in one direction and continuously circulated around the muscular ring. Mines (1913) reproduced this phenomenon using the ring-like strips of cardiac tissue, and applied the concept of re-entry to explain the mechanism of fibrillation.

The model of functional re-entry, as formulated in the "*leading circle*" concept, was developed by Allessie *et al.* (1977), who showed that tachyarrhythmia could be induced in isolated rabbit atria by premature electrical stimulation without involving any anatomical obstacle. In this model, the *functional* obstacle around which the wavefront is rotating is a small area of refractory but potentially excitable cardiac tissue that forms the *central core*. The state of depressed excitability in the core is attributed to Na^+ channel inactivation secondary to sustained depolarization induced by centripetal wavelets (Figure 3, panel B). Another important attribute of this model is a lack of the fully excitable spatial gap; because the rate of atrial activation is as rapid as it can be, the head of the circulating wavefront always encounters relatively refractory tissue.

When represented on a two-dimensional scale, for example, in a thin layer of epicardial tissue, the functional re-entry is driven by a *spiral wave*, or *rotor* (Pertsov *et al.* 1993). In this model (Figure 3, panel C), the central core could not be excited, because the wavefront at the tip of the spiral wave is very convex. This provokes the source-sink mismatch, and stops impulse propagation, despite the presence of excitable tissue ahead of it.

The re-entry in a three-dimensional excitable medium, for instance, in a thick LV wall, is maintained by a *scroll wave* (Figure 3, panel D). The scroll waves may be stationary or drifting from the site of their origin; these features determine the electrophysiological phenotype of cardiac arrhythmia (Pertsov *et al.* 1993; Fast & Kleber 1997). For example, monomorphic VT is typically caused by a stationary scroll wave that is anchored to a small anatomical obstacle, e.g. papillary muscle, whereas polymorphic VT, like torsade de pointes, is often caused by a drifting scroll wave. The drifting scroll waves are generally short-lived. They either extinguish spontaneously after a few rotations owing to collision with the tissue border, or degenerate to a more complex form of electrical activation such as ventricular

fibrillation. In VF, electrical activation is typically driven by multiple scroll waves that propagate randomly around islets of refractory tissue. The scroll waves may either emanate from a stable, high-frequency source of activation (the *mother rotor hypothesis*; for review see Jalife 2000, 2009; Pandit & Jalife 2013), or be formed as a result of instability of the original single spiral wave, which can spontaneously break up and produce several daughter wavelets (the *multiple wavelet hypothesis*; for review see Weiss *et al.* 2005).

4. Spatial repolarization gradients

Because amplified regional heterogeneities in repolarization importantly contribute to the substrate for re-entry, the assessments of spatial APD dispersion are receiving increasing attention in studies on inherited and acquired cardiac arrhythmia syndromes (for review see Burton & Cobbe 2001; Antzelevitch 2007a, b). The intrinsic non-uniformities in APD could be subdivided into *interventricular*, i.e. determined at corresponding RV vs. LV recording sites, and *intraventricular*, whereby APD variations are determined over the *apico-basal* axis or *transmural* (epicardial-to-endocardial) axis.

4.1 Interventricular repolarization heterogeneities

In human subjects, the duration of repolarization is longer in the LV than the RV chamber, as evidenced by non-invasive electrocardiographic imaging (Ramanathan *et al.* 2006), and electrogram recordings from multiple LV and RV endocardial sites (Bueno-Orovio *et al.* 2012). These repolarization heterogeneities are explained by greater RV than LV mRNA expression levels of HERG1 and KCNQ1, the proteins that encode the pore-forming α-subunit of I_{Kr} and I_{Ks} channels, respectively (Luo *et al.* 2008). The LV-to-RV dispersion of repolarization is transiently increased upon abrupt heart rate acceleration, an effect which is attributed to slower APD rate adaptation in the LV vs. RV chamber (Bueno-Orovio *et al.* 2012).

Similar to human studies, a greater LV than RV action potential duration has been determined at epicardial (Janse *et al.* 2005; Gallacher *et al.* 2007; Meijborg *et al.* 2015) and endocardial (Verduyn *et al.* 1997a, b; van Opstal *et al.* 2001) recording sites in anesthetized dogs. The LV-to-RV APD dispersion in canine hearts is attributed to the increased density of I_{Ks} in RV myocytes (Volders *et al.* 1999). Owing to the reduced repolarization reserve in LV cells, pharmacological blockade of I_{Ks} or I_{Kr} provokes a greater APD prolongation in the LV vs. RV chamber, thus increasing the interventricular repolarization gradient (Gallacher *et al.* 2007; Meijborg *et al.* 2015).

Apart from regional variations in final repolarization time, the interventricular heterogeneities are manifested in different configurations of the action potential in RV vs. LV cells. In canine epicardial and midmyocardial layers, action potential exhibits a deeper notch and therefore more pronounced spike-and-dome configuration in RV compared to LV myocytes, which is accounted for by a greater density of I_{to}, the transient outward K^+ current, in RV cells (Di Diego *et al.* 1996; Volders *et al.* 1999). The same pattern of I_{to} distribution is present in the rodent heart (Brunet *et al.* 2004). A greater expression of I_{to} in RV myocytes contributes to a shorter APD in the RV compared to the LV chamber in rats (Watanabe *et al.* 1983; Casis *et al.* 1998) and mice (Knollmann *et al.* 2001; Waldeyer *et al.* 2009; Martin *et al.* 2011).

In contrast, in the guinea-pig heart, epicardial APD is longer in RV vs. LV myocytes (Poelzing & Veeraraghavan 2007; Osadchii *et al.* 2009; but see Pandit *et al.* 2011), a difference which is attributed to the greater expression of I_{K1}, the inward rectifier K^+ current, in LV cells (Warren *et al.* 2003; Veeraraghavan & Poelzing 2008). In rabbit heart, the published data on interventricular difference in repolarization are quite inconclusive, with APD shown to be similar in both ventricular chambers (Myles *et al.* 2012; Smith *et al.* 2012;

Ovechkin *et al*. 2015), or to exhibit a greater value in either RV (Wolk *et al*. 1999b; Azarov *et al*. 2008) or LV myocytes (Qi *et al*. 2015).

A significant increase in interventricular APD dispersion could be induced upon no-flow global ischemia in rabbit (Kurz *et al*. 1993; Smith *et al*. 2012) and guinea-pig hearts (Pandit *et al*. 2011), an effect which is attributed to substantially greater APD shortening in the LV vs. RV chamber. This change is likely to be caused by activation of the ATP-sensitive K^+ channels (I_{KATP}), which is more prominent in LV myocytes owing to a larger I_{KATP} expression compared to RV cells (Pandit *et al*. 2011). These findings indicate that an amplified RV-to-LV repolarization gradient can importantly contribute to arrhythmogenesis in ischemic hearts.

4.2 Apico-basal repolarization heterogeneities

Apico-basal non-uniformities in APD have been most thoroughly characterized in guinea-pig (Kanai & Salama 1995; Laurita *et al*. 1996; Salama *et al*. 1998; Choi & Salama 2000; Restivo *et al*. 2004), rabbit (Choi *et al*. 2002; Liu *et al*. 2005; Mantravadi *et al*. 2007), mouse (Baker *et al*. 2000, 2004; London *et al*. 2007), and rat hearts (Weber dos Santos *et al*. 2009) using high-resolution optical mapping. In these animal species, APD is the shortest at the ventricular apex, and becomes progressively longer toward the base, with APD dispersion being greater in females than in males (Liu *et al*. 2005).

In canine hearts, although heterogeneities in repolarization along the apico-basal axis were determined in several studies, there is no common opinion regarding the direction of this gradient. In single ventricular myocytes, APD was found to be shorter in cells dissociated from apical compared to basal regions (Szentadrassy *et al*. 2005). Nevertheless, *in vivo* recordings in anesthetized dogs suggest the opposite, i.e. both APD and ERP are longer at the LV apex than at the base (Bauer *et al*. 2002; Janse *et al*. 2005; Stoll *et al*. 2008; Tsvetkova *et*

al. 2011; Izumi *et al.* 2012). These observations imply that cellular electrotonic interactions in electrically coupled cardiac tissue, as well as the presence of intact autonomic innervation, could be important in shaping the apico-basal gradient.

There are substantial interspecies differences in the nature of mechanisms contributing to apico-basal APD dispersion. In mouse (Brunet *et al.* 2004; London *et al.* 2007), and rat (Casis *et al.* 1998), the latter is attributed to the increased density of I_{to} in apical vs. basal myocytes. In contrast, in rabbit heart, APD variations along this axis are accounted for by the asymmetric distribution of I_{Kr} and I_{Ks}, with I_{Kr} density being two-fold higher at the ventricular apex vs. the base, whilst I_{Ks} is more abundant at the basal regions (Cheng *et al.* 1999; Ng *et al.* 2009). Therefore, there is a large apico-basal difference in the I_{Ks}-to-I_{Kr} ratio.

In canine hearts, the apico-basal repolarization heterogeneities are attributed to larger I_{Ks} and I_{to} expression at the apex vs. the base (Szentadrassy *et al.* 2005). The same study also revealed a similar pattern of apico-basal asymmetry in I_{Ks} and I_{to} expression in non-diseased human hearts. These findings are congruent with electrocardiographic repolarization imaging in healthy human subjects which demonstrates a progressive delay in repolarization from the epicardial apex toward the base (Ramanathan *et al.* 2006). Interestingly, in cardiac disease, this pattern is preserved in about 60% of patients, whereas the remainder shows the opposite direction of the gradient (Chauhan *et al.* 2006).

In cardiac disease, the apico-basal APD dispersion can be modulated by many factors including electrical remodeling, impaired cardiac autonomic regulation, and altered ventricular loads. The role played by downregulation of outward K^+ channels was addressed in studies that utilized the animal models of the long QT syndrome (Cheng *et al.* 1999; Baker *et al.* 2000; Choi *et al.* 2002; Liu *et al.* 2005; London *et al.* 2007). In rabbit heart, I_{Kr} blocker infusion has been shown to produce a greater APD lengthening in apical compared to basal

myocytes (Cheng *et al.* 1999; Choi *et al.* 2002; Liu *et al.* 2005; but see Guerard *et al.* 2014), thus reversing the repolarization gradient, and markedly increasing epicardial APD dispersion. In transgenic mice with a loss of the slowly inactivating 4-aminopyridine-sensitive K^+ current (I_{slow}) (Baker *et al.* 2000), or a lack of both slow and fast components of I_{to} (London *et al.* 2007), the long QT phenotype is associated with increased apico-basal gradient in APD and refractoriness, and increased susceptibility to torsade de pointes upon extrasystolic stimulation.

The apico-basal repolarization gradient is substantially modified by changes in cardiac autonomic balance. In rabbit hearts, sympathetic nerve stimulation evokes a significant APD reduction at the base, while producing no effect at the apex (Mantravadi *et al.* 2007). In contrast, bilateral vagal stimulation significantly prolongs APD at the apex, while producing no effect at the base. In both cases, autonomic stimulation reverses the direction of the repolarization gradient. Non-uniform APD shortening in apical vs. basal regions has been observed following the infusion of adrenergic agonist in human patients (Selvaraj *et al.* 2009), and upon stellate ganglia stimulation (Vaseghi *et al.* 2013) or vagal stimulation (Yamakawa *et al.* 2014) in anesthetized, open-chest pigs. These findings are likely accounted for by the non-uniform distribution of autonomic innervation and/or adrenergic and muscarinic receptors throughout the apico-basal axis.

The apico-basal disparities in repolarization are amplified in the presence of increased LV afterload and associated myocardial stretch. An acute LV pressure overload produced by aortic clamping in anesthetized, open-chest rabbits (Ovechkin *et al.* 2015) and pigs (Dean & Lab 1990), was found to evoke a greater shortening of APD and ERP at the apex compared to the base, thus resulting in an increased apico-basal gradient. Similar changes have been reported to develop in Langendorff-perfused rabbit hearts in response to acute LV dilatation

caused by an increase in the volume of a fluid-filled balloon anchored within LV (Reiter *et al.* 1988). These experimental works suggest a role played by accentuated apico-basal gradients in refractoriness in stretch-induced arrhythmias seen in clinical conditions associated with elevated cardiac loads, for instance, in heart failure.

4.3 Transmural repolarization heterogeneities

Repolarization heterogeneities across the LV wall are attributed to the presence of three electrophysiologically distinct cell types - epicardial, endocardial, and M cells (for review see Antzelevitch *et al.* 1991, 1999; Anyukhovsky *et al.* 1999; Wilson *et al.* 2011). The longest APD is determined in the M cells, and the shortest APD is measured in epicardial myocytes.

The M cells were originally described in studies on canine ventricular tissue, wherein they are identified as a unique subpopulation of myocytes that are distributed uniformly (Sicouri & Antzelevitch 1991, 1995; Yan *et al.* 1998) or in the form of circular islands (Akar *et al.* 2002) in deep subepicardial to midmyocardial layers (the M-region). The hallmark of the M cell is a disproportionately greater APD prolongation upon heart rate deceleration, or the infusion of pharmacological agents that delay ventricular repolarization, such as I_{Kr} blockers, compared to the relative APD changes at the epicardium or endocardium (Sicouri & Antzelevitch 1991; Drouin *et al.* 1995; El-Sherif *et al.* 1996; Antzelevitch *et al.* 1999; Yan *et al.* 2001b; Akar *et al.* 2002). The M cells exhibit lower levels of I_{Ks}, the slow component of the delayed rectifier (Liu & Antzelevitch 1995; Szabo *et al.* 2005), and a greater density of the late Na^+ current (Zygmunt *et al.* 2001), and the Na^+-Ca^{2+} exchange current ($I_{Na/Ca}$) (Zygmunt *et al.* 2000), compared to epicardial and endocardial myocytes. These features contribute to a larger net inward current during the plateau phase, which prolongs APD in M cells.

Distinct electrophysiological characteristics of the M cells play a pivotal role in the mechanism of arrhythmias associated with long QT syndrome (Akar *et al.* 2002; Antzelevitch 2005). In this setting, a reduction of the net repolarizing current causes much greater APD prolongation in M cells than in epicardial myocytes, thus increasing transmural dispersion of repolarization. The excessive APD lengthening markedly increases the propensity to develop EADs in M cells, especially at slow cardiac beating rates. These changes set a stage for intramural re-entry in canine hearts. Nevertheless, the clinical implications of these findings remain a matter of debate, as the presence of M cells within human LV wall was confirmed by some (Drouin *et al.* 1995; Li *et al.* 1998; Glukhov *et al.* 2010), but not other studies (Taggart *et al.* 2001; Conrath *et al.* 2004; Boukens *et al.* 2015).

Transmural heterogeneities, apart from regional variations in APD, also refer to the differences in action potential waveform in cells spanning the ventricular wall. Action potentials from canine epicardial myocytes, in contrast to endocardial cells, have smaller amplitude of phase 0, but a prominent phase 1 notch, and display a clear spike-and-dome configuration (Litovsky & Antzelevitch 1988; Liu *et al.* 1993). This feature is attributed to the much higher expression levels of I_{to}, the transient outward K^+ current, in epicardial myocytes. The M cells show a prominent spike-and-dome morphology of the action potential that is typical of epicardial myocytes.

The data in support of the role played by M cells in transmural repolarization heterogeneities were mostly obtained in studies on muscle strips shaved from the LV wall (Sicouri & Antzelevitch 1991; Drouin *et al.* 1995), or perfused ventricular wedge preparations (Yan *et al.* 1998; Akar *et al.* 2002; Akar & Rosenbaum 2003; Xu *et al.* 2012), or single cardiac myocytes (Liu & Antzelevitch 1995; Li *et al.* 1998; Zygmunt *et al.* 2000, 2001). These findings, however, are challenged by electrophysiological recordings from the intact

heart *in situ*, obtained in anesthetized, open-chest dogs (Anyukhovsky *et al.* 1996; Bauer *et al.* 1999, 2002; Janse *et al.* 2005; Voss *et al.* 2009), and in human patients during cardiac surgery (Taggart *et al.* 2001). These studies strongly suggest that under *in vivo* conditions, transmural repolarization heterogeneities are smoothed, owing to electrotonic current flow between adjacent myocytes. This causes a shortening of APD in M cells, while prolonging APD in epicardial and endocardial myocytes, thus decreasing the transmural gradient. The aforementioned studies are therefore lending support to the notion that transepicardial (e.g. apico-basal or interventricular) APD dispersion provides much greater contribution to the spatial repolarization heterogeneities in the intact heart compared to the transmural gradient (for review see Taggart *et al.* 2003b; Opthof *et al.* 2009; Janse *et al.* 2012), although this remains a matter of debate (Patel *et al.* 2009).

The contribution of M cells into genesis of the transmural repolarization gradient may depend on species-related differences. In contrast to canines, the largest transmural gradient in the heart of smaller animal species like rabbit (Yan *et al.* 2001a; Myles *et al.* 2010), guinea-pig (Bryant *et al.* 1998; Main *et al.* 1998; Wan *et al.* 2003), rat (Shipsey *et al.* 1997), and mouse (Wang *et al.* 2006) has been measured between the endocardium (the longest APD) and epicardium (the shortest APD). Although the M cells were found in guinea-pig ventricular muscle strips (Sicouri *et al.* 1996), subsequent studies on single myocytes dissociated from different transmural layers reported similar APD values in the subendocardial and midmyocardial cells (Main *et al.* 1998), or even longer APD measured at the subendocardium (Bryant *et al.* 1998; Wan *et al.* 2003). Furthermore, Main *et al.* (1998) found no difference in either I_{Kr} or I_{Ks} current density in subendocardial vs. midmyocardial cells from guinea-pig ventricles. In rabbit, similar APD values were determined in endocardium compared to the M region in perfused LV wedge preparations (Yan *et al.* 2001a;

Myles *et al*. 2010), whereas in mice, APD was found to be greater in subendocardial than midmyocardial myocytes (Wang *et al*. 2006). These studies therefore challenge the role played by M cells in shaping the maximal transmural repolarization gradient in small animal species.

4.4 Modifications of the spatial repolarization gradients in cardiac disease

Heart failure is associated with electrical remodeling, the hallmark of which is the prolongation of ventricular APD. This change is accounted for by the downregulation of multiple repolarizing K^+ currents such as I_{to}, I_{Kr}, I_{Ks}, and I_{K1}, as well as the up-regulation of depolarizing currents, such as the late I_{Na}, I_{Ca}, and $I_{Na/Ca}$ (for review see Nabauer & Kaab 1998; Tomaselli & Marban 1999; Sipido *et al.* 2002; Nattel *et al.* 2007; Nass *et al*. 2008; Luo & Anderson 2013). The contributing molecular mechanisms are likely to be multifactorial, and related to impaired transcription, translation, subunit assembly, membrane trafficking, or degradation of ion channel proteins in cardiac cells.

APD prolongation in the diseased heart initially evolves as an adaptive effect aimed at prolonging the plateau phase of action potential, therefore increasing the overall time for Ca^{2+} entry. This allows to compensate for reduced contractility during early stages of cardiac disease (Michael *et al*. 2009). The APD prolongation nevertheless becomes maladaptive in the longer term, because it facilitates arrhythmogenic EADs, especially in the presence of APD prolonging agents, such as class III antiarrhythmic drugs. Furthermore, an increase in APD in diseased hearts tends to be non-uniform, thus contributing to abnormal spatial repolarization gradients. The latter could be categorized as two major electrophysiological phenotypes, including (i) amplified APD dispersion, and (ii) eliminated or even reversed physiological APD gradient.

Amplified spatial APD dispersion is a common finding in both clinical studies and in animal models of volume- and/or pressure-overload-induced cardiac hypertrophy and failure. An increase in apico-basal APD dispersion has been reported to occur in cardiac patients prone to tachyarrhythmia upon programmed electrical stimulation vs. those with no inducible arrhythmia (Chauhan *et al.* 2006). Likewise, a considerable enhancement in LV-to-RV APD dispersion has been observed in a canine model of biventricular hypertrophy induced by chronic AV block (Verduyn *et al.* 1997a, b; Volders *et al.* 1998; van Opstal *et al.* 2001), which is accounted for by a greater APD prolongation at LV compared to RV sites. In this model, the application of a "short-long-short" pacing modality in the presence of an I_{Kr} blocker has been found to induce torsade de pointes in more than 50% of dogs. The increased LV-to-RV dispersion in APD and ERP, and a greater vulnerability to ventricular fibrillation, have been found in a feline aortic-banding model of cardiac hypertrophy (Kowey *et al.* 1991), and in rat RV hypertrophy induced by pulmonary hypertension (Benoist *et al.* 2011). Over the transmural plane, APD dispersion is amplified in cardiac hypertrophy induced by chronic AV block (Kozhevnikov *et al.* 2002), and rapid cardiac pacing (Akar & Rosenbaum 2003; Pajouh *et al.* 2005; Zhou *et al.* 2012) in dogs, and in renovascular hypertension-induced cardiac hypertrophy in rabbit (Yan *et al.* 2001a). A greater APD lengthening in M cells than epicardial myocytes upon I_{Kr} blocker infusion seen in normal hearts, appears to be markedly accentuated in hypertrophied myocardium, thus amplifying transmural dispersion of repolarization and increasing the occurrence of torsades de pointes (Kozhevnikov *et al.* 2002; Milberg *et al.* 2011).

Collectively, the aforementioned studies strongly suggest that amplified spatial gradients in APD, either over the transepicardial or the transmural plane, serve as an important proarrhythmic determinant in cardiac disease.

Nevertheless, another line of evidence points out the role played by eliminated or reversed repolarization gradients in diseased hearts. Spatial repolarization gradients existing in the normal heart can be important for setting an ordered sequence of ventricular activation and relaxation, hence maintaining optimal mechanical function (Sengupta *et al.* 2006; Zhu *et al.* 2009; Odening *et al.* 2013). This implies that elimination, or even reversal, of the physiological APD difference at distinct myocardial regions may lead to impaired contractile performance, and provoke arrhythmia via mechanisms related to mechano-electrical feedback. In connection with this principle, the greater LV than RV APD difference seen in normal human heart (Ramanathan *et al.* 2006; Bueno-Orovio *et al.* 2012), was shown to be reversed in patients with RV hypertrophy associated with pulmonary hypertension (Hardziyenka *et al.* 2009). With regards to transmural heterogeneities in failing human hearts, APD was found to be prolonged to a greater extent in subepicardial than midmyocardial or endocardial myocytes, thus leading to a considerable reduction of APD dispersion across the LV wall (Glukhov *et al.* 2010, 2012). It is also noteworthy that although M-cell islands, the major determinants of transmural gradient, can be frequently found in *non-failing* human ventricles (Glukhov *et al.* 2010; Lou *et al.* 2011), they are very uncommon in LV wedge preparations from *failing* hearts (Yu *et al.* 2015). A reduction in transmural APD dispersion has been observed in various animal models, including pressure overload-induced cardiac hypertrophy in mouse (Wang *et al.* 2006, 2007), rat (Volk *et al.* 2001; McCrossan *et al.* 2004), guinea-pig (Bryant *et al.* 1997), and rabbit (McIntosh *et al.* 1998; Wolk *et al.* 2000), and tachypacing-induced heart failure in dog (Li *et al.* 2002) and pig (Lacroix *et al.* 2002). Both RV-to-LV transepicardial and LV epicardial-to-endocardial APD difference were found to be lost in rodent models of cardiomyopathy induced by chronic adrenergic over-activation (Shipsey *et al.* 1997; Bryant *et al.* 1999; Soltysinska *et al.* 2011).

Shi *et al*. (2013) recently suggested that distinct electrophysiological phenotypes seen in heart failure, that is, either an increase or attenuation with the subsequent reversal of spatial repolarization gradients, may well reflect the temporal evolution of electrical remodeling upon progression of the cardiac disease from early hypertrophic to the late failing stage. In the aortic banded mouse model, both downregulation of outward K^+ currents and the resulting APD prolongation have been shown to evolve in a non-parallel manner in subepicardial and subendocardial myocytes, which leads to a reduction in transmural APD dispersion at the stage of cardiac hypertrophy, followed by a subsequent increase in repolarization gradients whilst developing heart failure.

5. Activation-repolarization coupling

At the whole heart level, the final repolarization time at any recording site represents a sum of the local activation latency and the action potential duration. Therefore, with a given intrinsic difference in APD between the two myocardial regions, a spatial disparity in the final repolarization time could be either amplified or attenuated depending on whether these regions are activated at the same time point, or the activation in one region is postponed relative to the other. High-resolution mapping of ventricular depolarization in human subjects (Franz *et al*. 1987; Cowan *et al*. 1988; Yuan *et al*. 2001; Yue *et al*. 2005; Chauhan *et al*. 2006; Hanson *et al*. 2009; Selvaraj *et al*. 2009; Subramanian *et al*. 2011) and various animal models (dog: Osaka *et al*. 1987; Furukawa *et al*. 2000; rabbit: Costard-Jackle *et al*. 1989; pig: Gepstein *et al*. 1997; Yuan *et al*. 2001; rat: Walton *et al*. 2013) suggests the presence of close relationships between the sequence of electrical activation and the distribution of APD along the propagation path. Under physiological conditions, the impulse propagation from the site of earliest activation is associated with progressive shortening of APD, which is attributed to electrotonic interactions between adjacent cardiac cells. Once the excitation wavefront

spreads over, the upstream myocytes repolarize earlier, and hence exhibit more negative membrane voltage during phase 3 of the action potential, compared to the cells in the surrounding area. For instance, in guinea-pig epicardium, the myocytes along the propagation path exhibit membrane potential values ranging from -60 mV in cells close to the pacing electrode, to -5 mV in remote cells (Laurita *et al.* 1997). This voltage difference generates a local depolarizing circuit current that flows from the downstream to upstream myocytes, and contributes to APD lengthening in the latter (Osaka *et al.* 1987; Laurita *et al.* 1997; Furukawa *et al.* 2000). As a result, during electrical activation, the longest APD is invariably determined in cells at the pacing site, and the APD in more peripheral cells is reduced upon increasing the activation delay. The inverse correlation between local APD and activation time seen in normal hearts is called the *activation-repolarization coupling*.

The presence of the activation-repolarization coupling has been validated by reconstructing the epicardial (Franz *et al.* 1987; Osaka *et al.* 1987; Cowan *et al.* 1988; Costard-Jackle *et al.* 1989; Chauhan *et al.* 2006; Subramanian *et al.* 2011; Walton *et al.* 2013), endocardial (Franz *et al.* 1987; Gepstein *et al.* 1997; Yuan *et al.* 2001; Yue *et al.* 2005; Hanson *et al.* 2009; Selvaraj *et al.* 2009; Subramanian *et al.* 2011), and transmural (Myles *et al.* 2010; Boukens *et al.* 2015) maps of ventricular activation. Quantitatively, it could be assessed by plotting APD_{90} values measured in distinct ventricular recording sites vs. corresponding activation times (Figure 4), and calculating the linear slope value of the APD_{90} to activation time relationship. A negative slope indicates an *inverse* relationship, wherein the intrinsic difference in APD between two myocardial regions is compensated for by setting an appropriate difference in activation time between them. Consequently, the spatial dispersion of final repolarization time tends to be less than the intrinsic difference in APD. In this way, the activation-repolarization coupling allows to minimize the spatial repolarization

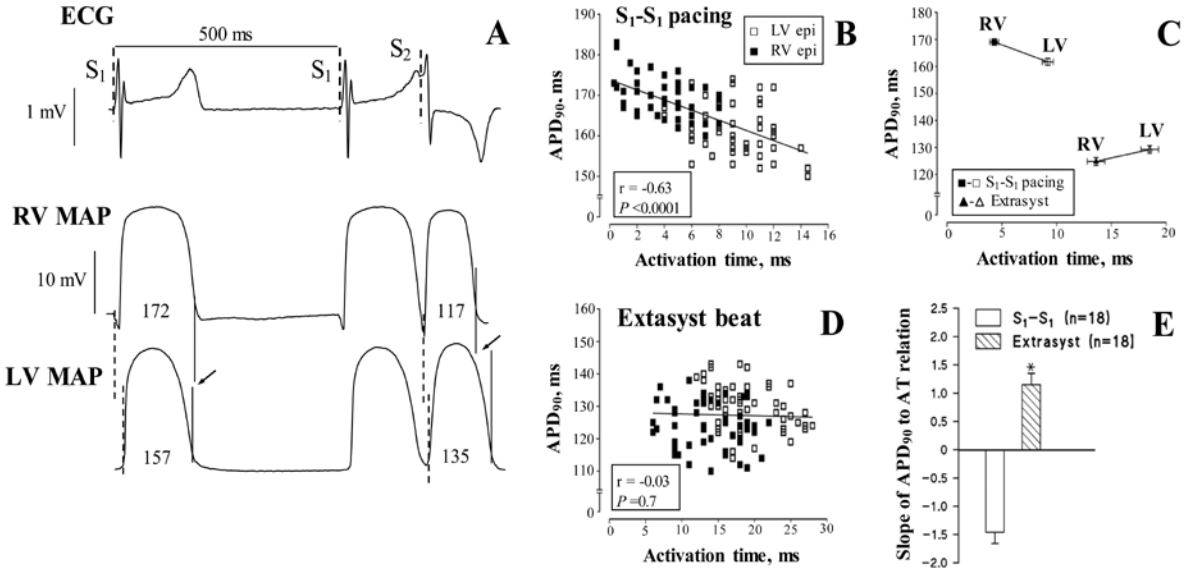

Figure 4. Epicardial action potential duration to the activation time relationships determined upon regular pacing and extrasystolic stimulation in perfused guinea-pig hearts

Panel A shows representative recordings of the volume-conducted ECG (upper trace), right ventricular (RV) monophasic action potential (MAP) (middle trace), and left ventricular (LV) monophasic action potential (lower trace). The dashed lines on ECG show the moments of regular (S_1) and premature (S_2) stimulus application. On the MAP recordings, the dashed lines indicate the beginning of the MAP upstroke, the solid lines indicate 90% repolarization time point (APD_{90}), and the numbers under the trace indicate APD_{90} (in ms) in the RV and the LV chamber. Panels B and D show linear regression relations obtained by plotting individual APD_{90} values vs. corresponding activation times determined at six epicardial recording sites (three sites in each chamber) in 18 perfused heart preparations, during regular pacing (panel B) and upon extrasystolic stimulation (panel D). Panel C shows the mean RV and LV APD_{90} plotted vs. corresponding mean activation times. Panel E shows the slope values of APD_{90} to the activation time relationships during regular pacing and upon extrasystolic stimulation. Adapted from study X (Osadchii, O.E. 2014. Impaired epicardial activation-repolarization coupling contributes to the proarrhythmic effects of hypokalemia and dofetilide in guinea-pig ventricles. *Acta Physiol (Oxf.)* 211, 48-60), with permission from publisher.

heterogeneities, and therefore to eliminate the arrhythmic substrate. In contrast, a positive slope indicates a *direct* APD to activation time relationship, meaning that a ventricular region with delayed activation exhibits a longer APD compared to the region with the earliest

activation. In this situation, the dispersion of final repolarization time originating from the intrinsic APD difference is further accentuated owing to the conduction delay along the activation path. Impaired activation-repolarization coupling may develop as a consequence of electrical remodeling in cardiac disease, as evidenced by electrophysiological assessments in cardiac patients (Cowan *et al.* 1988; Chauhan *et al.* 2006), and in animal model of heart failure (Soltysinska *et al.* 2011).

Overall, these considerations suggest that the proarrhythmic tendency is likely to be increased upon elimination of the inverse correlation between the local APD and activation time values in a given myocardial region. This applies to interventions that either flatten the negative slope of the activation-repolarization coupling, or even reverse its direction (i.e. impose a positive slope value). One proarrhythmic challenge that could be used to illustrate this principle is the closely coupled extrasystolic stimulation. Figure 4 shows the data based on assessments of the activation-repolarization coupling in perfused guinea-pig hearts (Osadchii 2014c: study X), wherein local monophasic APDs and corresponding activation times were measured at multiple epicardial recording sites, both during steady-state pacing and extrasystolic stimulations. The pacing protocols were applied at RV endocardium, the stimulation site that is commonly used in a clinical setting. During regular pacing (the first beat in panel A), the final repolarization times in RV and LV epicardium are synchronized (as highlighted by an arrow), because the site with shorter APD (LV) is activated later than the site with longer APD (RV). This indicates the inverse APD to activation time relationships (panel B and the upper line in panel C). However, a positive RV-to-LV APD_{90} difference (+15 ms) determined upon S_1-pacing (first beat in panel A) is reversed to a negative value (-18 ms) during premature activation (last beat in panel A) owing to greater APD shortening in the RV than the LV epicardium. This contributes to the markedly increased spatial difference

in the final repolarization time (highlighted by arrow), which results from impaired activation-repolarization coupling (panel D and the lower line in panel C). The negative slope of APD to activation time relationship determined during regular pacing is reversed to a positive slope in the extrasystolic beat (panel E). Similar changes have been shown to develop upon extrasystolic stimulation in human patients (Yue *et al.* 2005; Hanson *et al.* 2009; Subramanian *et al.* 2011), which underscores the clinical value of the impaired activation-repolarization coupling as the arrhythmogenic mechanism.

The impaired activation-repolarization coupling may also contribute to abnormal repolarization gradients during steady-state beating, an effect that has been invoked to explain proarrhythmic effects produced by adrenergic agonist infusion (Selvaraj *et al.* 2009), hypokalemia (Osadchii 2014c: study X), and class I and III antiarrhythmic drugs (Osadchii 2012e, 2014a, 2014c: studies VI, VIII and X, respectively). Collectively, these findings raise the point that the slope of APD to activation time relationships may serve as a sensitive metric of arrhythmic susceptibility, and the therapies that improve activation-repolarization coupling are likely to prevent arrhythmia.

6. Electrical restitution

Electrical restitution refers to the shortening of ventricular APD immediately upon a reduction in cardiac cycle length. This change contributes to prolongation of the following diastolic interval, which allows to maintain an appropriate ventricular filling and coronary flow in diastole at fast beating rates. Although the adaptive effects related to APD restitution have been recognized for decades, its implication in arrhythmia research has started to gain a growing attention only during the last years. This led to formulation of the *restitution hypothesis*, which postulates that increased steepness of APD rate adaptation may promote a wave break, and initiate VF, even in the absence of pre-existing structural or electrical

heterogeneities. Flattening the electrical restitution may therefore constitute a novel antiarrhythmic principle (Riccio *et al.* 1999; Garfinkel *et al.* 2000; Omichi *et al.* 2002).

6.1 Electrical restitution slope, repolarization alternans, and ventricular fibrillation

The APD rate adaptation could be assessed by measuring the amount of APD shortening evoked either by a single premature activation, or a sustained increase in cardiac beating rate. These assessments are performed using the *standard* and the *dynamic* cardiac pacing protocols (Figure 5). With the standard protocol, a train of regular (S_1) stimuli is followed by a premature pulse (S_2) applied at progressively reducing coupling stimulation intervals (S_1-S_2) until refractoriness is reached (Figure 5, panel A). With the dynamic protocol, the heart is paced at progressively increasing regular rates until a 2:1 conduction block is induced at pacing intervals shorter than ERP (Figure 5, panel B). The electrical restitution curve obtained from these stimulations shows exponential APD shortening upon reduction of the preceding diastolic interval (Figure 5, panel C). At any segment of the curve, the *slope* of electrical restitution reflects the relative amount of APD shortening, and its maximum value is attained at the shortest diastolic interval (Figure 5, panel D).

According to the restitution hypothesis (for review see Weiss *et al.* 1999, 2005; Qu & Weiss 2006), a steep (greater than 1) APD restitution slope contributes to initiation of VF by promoting repolarization alternans at rapid cardiac activation rates (Figure 6). Repolarization alternans is the regular beat-to-beat oscillations in APD, which create a sequence of pairs of long-short, or short-long, action potentials. The genesis of repolarization alternans is attributed to the reciprocal relations between APD and the following diastolic interval, as originally determined by Nolasco & Dahlen (1968). Once the heart is paced at a constant cycle length, a sudden APD shortening in one beat contributes to prolongation of the following diastolic interval, and therefore, increased APD in the next beat. This, in turn, is

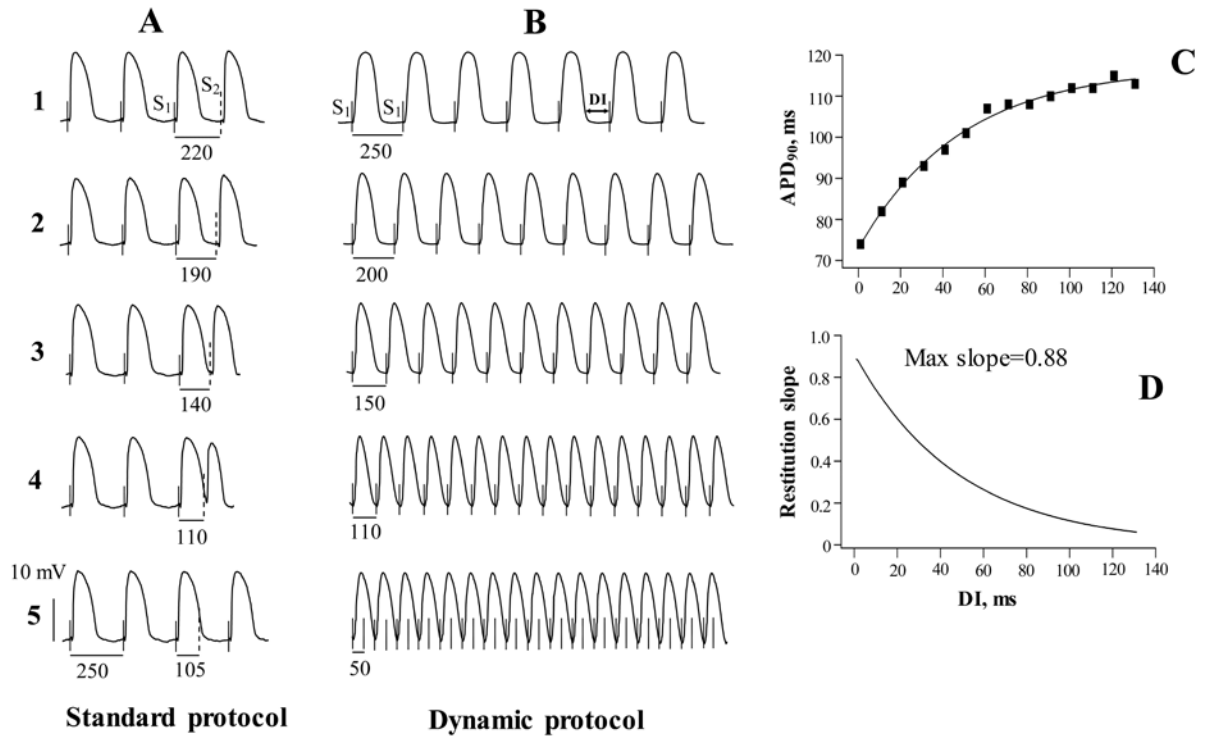

Figure 5. Methodology of electrical restitution assessments in perfused guinea-pig hearts

Panel A and B show representative LV epicardial monophasic action potential recordings obtained upon extrasystolic stimulation (standard protocol) and progressive increase in steady-state pacing rate (dynamic protocol). The vertical dotted lines show the moments of regular stimulus (S_1) application, and the dashed lines show the moments of premature pulse (S_2) application. The numbers below each trace indicate the duration (ms) of S_1-S_2 and S_1-S_1 stimulation intervals. With standard protocol (A), a premature pulse is applied after a train of regular stimuli at progressively reduced coupling stimulation intervals (fragments 1-4) until the effective refractory period is reached (fragment 5). With dynamic protocol (B), the steady-state pacing rate is progressively increased (fragments 1-4) until 2:1 conduction block is induced at S_1-S_1 intervals shorter than the effective refractory period (fragment 5). S_2-evoked APD_{90} values from stimulations shown in panel A were plotted as a function of preceding diastolic interval (DI) in panel C. The restitution plot was fitted by a double-exponential function, and then differentiated in order to assess changes in APD_{90} restitution slope value over the range of diastolic intervals used (panel D).

followed by reduced diastolic interval and hence shortened APD in the following beat, thus initiating repolarization alternans. If the APD restitution slope is greater than 1, then the amplitude of repolarization alternans is incrementally amplified over successive cardiac cycles. As a result, in one of those cycles, the APD could be prolonged to such an extent, that

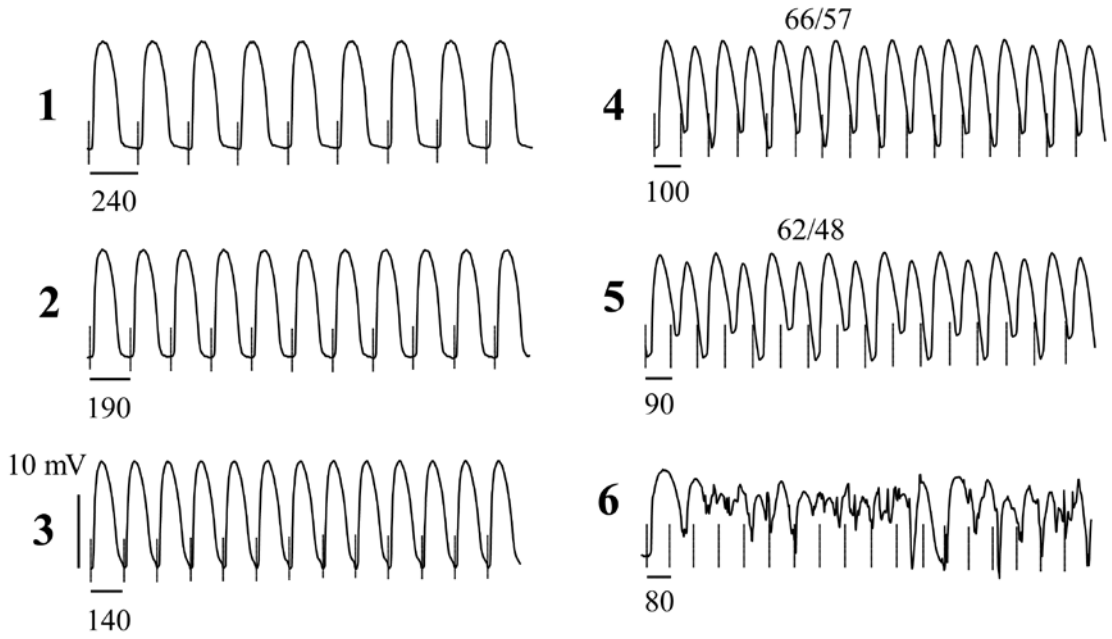

Figure 6. Repolarization alternans and ventricular tachyarrhythmia induced by rapid cardiac pacing in perfused guinea-pig hearts

Panels 1-6 show left ventricular epicardial monophasic action potential recordings obtained upon reducing the S_1-S_1 pacing interval from 240 to 80 ms. The heart preparation was perfused with hypokalemic (2.5 mM K^+) solution in order to increase arrhythmic susceptibility. Note that tachyarrhythmia induction (panel 6) is preceded by alternans in action potential duration (panels 4-5), which amplitude is increasing upon reduction in pacing interval from 100 to 90 ms. The numbers above traces in panels 4-5 indicate the action potential duration at 60% repolarization (APD_{60}, ms) in each pair of long-short monophasic action potentials. Adapted from study I (Osadchii, O.E. *et al.* 2010. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. *Am J Physiol Heart Circ Physiol* 298, H210-H220).

the following diastolic interval is totally eliminated, meaning that the next depolarizing pulse would fall in the refractory period of the preceding beat. This leads to the local conduction block at some point of the excitation wavefront, which precipitates wavefront fragmentation and hence initiates VF. In contrast, low values (<1) of the restitution slope prevent the induction of persistent repolarization alternans, because the initial APD change will be dampened over the following cardiac cycles due to the flat relationships between APD and the preceding diastolic interval.

High resolution optical mapping in perfused guinea-pig hearts revealed a characteristic pattern of developing APD alternans upon progressive increase in pacing rate (Pastore *et al.* 1999). Submaximal heart rate acceleration first promotes *concordant* APD alternans whereby APD in each beat is either simultaneously prolonged or shortened at all epicardial sites. A further increase in pacing rate may promote *discordant* alternans whereby APDs recorded at LV base alternate in a long-short pattern, and APDs recorded at LV apex alternate in a short-long pattern (Pastore *et al.* 1999). When APDs alternate with the opposite phase, spatial dispersion of repolarization is increased up to 13-fold, which facilitates unidirectional conduction block and re-entry.

6.2 Ionic basis for the restitution of action potential duration

Restitution of APD is attributed to the incomplete recovery of ionic currents after previous excitation (for review see Boyett & Jewell 1980; Carmeliet 2004). An electrical pulse applied shortly after previous beat recruits less inward current due to the incomplete recovery of I_{Ca} from inactivation. Furthermore, due to slow time decay of outward K^+ currents, a premature pulse activates more I_{Kr} and I_{Ks}. These effects account for APD abbreviation in extrasystolic beats or upon a sustained increase in cardiac activation rate. The APD restitution also partly relies on recovery of I_{to} (Litovsky & Antzelevitch 1989) and $I_{Na/Ca}$ current (Janvier *et al.* 1997).

In guinea-pig ventricular myocytes, the activation of the delayed rectifier K^+ current was shown to be the most important determinant of APD restitution over a range of short diastolic intervals, corresponding to the initial steep portion of the restitution curve (Zeng *et al.* 1995). The relative APD change depends on interplay between the fast and the slow component of the delayed rectifier, and an increase in I_{Kr}-to-I_{Ks} ratio contributes to a greater maximum APD restitution slope (Zeng *et al.* 1995). Another important determinant of the

maximum slope is availability of the L-type Ca^{2+} current. Elimination of I_{Ca} restitution (i.e., making I_{Ca} recovery from inactivation instantaneous) contributes to a decreased maximal slope of APD restitution (Qu *et al.* 1999; Fox *et al.* 2002), whereas an increase in conductance of the L-type Ca^{2+} channel is associated with steepened restitution and enhanced amplitude of APD alternans (Xie *et al.* 2001; Fox *et al.* 2002). Consistently, Ca^{2+} channel blockers have been found to flatten the restitution slope in Purkinje cells (Colatsky & Hogan 1980; Elharrar *et al.* 1984; Saitoh *et al.* 1988) and ventricular myocytes (Riccio *et al.* 1999; Wu *et al.* 2002; Tolkacheva *et al.* 2006), and suppress APD alternans during rapid cardiac pacing (Saitoh *et al.* 1988; Riccio *et al.* 1999; Gelzer *et al.* 2008).

6.3 Spatial heterogeneities in electrical restitution

With significant heterogeneities in the expression of repolarizing ionic currents (see sections 4.1 to 4.3), it is conceivable that the degree of APD shortening upon rate adaptation is not uniform at distinct ventricular sites. Study I (Osadchii *et al.* 2010) showed that in perfused guinea-pig hearts, spatial heterogeneities in electrical restitution exist both over the RV-to-LV transepicardial plane, and transmurally across the ventricular wall. Monophasic APD during steady-state pacing has been found to be longer at RV compared to LV epicardium. During extrasystolic stimulations, the APD restitution curve obtained from RV epicardium is shifted upwards, and shows a larger maximum-to-minimum APD change, and a greater maximum slope value compared to that from the LV epicardium (Figure 7, panels A, B and C). Over the transmural axis, the maximum slope of APD restitution is greater at the endocardium than the epicardium (Figure 7, panels D, E and F). The same differences were determined when exploring restitution of the effective refractory period (Figure 8).

In rabbit heart, the basal APD is longer, and the slope of APD rate adaptation is steeper in the LV compared to the RV chamber (Qi *et al.* 2015). These dissimilarities are likely

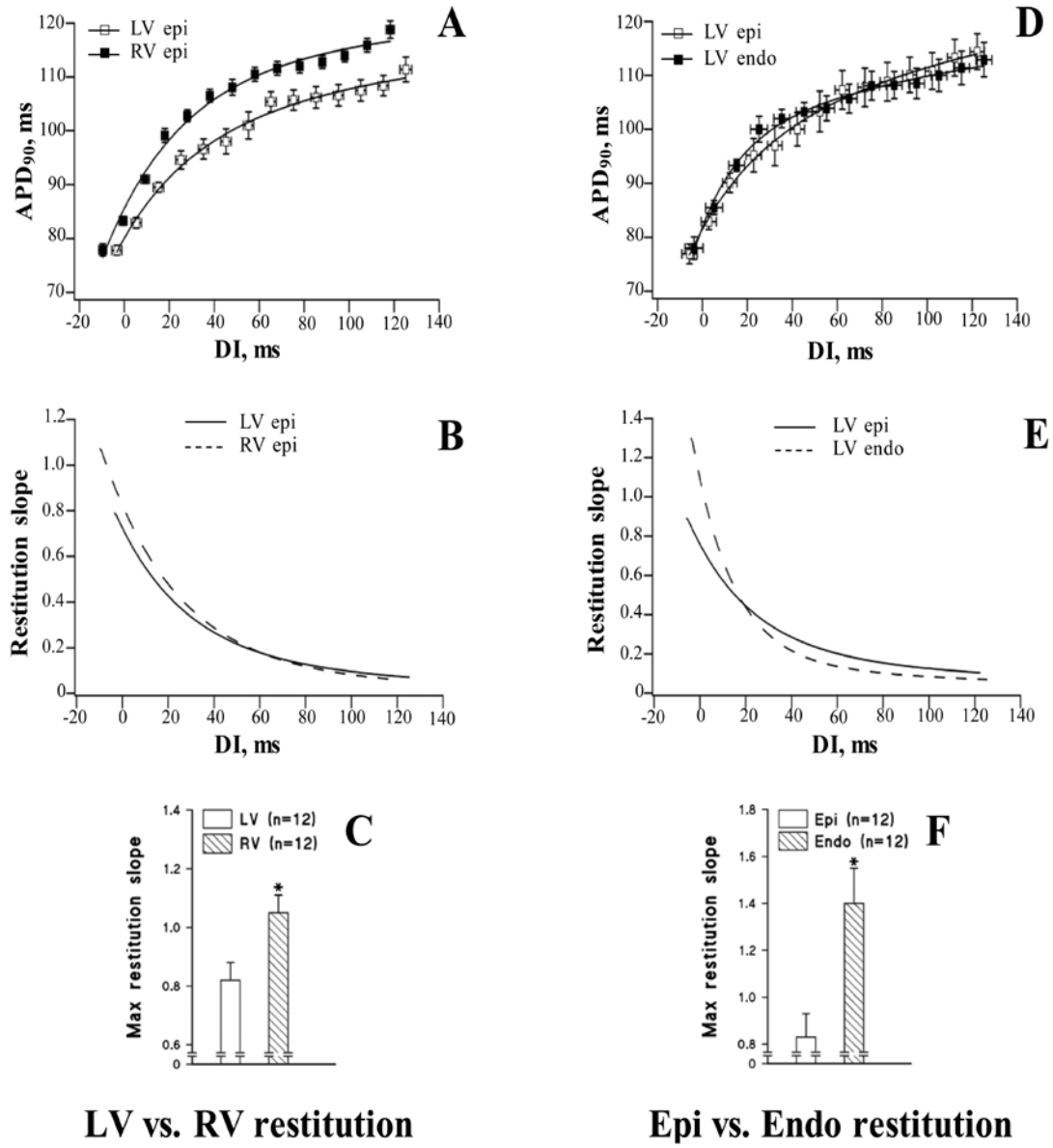

Figure 7. Restitution of monophasic action potential duration (APD_{90}) assessed at distinct ventricular sites upon extrasystolic stimulations applied in perfused guinea-pig hearts

Average APD_{90} values determined at three epicardial recording sites in each ventricular chamber (panel A), or epicardial and endocardial APD_{90} determined at two opposite recording sites across the LV wall (panel D) were plotted as a function of the preceding diastolic interval (DI). Changes in the restitution slope over the range of DIs used (panels B and E) were assessed by differentiating the corresponding restitution curves shown in panels A and D. Mean values of the maximum restitution slope (panels C and F) were calculated from individual measurements obtained in each experiment. *P<0.05 vs. LV epi value (in panels C and F). Adapted from study I (Osadchii, O.E. *et al.* 2010. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. *Am J Physiol Heart Circ Physiol* 298, H210-H220).

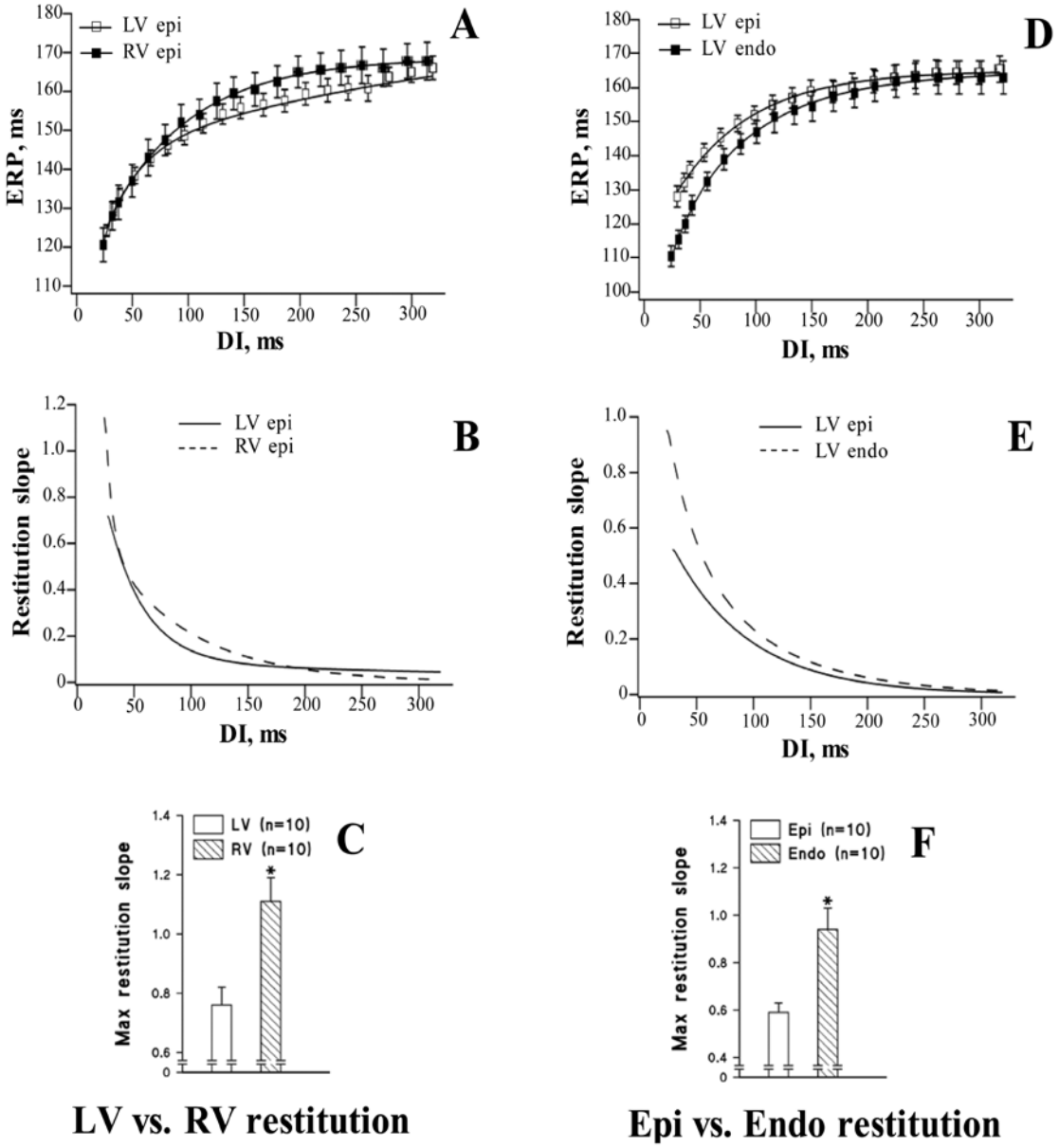

Figure 8. Restitution of the effective refractory period assessed at distinct ventricular sites in perfused guinea-pig hearts

The data shown were obtained in experiments wherein a complete atrioventricular block was induced mechanically in order to allow a stable pacing over a wide range of S_1-S_1 intervals without escaped beats. Effective refractory periods (ERP) were assessed at different S_1-S_1 intervals at the LV epicardium and RV epicardium (panel A), and at the LV epicardium and LV endocardium (panel D), and plotted as a function of the preceding diastolic interval (DI). The details for panels B, C, E and F are the same as indicated in the legend for Figure 7. Adapted from study I (Osadchii, O.E. *et al.* 2010. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. *Am J Physiol Heart Circ Physiol* 298, H210-H220).

attributed to a greater density of the late Na^+ current in LV myocytes. Overall, the findings from both the guinea-pig and rabbit models demonstrate that ventricular sites with longer basal APD exhibit a greater percentage APD shortening, and hence a steeper restitution, upon changes in the cardiac activation rate, compared to the sites with shorter basal APDs, as originally proposed by Laurita *et al.* (1996).

In human heart, the presence of spatial heterogeneities in electrical restitution was validated by using multielectrode ventricular mapping (Yue *et al.* 2005; Nash *et al.* 2006). On a global scale, the APD restitution slopes were found to be distributed non-uniformly throughout both the epicardial and endocardial surface, with 25-to-45% of recording sites showing slope values greater than 1. The maximum restitution slope was shown to be greater in the LV than the RV chamber, with no difference determined between the apex and the base. Spatial dispersion in APD restitution slope value is markedly increased in cardiac patients compared to controls (Pak *et al.* 2004).

6.4 Modifications of electrical restitution in cardiac disease

Although vulnerability to VF is increased in diseased hearts, it is uncertain as to whether this change could be accounted for by steepened electrical restitution. Morgan *et al.* (1992) have shown that the maximal shortening of ventricular APD upon extrasystolic stimulation is greatly exaggerated in patients with structural heart disease compared to control subjects, indicating an increased steepness of electrical restitution in the former. The electrical restitution slope appears to be steeper in a subgroup of high-risk patients susceptible to VT upon programmed stimulation compared to low-risk patients (Selvaraj *et al.* 2007). Cardiac patients also exhibit a reduced threshold for APD alternans induction by rapid pacing, a greater amplitude of APD alternans, and a wider range of diastolic intervals over which the alternans is present (Koller *et al.* 2005; Bayer *et al.* 2010). In contrast, transmural APD

recordings in perfused human ventricular wedge preparations demonstrate that electrical restitution slope is *flattened* in failing vs. non-failing hearts (Glukhov *et al.* 2010). Narayan *et al.* (2007) have shown that the maximum restitution slope is similar in heart failure patients and control subjects, and in the study-group, the slope values were the same in patients with and without inducible arrhythmia. Finally, neither steepness of electrical restitution slopes nor the width of the range of diastolic intervals over which the slope value is greater than 1 were found to have prognostic value regarding long-term survival in cardiac patients (Narayan *et al.* 2007; Dorenkamp *et al*. 2013).

These contrasting observations could be partly attributed to a variety of factors that may potentially affect the APD rate adaptation in cardiac disease. Downregulation of repolarizing K^+ currents in the diseased heart is likely to contribute to the increased steepness of electrical restitution. Indeed, in a canine model, the slope of APD rate adaptation is significantly increased upon I_{Kr} blocker administration (Yamauchi *et al.* 2002). The same effect is produced by sympathetic activation; catecholamine infusion in human patients (Taggart *et al.* 2003a) as well as sympathetic nerve stimulation in perfused rabbit hearts (Ng *et al.* 2007) have been shown to considerably increase the maximum restitution slope, and facilitate occurrence of APD alternans. Steepening of the electrical restitution in the diseased heart can also be caused by increased LV loading and myocardial stretch (Horner *et al.* 1996). In contrast, the electrical restitution curve is flattened by myocardial ischemia (Dilly & Lab 1988; Lukas & Antzelevitch 1993; Kurz *et al.* 1994; Taggart *et al.* 1996), and some medications commonly used in heart failure, such as beta-blockers (Pak *et al.* 2003; Hao *et al.* 2003), class I antiarrhythmics (Varro *et al.* 1985, 1987), and amiodarone (Omichi *et al.* 2002). Downregulation of L-type Ca^{2+} channels, which is often observed in advanced heart failure (Horiuchi-Hirose *et al.* 2011), contributes to the reduced APD restitution slope (Qu *et al.*

1999; Xie *et al.* 2001; Fox *et al.* 2002). It is therefore plausible to assume that when the aforementioned factors are acting in combination, their contrasting effects may translate to variable profiles of APD rate adaptation in cardiac patients, ranging from steepening to no change or even flattening the restitution slope, depending on which factors prevail.

6.5 The relative value of standard and dynamic pacing protocols in electrical restitution assessments

In cardiac patients, the electrical restitution is invariably assessed by extrasystolic stimulations (Taggart *et al.* 1996, 2003a; Nash *et al.* 2006; Narayan *et al.* 2007; Selvaraj *et al.* 2007), because dynamic pacing at rapid rates may increase myocardial oxygen demands and precipitate acute ischemia. These assessments largely rely on the assumption that when an increased APD restitution slope is seen with extrasystolic stimulations, the same outcome would have been obtained upon implementing the dynamic pacing. The latter, however, disagrees with studies (Goldhaber *et al.* 2005) which suggest that cellular electrophysiological changes invoked with these pacing protocols are not the same. A sustained increase in cardiac activation rate, as replicated with the dynamic protocol, is associated with significantly increased cytosolic Ca^{2+} levels, an effect that may abbreviate APD via either increased Ca^{2+}-dependent outward K^+ currents, or inactivation of the L-type Ca^{2+} current, or both (for review see Eisner 2014). These effects are of much less relevance to the standard protocol, whereby a reduction in cardiac cycle is imposed in just a single beat. Hence it is reasonable to expect that electrical restitution kinetics may show dissimilar patterns when assessed by dynamic pacing compared to extrasystolic stimulations. In canine endocardial slices and isolated Purkinje fibers, a profibrillatory tendency was found to be well-predicted by dynamic APD restitution curve because it shows a steep (>1) maximum slope, whereas standard extrasystolic protocol was much less informative as it elicited flat

APD restitution (Koller *et al.* 1998; Riccio *et al.* 1999). Nevertheless, it may be questioned as to whether the same relations exist at the whole heart level wherein electrical restitution is influenced by a variety of physiological factors including spatial repolarization gradients, electrotonic effects, and mechanoelectrical feedback.

These issues were addressed in study III (Osadchii 2012b). Figure 9 (panel A) shows that in the perfused guinea-pig heart, APD measured at any given point over a range of diastolic intervals less than 150 ms is shorter during dynamic pacing compared to extrasystolic stimulation. The maximum slope of APD restitution is steeper when assessed by extrasystolic stimulation than dynamic pacing (Figure 9, panels B and C). This difference was related to the impact of the previous pacing history (to be discussed in section 6.6.2), and to the ability to preserve LV capture at shorter minimum diastolic intervals during extrasystolic stimulation. An increase in the ERP-to-APD_{90} ratio upon administration of flecainide, a Na^+ channel blocker, was found to flatten the electrical restitution curve obtained by extrasystolic stimulation, while producing no change in the dynamic APD restitution. The two stimulation protocols also had a dissimilar value in predicting hypokalemia-induced arrhythmogenicity, wherein a reduction in the VF threshold was associated with the increased steepness of the dynamic restitution curve, while no change in the maximum restitution slope was revealed by extrasystolic stimulations.

Taken together, these findings demonstrate that changes in APD restitution revealed by extrasystolic stimulation may dissociate from those obtained upon dynamic pacing. Importantly, flattening of the standard restitution curve may not necessarily indicate a reduced arrhythmogenic tendency. For instance, a decrease in the maximum APD restitution slope during extrasystolic stimulation has been observed in acute myocardial ischemia (Dilly & Lab 1988; Lukas & Antzelevitch 1993; Kurz *et al.* 1994; Taggart *et al.* 1996), and in a heart

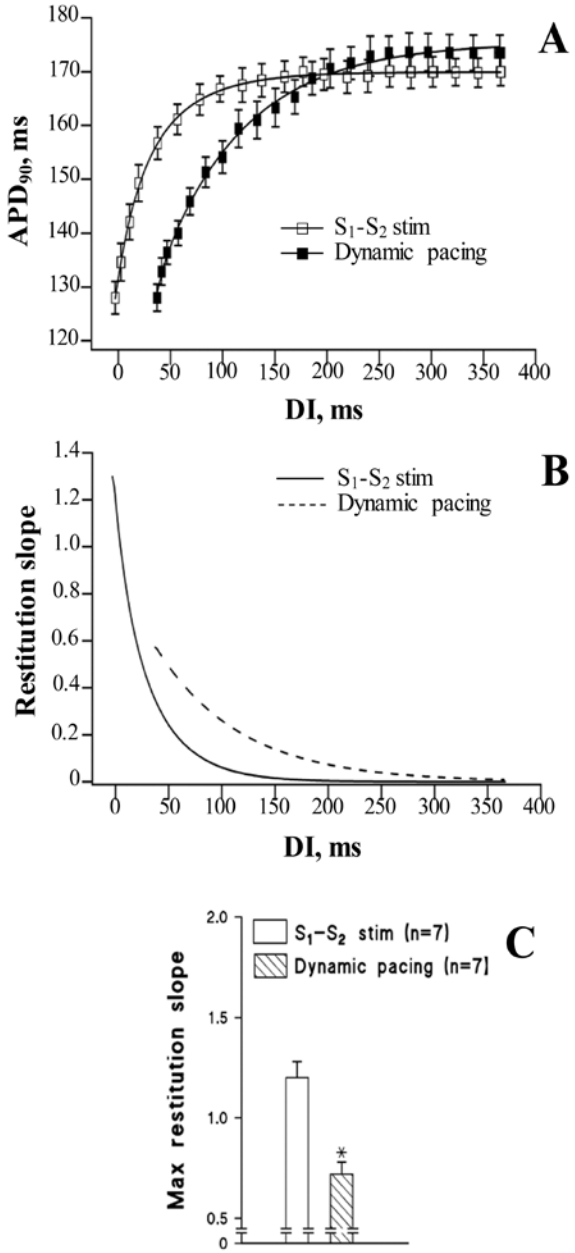

Figure 9. Impact of the pacing protocol on electrical restitution assessments in perfused guinea-pig hearts

Epicardial monophasic action potential duration at 90% repolarization (APD_{90}) was determined over a wide range of diastolic intervals (DI) using extrasystolic stimulations (S_1-S_2 stim; basic drive cycle length of 550 ms) and dynamic pacing. The details related to panels B and C are the same as indicated in the legend for Figure 7. Adapted from study III (Osadchii, O.E. 2012. Effects of ventricular pacing protocol on electrical restitution assessments in guinea-pig heart. *Exp Physiol* 97, 807-821), with permission from publisher.

failure model induced by chronic adrenergic over-activation (Soltysinska *et al.* 2011), despite a marked increase in arrhythmic susceptibility in both settings. These findings therefore challenge the value of electrical restitution assessments by extrasystolic stimulation alone, which is commonly used to evaluate the proarrhythmic risk in cardiac patients.

6.6 Other determinants of the dynamic instability

Apart from a steep slope of APD rate adaptation, instability of the spiral wave can be caused by other factors, including conduction velocity restitution, cardiac memory, electrotonic currents, and abnormal Ca^{2+} cycling (Weiss *et al.* 2005; Qu & Weiss 2015).

6.6.1 Conduction velocity restitution

Conduction velocity restitution is attributed to incomplete recovery from inactivation of the fast Na^{+} channels when a premature pulse is applied during phase 3 repolarization. This accounts for reduced V_{max}, and hence slowed conduction in extrasystolic beats. Because the excitation wavelength is a product of APD and conduction velocity, the wavelength oscillations leading to the wave break could be produced by steepening conduction velocity restitution, an effect that may develop independently of APD restitution kinetics (Qu *et al.* 1999; Cherry & Fenton 2004; Mironov *et al.* 2008). In particular, when spontaneous wave break is prevented by flattening APD restitution, an increase in the slope of the conduction velocity restitution may restore the wavefront fragmentation (Wu *et al.* 2002). Therefore, it appears that VF could be promoted both by instability of the waveback, i.e. repolarization phase (promoted by steep APD restitution) and instability of the wavefront, i.e. action potential upstroke (promoted by steep conduction velocity restitution).

These relations play a role in shaping the electrophysiological phenotype of VF. Depending on the frequency of electrical activation, VF could be categorized as *fast* and *slow* (Wu *et al.* 2002; Chen *et al.* 2003). The fast (or type I) VF is associated with a steep APD

restitution, normal ventricular excitability, and a flat restitution of conduction velocity. In contrast, the slow (or type II) VF is associated with flat APD restitution, depressed tissue excitability, and steep restitution of conduction velocity. The fast VF could be converted to slow VF by reducing tissue excitability, e.g. upon infusion of the I_{Na} blocker. In ischemic hearts, both types of VF may co-exist (Liu *et al.* 2004), suggesting that steep APD restitution and depressed tissue excitability can act synergistically in promoting wavebreaks.

6.6.2 Short-term cardiac memory

During changes in the cardiac activation rate, APD alterations may not necessarily be solely determined by the preceding diastolic interval, as prescribed by the restitution hypothesis. APD restitution also depends on the past pacing history, an effect called *short-term cardiac memory* (Gilmour *et al.* 1997; Watanabe & Koller 2002; Choi *et al.* 2004; Wu & Patwardhan 2004). Specifically, the degree of APD shortening in extrasystolic beat is influenced by APD in the preceding regular beat – for a given diastolic interval, extrasystolic APD is abbreviated to a lesser extent if it is generated after a long APD, and vice versa. These relations can be illustrated by assessments of electrical restitution with extrasystolic stimulations applied at two different S_1-S_1 intervals (550 vs. 300 ms) in a train of regular pulses (Figure 10). At a given S_1-S_2 coupling interval (panel A: S_1-S_2=400 ms; panel B: S_1-S_2=260 ms), APD in the extrasystolic beat is longer (panel A: 167 ms vs. 146 ms; panel B: 160 ms vs. 137 ms) when S_2 is applied after a train of long S_1-evoked action potentials (S_1-S_1=550 ms) compared to a train of short APDs (S_1-S_1=300 ms).

In a graphical form, the impact of past stimulation history is illustrated in Figure 11 (panel A), which shows that the plateau portion of the standard restitution curve is progressively shifted downwards following a reduction in pacing interval in a train of S_1 pulses (Osadchii 2012b: study III). This change contributes to the reduced maximum

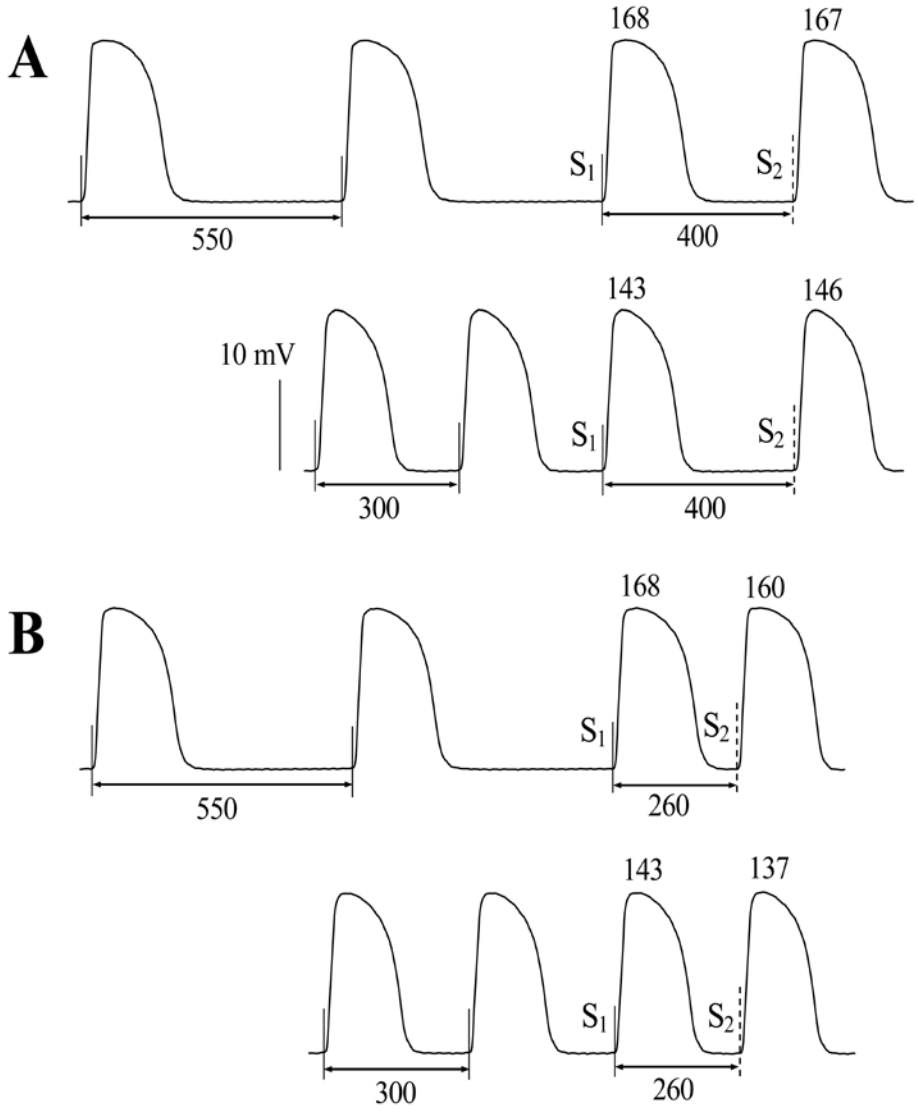

Figure 10. Extrasystolic stimulation protocol applied at variable basic drive cycle lengths in perfused guinea-pig hearts

Left ventricular epicardial monophasic action potentials were recorded upon extrasystolic (S_2) stimulations applied after a train of regular (S_1 pulses). The S_1-S_1 interval in a basic drive train varied from 550 ms (upper fragment in each panel) to 300 ms (lower fragment in each panel). The numbers (ms) above recordings indicate action potential duration at 90% repolarization (APD_{90}) in regular and extrasystolic beats. Note that APD_{90} in regular beats is longer while pacing at S_1-S_1 cycle length of 550 ms as compared to 300 ms. Also note that at comparable S_1-S_2 coupling stimulation intervals (panel A: 400 ms; panel B: 260 ms), APD_{90} in extrasystolic beat is greater when S_2 is applied after a train of pulses with S_1-S_1=550 ms, as compared to stimulations at S_1-S_1=300 ms. Adapted from Osadchii, O.E. 2016. Flecainide attenuates rate adaptation of ventricular repolarization in guinea-pig heart. *Scand Cardiovasc J* 50, 28-35, with permission from Taylor & Francis Ltd (www.tandfonline.com).

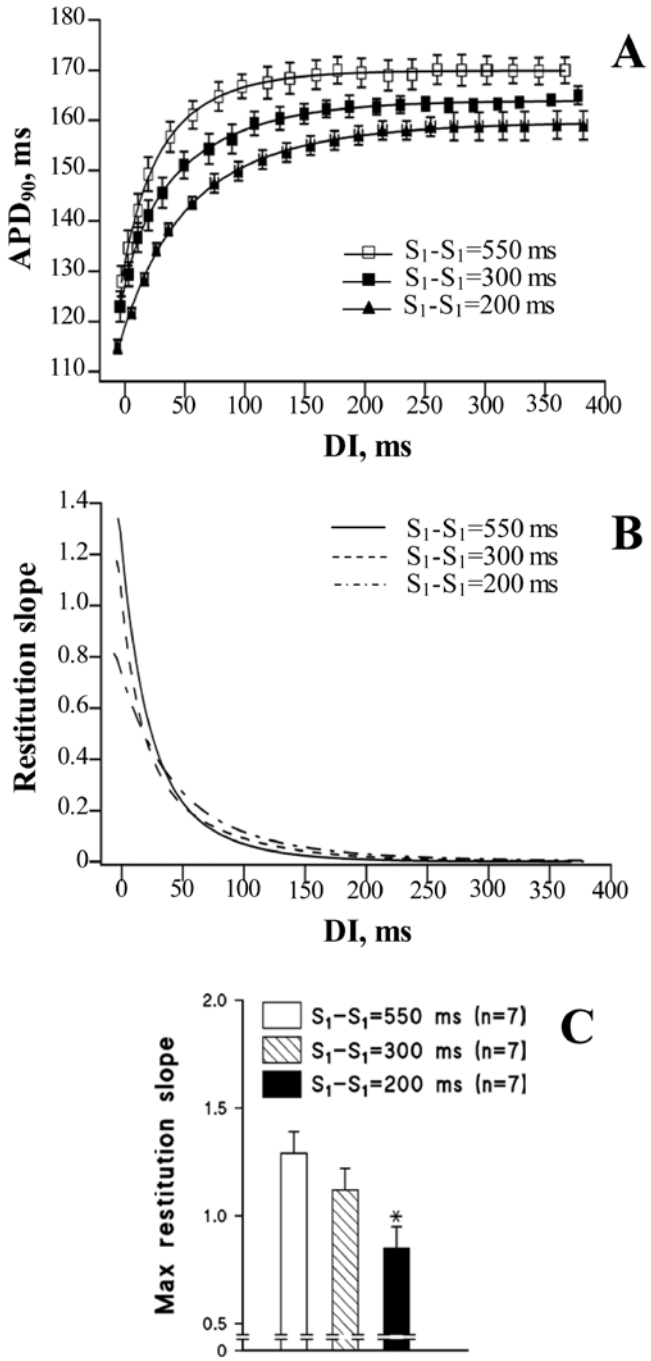

Figure 11. Electrical restitution plots obtained when using variable basic drive cycle lengths during extrasystolic stimulations in perfused guinea-pig hearts

Restitution of monophasic action potential duration (APD_{90}) was assessed at the LV epicardium by using the standard pacing protocol applied at basic drive stimulation intervals of 550 ms, 300 ms and 200 ms. In panel C, **P*<0.05 vs slope value measured at S_1-S_1=550 ms. Adapted from study III (Osadchii, O.E. 2012. Effects of ventricular pacing protocol on electrical restitution assessments in guinea-pig heart. *Exp Physiol* 97, 807-821), with permission from publisher.

restitution slope (Figure 11, panels B and C). Overall, these measurements suggest that apart from the previous diastolic interval, APD change upon sudden reduction in the activation cycle length is determined by other physiological factors.

The mechanisms involved in short-term cardiac memory are thought to be related to slowly developing alterations in ionic concentrations on both sides of the myocyte membrane, which result from changes in the cardiac activation rate (for review see Boyett & Jewell 1980; Carmeliet 2004; Eisner *et al.* 2009). Fast activation rates are associated with K^+ accumulation in extracellular clefts, which may accentuate APD shortening in the extrasystolic beat owing to the increased conductance of I_{Kr} and I_{K1} channels (Scamps & Carmeliet 1989; Sanguinetti & Jurkiewicz 1992). Rapid S_1-S_1 pacing also increases intracellular Na^+ levels, which stimulate the reversed mode of the Na^+-Ca^{2+} exchange and Na^+-K^+ pump, thus contributing to enhanced APD shortening in the S_2-beat. The same effect could be produced owing to faster inactivation of I_{Ca} in response to elevated intracellular Ca^{2+} concentrations in rapidly paced LV (Goldhaber *et al.* 2005; Eisner 2014). In summary, it appears that these ionic alterations contribute to a greater net repolarizing current across the cardiac myocyte sarcolemma, which explains why, for a given diastolic interval, the APD shortening in the premature beat is greater when it is preceded by a train of pulses with short compared to long S_1-S_1 intervals.

Memory effects have important implications in cardiac arrhythmia. VF can be precipitated by excessive APD shortening upon transient cardiac acceleration, because a segment of the wavefront with critically short APD carries insufficient current strength to depolarize downstream cardiac cells (Kim *et al.* 1999; Harada *et al.* 2011). This translates to the source-sink mismatch, which causes local conduction block and leads to wave fragmentation. From this perspective, preventing an excessive APD shortening via short-term memory can contribute to the antiarrhythmic effect. Nevertheless, the short-term memory may

also have proarrhythmic consequences. Memory effects contribute to protracted QT interval adaptation to sudden changes in heart rate, for example those associated with the onset or termination of atrial fibrillation (Grom *et al.* 2005; Pueyo *et al.* 2010; Bueno-Orovio *et al.* 2012). This may result in a "too long" or "too short" QT interval value for a given heart rate, which would transiently increase arrhythmic susceptibility by facilitating EADs and increasing spatial repolarization gradients.

6.6.3 Electrotonic effects

In contrast to isolated myocytes, wherein APD restitution is primarily determined by the recovery of repolarizing ionic currents, in a coupled cardiac tissue, APD restitution is also influenced by the axial current flow from surrounding cells. This can account for variations in shape of the APD restitution curve at a given recording site depending on its proximity to the pacing electrode. When the pacing site is remote, APD exhibits exponential shortening upon progressive reduction of preceding diastolic interval (Laurita *et al.* 1997). However, when the pacing site is close (within 3 mm) to the recording site, the APD change at short diastolic intervals deviates from the expected exponential course ("atypical restitution"), thus causing the maximum restitution slope to flatten (Laurita *et al.* 1997). Because the axial current flow is proportional to the membrane voltage difference in coupled myocytes, the most pronounced changes of APD restitution kinetics are likely to occur in cells located close to the steep repolarization gradients imposed by ectopic activation.

6.6.4 Abnormal Ca^{2+} cycling

APD alternans, in addition to being linked to the steep slope of electrical restitution, could also result from abnormal Ca^{2+} cycling (for review see Clusin 2008; Laurita & Rosenbaum 2008; Qu *et al.* 2010; Merchant & Armoundas 2012; Qu & Weiss 2015). Rapid cardiac activation rates are associated with increased systolic Ca^{2+} fluxes that may perturb the

activity of Ca^{2+}-dependent sarcolemmal ion channels and exchangers, and impair SR Ca^{2+} handling via effects on SERCA and Ca^{2+}/Calmodulin-dependent kinase II. These changes may cause beat-to-beat oscillations in cytosolic Ca^{2+} levels, i.e. *Ca^{2+} transient alternans*, which then promotes beat-to-beat APD changes via either positive or negative coupling (for review see Weiss *et al*. 2005). A large Ca^{2+} transient in a given beat may cause APD to prolong (*positive coupling*) by activating the forward mode of Na^{+}-Ca^{2+} exchange, or may cause APD to shorten (*negative coupling*) by inducing the Ca^{2+}-dependent inactivation of I_{Ca}. It is noteworthy that the membrane voltage to intracellular Ca^{2+} relations are bidirectional – the APD prolongation in a current cardiac cycle contributes to increased time for Ca^{2+} entry, which primes SR Ca^{2+} stores, and thus ensures a large Ca^{2+} transient in the following beat (Pruvot *et al.* 2004).

7. TRIaD concept

7.1 The relevance of TRIaD as a surrogate marker of arrhythmic liability

In cardiac patients, life-threatening VT, such as torsade de pointes, can be promoted by drugs that possess I_{Kr} blocking effects and hence prolong ventricular repolarization. Therefore, at a preclinical stage of drug development, the potential torsadogenic risks are commonly assessed by using I_{Kr} channel binding assays and detecting QT interval prolongation in animal models. Nevertheless, clinically, not all drugs that prolong the QT interval can cause arrhythmia, and the absence of QT interval lengthening does not necessarily indicate a good safety profile (for review see Shah & Hondeghem 2005; Hondeghem 2008). This indicates that assessments based solely on QT interval measurements are likely to reject some safe agents and overlook those that are proarrhyrhmic.

Hondeghem *et al.* (2001a) proposed that arrhythmogenic risks should be evaluated based on more refined criteria that include action potential triangulation (T), reverse use

dependence (R), instability (I), and dispersion (D). Taken together, these modifications form the TRIaD approach for an integrated assessment of torsadogenic tendency (Hondeghem & Hoffmann 2003; Hondeghem *et al.* 2003; Shah & Hondeghem 2005). APD (and QT interval) prolongation in the absence of TRIaD is antiarrhythmic; in contrast, agents inducing TRIaD are likely to promote arrhythmia, regardless of whether they prolong the QT interval or not. The associated change in QT interval, however, may determine the phenotype of drug-induced arrhythmia – TRIaD in association with *prolonged* QT typically leads to torsade de pointes, whereas TRIaD in association with *shortened* QT is most likely to promote ventricular fibrillation.

7.2 Action potential triangulation

APD prolongation could be produced in different ways. It may involve lengthening of the plateau, without any change in phase 3, which would contribute to the rectangular action potential waveform (Figure 12). This modification is thought to be antiarrhythmic (Hondeghem *et al.* 2001a, b), because it contributes to prolonged ERP and hence increased excitation wavelength, changes that eliminate a substrate for re-entry. Alternatively, APD could be prolonged by slowing phase 3, without any change in plateau, which would render the action potential more triangular (Figure 12). This modification is proarrhythmic, because it contributes to the increased difference between the total APD and the end of ERP, thus widening the time window over which the left ventricle could be re-excited in final repolarization. Furthermore, slowing phase 3 contributes to increased time spent over the voltage window for I_{Ca} reactivation, which increases the propensity for arrhythmogenic EADs (Lu *et al.* 2002; Guo *et al.* 2007; Guerard *et al.* 2008).

It is noteworthy that triangulation should be regarded as characteristics of the action potential configuration rather than an attribute of prolonged APD. Indeed, although

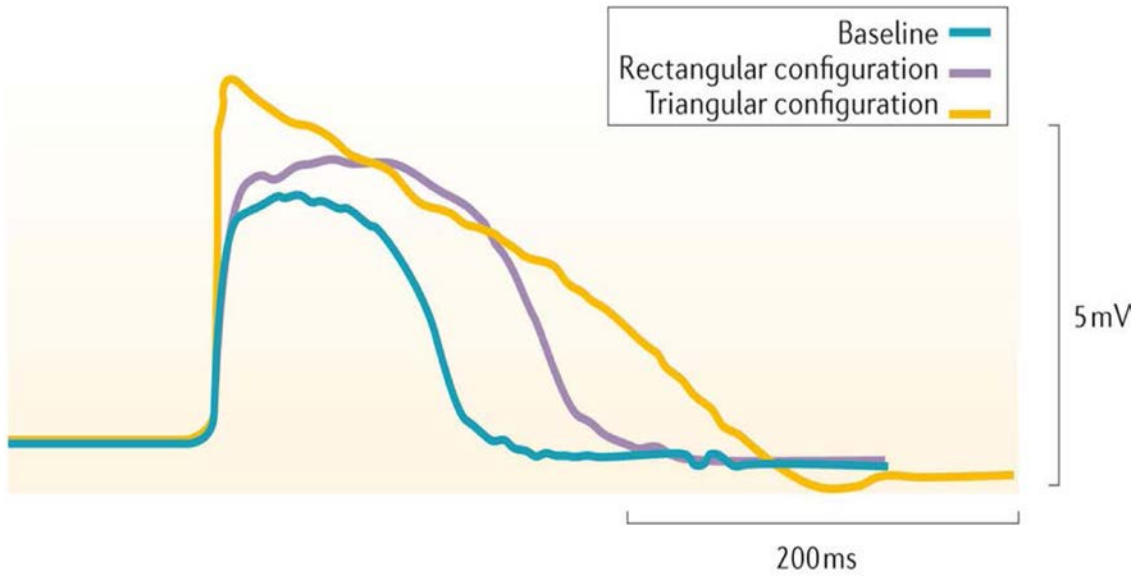

Figure 12. Antiarrhythmic and proarrhythmic modifications of the ventricular action potential waveform upon drug administration

Representative monophasic action potential recordings were taken from perfused rabbit heart preparations at baseline (blue) and following administration of sotalol (yellow) and amiodarone (violet). A triangular action potential configuration is associated with proarrhythmia, whereas a rectangular configuration contributes to antiarrhythmic effect. Adapted from Frommeyer, G. & Eckardt, L. 2016. Drug-induced proarrhythmia: risk factors and electrophysiological mechanisms. *Nat Rev Cardiol* 13, 36-47, with permission from publisher.

triangulation is often associated with increased APD, it could also be induced by drugs that produce no change, or even shorten APD. The extent of action potential triangulation can be assessed by calculating the difference between APD measured at 30% and 90% repolarization time points; the larger APD_{90} to APD_{30} difference, the greater the triangulation.

Increased action potential triangulation has been found to occur upon the administration of agents with proarrhythmic profiles of action, such as I_{Kr} blockers (Hondeghem *et al.* 2001a, b; Lu *et al.* 2002; Frommeyer *et al.* 2011; Milberg *et al.* 2004, 2012a), activators of the late Na^+ current (Lu *et al.* 2002), and torsadogenic non-cardiovascular drugs like quinolone or

macrolide antibiotics (Milberg *et al.* 2002, 2007b). In contrast, a rapid phase 3 repolarization is preserved upon the infusion of safe antiarrhythmics like amiodarone and dronedarone (Frommeyer *et al.* 2011; Milberg *et al.* 2004, 2012a).

7.3 Reverse use dependence

A safe antiarrhythmic drug is expected to significantly prolong APD, and hence refractory period, at *fast* beating rates, while producing no effect on repolarization in normal or slowed heart rates. In contrast, the reverse use dependency refers to the preferential APD lengthening at *slow* heart rates, with a minimum effect produced in tachycardia. Most clinically used class III antiarrhythmic agents increase APD in a reverse use-dependent manner, which limits their therapeutic efficacy in tachycardia, and may facilitate arrhythmogenic EADs upon sudden prolongation of the cardiac cycle, for example, during post-extrasystolic pause, or upon conversion of atrial fibrillation to sinus rhythm (for review see Hondeghem & Snyders 1990; Dorian & Newman 2000).

The reverse use dependency could not be ascribed to changes in drug affinity to the I_{Kr} channel upon variations in cardiac pacing rate. In guinea-pig (Jurkiewicz & Sanguinetti 1993) and rabbit (Carmeliet 1992) ventricular myocytes, the sensitivity of I_{Kr} to the inhibiting effects of dofetilide, a selective I_{Kr} blocker, was found to be rate-independent. Instead, the attenuated APD response to class III antiarrhythmic agents in tachycardia could be attributed to K^+ accumulation in extracellular clefts, which may reduce the drug potency at inhibiting I_{Kr} (Yang & Roden 1996). Alternatively, the reverse rate-dependency could be explained by rapid pacing-induced accumulation of I_{Ks}, the slow component of the delayed rectifier, an effect accounted for by incomplete deactivation of I_{Ks} at short inter-pulse intervals (Jurkiewicz & Sanguinetti 1993; Lu *et al.* 2001). The increased magnitude of I_{Ks} then may partially offset drug-induced APD prolongation resulting from I_{Kr} block. The putative contribution of I_{Ks} to

the reverse use-dependency, however, is likely to be variable, being of much higher physiological relevance in animal species with slow I_{Ks} deactivation kinetics, such as guinea-pig (Jurkiewicz & Sanguinetti 1993; Lu *et al.* 2001) compared to those (e.g. canine, human, rabbit) that show a rapid I_{Ks} deactivation (Gintant 1996; Stengl *et al.* 2003), or exhibit only low (relative to I_{Kr}) I_{Ks} expression in cardiac myocytes (e.g. rabbit) (Lu *et al.* 2001).

Other factors contributing to the reverse use dependency include the rate-dependent variations in inward currents, such as I_{Ca}, $I_{Na/Ca}$, and late I_{Na} (Bril *et al.* 1998; Gjini *et al.* 1998; Wu *et al.* 2011), and the positive feedback relations between the I_{Kr} or I_{K1} currents and the rate of phase 3 repolarization (Virag *et al.* 2009). There is also the opinion that reverse use-dependency represents an intrinsic property of cardiac cells (Banyasz *et al.* 2009; Barandi *et al.* 2010), meaning that drug-induced APD change, either an increase or reduction, is always greater in the presence of longer basal APD value, independently of the type of ion channel pharmacologically blocked or activated.

7.4 Temporal instability of action potential duration

In cardiac patients, a spontaneous episode of VT is typically preceded by a short-long-short sequence of cardiac cycles and associated beat-to-beat variations in QT interval (Kay *et al.* 1983; Lewis *et al.* 1983; Viskin *et al.* 1996). The temporal instability of repolarization is therefore recognized as an important marker of proarrhythmia. The drugs that increase APD instability, as evidenced by significant variations in APD in successive beats, are usually highly arrhythmic (for review see Varkevisser *et al.* 2012). The APD instability, also called short-term APD variability, can be quantified by constructing a Poincare plot wherein APD in each following beat is plotted vs. APD in the preceding beat. In the absence of instability, all APD pairs are identical and hence project to a single point. In contrast, if the APD in successive beats is highly variable, the plot represents a cluster of points which markedly

deviate from the diagonal line, and form complex polygons when connected. Random beat-to-beat variations in APD could be detected at different levels, i.e. from recording transmembrane action potentials in single cardiac myocytes to assessments of repolarization in the whole heart or measuring QT intervals on the body surface ECG. The contributing mechanism is thought to be related to stochastic fluctuations in ionic currents that govern ventricular repolarization (Zaniboni *et al.* 2000; Wu *et al.* 2008, 2011).

Repolarization variability is considerably increased in conditions associated with reduced repolarization reserve, such as I_{Kr} blockade or an increase in late I_{Na}, with the effect being further accentuated at slow pacing rates (Zaniboni *et al.* 2000; Thomsen *et al.* 2004; Wu *et al.* 2006, 2008, 2011; Johnson *et al.* 2010; Szentandrassy *et al.* 2015). These changes are more prominent in M cells compared to epicardial and endocardial myocytes. Strengthening of repolarization reserve upon administration of $I_{K,ATP}$ opener levcromakalim both eliminates an increase in short-term APD variability and suppresses torsade de pointes caused by I_{Kr} blocker (Thomsen *et al.* 2004).

Increased repolarization variability is a better predictor of arrhythmogenic risk compared to QT interval prolongation alone. Based on a comparison of the electrophysiological effects of 702 chemicals with I_{Kr} blocking properties in perfused rabbit hearts, dynamic APD instability was found to precede drug-induced proarrhythmia in >69% of cases (Hondeghem *et al.* 2001a). Likewise, short-term APD variability was shown to accurately discriminate between safe and proarrhythmic drugs when testing in the canine model of chronic AV block (Thomsen *et al.* 2004, 2006a,b; Oros *et al.* 2010; Varkevisser *et al.* 2012).

In addition to being used for drug safety assessments, dynamic APD instability, and its clinical counterpart, QT interval variability, have been implicated as a marker of increased

arrhythmic susceptibility in patients with organic cardiac disease (Berger *et al.* 1997; Atiga *et al.* 1998; Hinterseer *et al.* 2010; Orosz *et al.* 2015), and congenital or drug-induced long QT syndrome (Hinterseer *et al.* 2008, 2009).

7.5 Spatial dispersion of action potential duration

In perfused canine ventricular wedge preparations, drug-induced increase in transmural dispersion of repolarization, but not QT interval prolongation, was found to discriminate proarrhythmic agents from those with a safe profile (Said *et al.* 2012). Drug-induced arrhythmia is also often associated with increased interventricular repolarization gradients (Verduyn *et al.* 1997a, b; van Opstal *et al.* 2001; Milberg *et al.* 2002; Osadchii 2012e, study VI; 2013, study VII; 2014a, study VIII; 2014b, study IX), and apico-basal APD dispersion (Choi *et al.* 2002; Liu *et al.* 2005). Nevertheless, an increase in transmural APD dispersion is likely to be more arrhythmogenic, because in this case, APD changes occur over a shorter distance, determined by the thickness of LV wall, thus leading to considerably steepened repolarization gradient.

In contrast, spatially uniform APD prolongation is safe. For instance, torsade de pointes does not occur in the setting of QT prolongation evoked by I_{Ks} blockers (Shimizu & Antzelevitch 1998; Burashnikov & Antzelevitch 2002), because these agents produce a homogenous APD lengthening across the LV wall. When a drug decreases the transmural dispersion of repolarization by producing a greater APD lengthening in epicardial and endocardial myocytes than in M cells, torsadogenic risks can be markedly reduced, even in the presence of a prolonged QT interval. This profile of action is often observed with agents that inhibit multiple ionic currents (e.g. I_{Kr}, I_{Ks}, I_{Na}, I_{Ca}); examples of this are sodium pentobarbital (Shimizu *et al.* 1999), amiodarone (Sicouri *et al.* 1997; Drouin *et al.* 1998), and ranolazine (Antzelevitch *et al.* 2004). In the setting of long QT syndrome, amplified spatial

APD dispersion could be reduced by I_{Ca} blockers (Milberg *et al.* 2005, 2012b) or inhibitors of the Na^{+}-Ca^{2+} exchanger (Milberg *et al.* 2008).

8. Electromechanical window

The *electromechanical window* refers to the time difference between the end of ventricular contraction and the end of the QT interval on ECG (Figure 13). In healthy human subjects, the duration of mechanical systole is greater than the duration of ventricular repolarization, thus contributing to the positive electromechanical window (Boudoulas *et al.* 1981; De Caprio *et al.* 1984; Ter Bekke *et al.* 2015). These relationships, however, could be reversed in cardiac disease, owing to either QT interval lengthening, or shortened ventricular systole, or both. Consequently, cardiac patients often present with a negative electromechanical window, wherein the QT interval exceeds the duration of ventricular contraction. The latter has been observed in various cardiovascular conditions including mitral leaflet prolapse (Chambers & Ward 1987), coronary artery disease (Boudoulas *et al.* 1982), and congenital long QT syndrome (Vincent *et al.* 1991; Ter Bekke *et al.* 2015). Temporal mismatch between electrical and mechanical systole may develop as a consequence of electrical remodeling in diseased hearts, as suggested by studies on the canine model of LV hypertrophy induced by chronic AV block (Stams *et al.* 2014).

The negative electromechanical window is thought to facilitate cardiac electrical instability via the mechanisms related to abnormal Ca^{2+} handling (for review see Ter Bekke & Volders 2012). When electrical systole (i.e. QT interval) exceeds the duration of ventricular contraction, Ca^{2+} ions can continue to enter cardiac cells and trigger SR Ca^{2+} release, after completing muscular shortening. This leads to Ca^{2+} overload, thus facilitating both EADs and DADs. The negative electromechanical window, therefore, has been recently proposed as a novel preclinical marker of increased propensity to VT, especially in the setting of prolonged

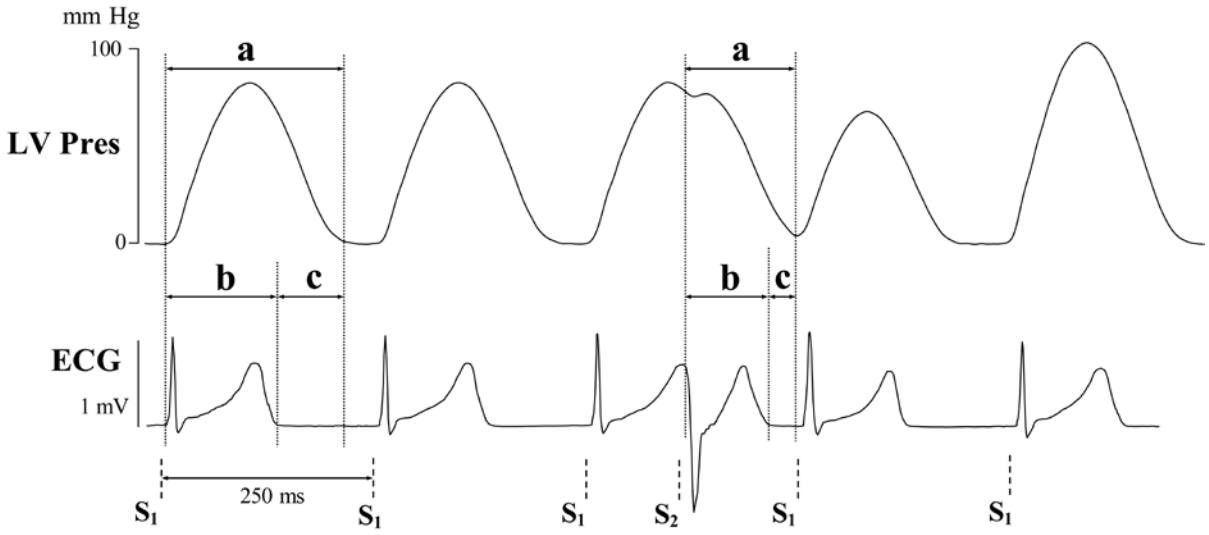

Figure 13. Methodology of the electromechanical window assessments in perfused guinea-pig hearts Left ventricular (LV) developed pressure (Pres) and volume-conducted ECG were recorded in paced heart preparation. The dashed lines under the ECG trace indicate the moments of electrical stimuli application at the LV epicardium. In third cardiac cycle, the premature (S_2) pulse was interpolated in between two regular (S_1) pulses. The S_1-S_2 interval was chosen to exceed the effective refractory period by 5 ms, in order to evoke extrasystolic beat in early diastole immediately upon recurrence of excitability. Electromechanical window (c) in the regular and extrasystolic beats is determined by calculating the difference between the duration of mechanical contraction (a) and QT interval (b). Adapted from study XI (Osadchii, O.E. 2014. Impact of hypokalemia on electromechanical window, excitation wavelength and repolarization gradients in guinea-pig and rabbit hearts. *PLoS* One 9, e105599).

cardiac repolarization (van der Linde *et al.* 2010; Guns *et al.* 2012a, b).

There are several lines of evidence in support of the role played by disturbed relationships between electrical and mechanical systole in the risk for sudden cardiac death. In patients with healed myocardial infarction, the long-term survival rate was found to be 2.6-fold lower in a patient subgroup with a negative electromechanical window (Boudoulas *et al.* 1982). Furthermore, in the setting of congenital long QT syndrome, the electromechanical window is more negative in symptomatic than arrhythmia-free patients (Ter Bekke *et al.* 2015). These clinical findings are strongly supported by animal studies, which demonstrate

that the negative electromechanical window markedly increases susceptibility to VT upon infusion of drugs with I_{Kr}/I_{Ks} blocking properties, especially in the presence of concomitant β-adrenergic receptor stimulation that shortens ventricular systole (van der Linde *et al.* 2010; Guns *et al.* 2012a, b). In a canine model of acquired long QT1 syndrome, a negative electromechanical window was found to be a prerequisite for initiating torsade de pointes; in contrast, the arrhythmia could have been prevented by agents (e.g. β-blocker or L-type Ca^{2+} channel antagonist) that made the electromechanical window less negative (van der Linde *et al.* 2010). In anesthetized guinea-pigs, the administration of drugs with known high proarrhythmic potential, such as quinidine, haloperidol, and domperidone, has been shown to induce a negative electromechanical window, whereas clinically safe antiarrhythmics such as amiodarone, verapamil and diltiazem produced no effect (Guns *et al.* 2012a, b). These observations suggest that assessments of the electromechanical window could be important in safety studies dealing with compounds that prolong the QT interval.

The contribution of the negative electromechanical window to the arrhythmic substrate has nevertheless been challenged in several studies. For example, it was shown that in perfused mini-pig and canine hearts, the electromechanical window remains positive even in the setting wherein I_{Kr} blockade, which prolongs QT interval, is combined with β-adrenoreceptor agonist challenge, which abbreviates mechanical systole (Laursen *et al.* 2011). In the canine model of chronic AV block, although I_{Kr} blocker infusion causes the electromechanical window to change from a positive to a negative value, the subgroups of dogs with inducible vs. non-inducible VT could not be discriminated by measuring the electromechanical window (Stams *et al.* 2014). Also, because a reduction in the electromechanical window in this setting is entirely accounted for by the QT interval prolongation, without any change in duration of mechanical contraction, it appears that

electromechanical window does not add more specificity to assessments of proarrhythmic tendency compared to measuring the QT interval. Finally, recent studies suggest that the reversed relationships between the duration of electrical and mechanical systole do not contribute to arrhythmic substrate in the setting of hypokalemia (Osadchii 2014d: study XI). In perfused guinea-pig and rabbit hearts, although hypokalemia has been found to prolong repolarization and increase the occurrence of tachyarrhythmia, the duration of mechanical systole remained invariably longer compared to the QT interval, thereby contributing to the positive electromechanical window, both during regular beating and upon extrasystolic stimulation. Instead, in hypokalemic hearts, the abnormal changes in ventricular conduction times, refractoriness, excitation wavelength, and spatial repolarization gradients were found to be more important mechanistic determinants of arrhythmia, compared to the changes in electromechanical window.

Taken together, the aforementioned studies indicate that a *negative* electromechanical window is not always proarrhythmic, and vice versa, a *positive* electromechanical window does not necessarily exclude the chance to develop tachyarrhythmia. Therefore, in assessments of arrhythmogenic risks, this parameter should preferably be evaluated in combination with other surrogate markers of cardiac electrical instability.

9. Impact of ventricular pacing site on arrhythmic vulnerability

The initiation of tachyarrhythmia is markedly facilitated when electrical stimulations are applied to the vulnerable pacing site. In a canine model of myocardial infarction, when programmed stimulation is applied at multiple LV and RV sites, the inducibility of sustained VT may vary from 44% to 19% (Michelson *et al.* 1981a). In human patients, electrical stimulations are more successful in provoking VT when at least two RV pacing sites (e.g. the apex and the outflow tract) are evaluated (Doherty *et al.* 1983, 1984; Somberg *et al.* 1987;

Klein *et al.* 1991; Martinez-Rubio *et al.* 1995). Moreover, in some patients with clinically documented arrhythmia, the LV stimulation is required in addition to RV testing, in order to reproducibly initiate VT (Michelson *et al.* 1979; Robertson *et al.* 1981; Page *et al.* 1983).

The most striking differences in arrhythmic vulnerability are observed when electrical stimulations are applied at the endocardium vs. the epicardium, and in the RV vs. the LV chamber.

9.1 Endocardial vs. epicardial pacing

9.1.1 Animal studies

In perfused guinea-pig hearts, the inducibility of APD alternans and tachyarrhythmia is higher when electrical stimulations are applied at endocardium compared to epicardium, in both RV and LV chamber (Osadchii *et al.* 2011; Osadchii 2012a: study II). Endocardial stimulations also require lower threshold current strength to induce VF. In previous works, the same difference was determined in canine LV (Horowitz *et al.* 1981). The epicardial-to-endocardial difference in arrhythmic susceptibility is partly accounted for by transmural heterogeneities in the distribution of Na^+ channels. The expression levels of $Na_v1.5$ and/or I_{Na} current density are greater at the endocardium than the epicardium in guinea-pig (Osadchii *et al.* 2011), rat (Ashamalla *et al.* 2001; Rosati *et al.* 2006), and mouse (Remme *et al.* 2009), as well as in non-diseased human hearts (Gaborit *et al.* 2007). Consequently, tissue excitability is higher at the endocardium, as evidenced by the lower ventricular capture thresholds compared to the epicardium (Borggrefe *et al.* 1988). In the presence of higher tissue excitability, an ectopic pulse of a given strength may excite more ventricular myocytes, produce greater voltage gradients, and create a large area of slowly propagating graded responses, thus facilitating re-entry (Frazier *et al.* 1989). The enhanced tissue excitability also contributes to the decreased size of a central core around which the spiral wave rotates during

VF (Mandapati *et al.* 1998; Pandit *et al.* 2010). This reduces the rotation period of the spiral wave, thus maintaining high-frequency activation in the fibrillating ventricle. Collectively, these mechanisms may account for direct correlation between the diastolic excitability threshold and the minimum current strength required to induce VF (Gaum *et al.* 1977; Chen 1992).

A greater availability of Na^+ channels contributes to the recurrence of excitability at earlier time points during repolarization, as evidenced by a lower ERP-to-APD ratio determined at the endocardium compared to the epicardium in guinea-pig heart (Osadchii 2012a: study II). This ensures an increased window over which the LV can be re-excited by a premature ectopic pulse, thus facilitating VT initiation at the endocardium. Likewise, when a premature action potential is generated at earlier repolarization time points, it propagates with a longer conduction delay owing to incomplete recovery of I_{Na} from inactivation. Accordingly, transmural conduction time is longer when extrasystolic stimulations are applied at the endocardium compared to the epicardium (Osadchii 2012a: study II), an effect that can facilitate re-entry by reducing the excitation wavelength.

In addition to spatial non-uniformities in I_{Na} expression, a greater arrhythmic susceptibility upon endocardial pacing could be accounted for by electrotonic modulation of the intrinsic transmural repolarization gradient. Electrical stimulations are associated with a "downstream effect" wherein APD in cardiac myocytes close to the pacing electrode tends to be prolonged by electrotonic depolarizing current generated by more peripheral cells (Osaka *et al.* 1987, Laurita *et al.* 1997, Furukawa *et al.* 2000). This contributes to the longest APD at the pacing site, which then progressively reduces along the propagation path (see chapter 5 for more discussion). When these relationships are applied to LV wall (Figure 14), it follows that during endocardial stimulations, endocardial APD tends to be longer, and epicardial APD

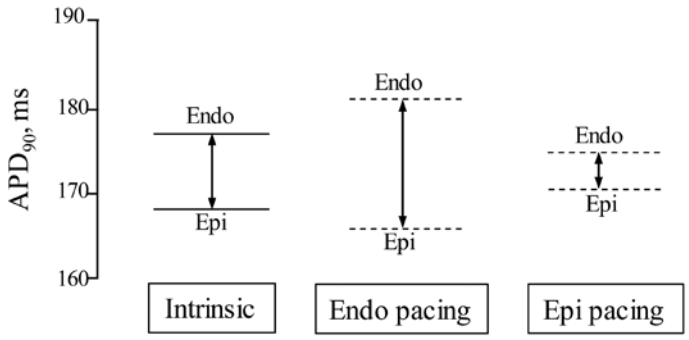

Figure 14. Putative changes in the transmural repolarization gradient during endocardial (endo) and epicardial (epi) pacing

Action potential duration (APD_{90}) in spontaneously beating hearts is greater at the endocardium than epicardium. The magnitude of this intrinsic difference (as shown by double arrow) is subjected to modulation by electrotonic effects initiated upon endo and epi pacing. In both cases, APD_{90} at a given ventricular site tends to be longer than its intrinsic value when stimulations are applied close to the recording electrode, and tends to be shorter than its intrinsic value when stimulations are introduced from the opposite side of the ventricular wall. According to these relations, the intrinsic transmural difference in APD_{90} is amplified during endocardial stimulations, but reduced during epicardial pacing. Adapted from study II (Osadchii, O.E. 2012. Electrophysiological determinants of arrhythmic susceptibility upon endocardial and epicardial pacing in guinea-pig heart. *Acta Physiol (Oxf.)* 205, 494-506), with permission from publisher.

tends to be shorter, compared to their intrinsic values determined during spontaneous beating. In contrast, epicardial pacing is associated with increased APD at epicardium, but reduced endocardial APD. Transmural APD dispersion therefore becomes greater when switching from epicardial to endocardial pacing (Osadchii 2012a: study II). In line with these findings, optical mapping of repolarization in rabbit hearts has revealed that transmural APD patterns are strongly determined by the activation sequence; even though a positive endocardial-to-epicardial APD difference is invariably seen during endocardial pacing, the gradient is reversed when activation proceeds from the epicardium (Myles *et al.* 2010).

Finally, endocardial-to-epicardial difference in arrhythmic susceptibility could be

explained by a steeper slope of APD rate adaptation at the endocardium. The minimum pacing interval that allows 1:1 LV capture is shorter during endocardial stimulations, which contributes to the increased slope of the initial steep portion of the restitution curve (Osadchii *et al.* 2011; Osadchii 2012a: study II). The latter, according to the restitution hypothesis (see section 6.1), facilitates APD alternans and increases propensity to VF.

In sharp contrast to these findings from the guinea-pig model, studies on perfused ventricular wedge preparations from dog (Di Diego *et al.* 2003; Fish *et al.* 2004, 2005; Ueda *et al.* 2004; Xu *et al.* 2012) and rabbit (Medina-Ravell *et al.* 2003; Fish *et al.* 2005) have shown that the inducibility of tachyarrhythmia is higher when electrical stimulations are applied to the *epicardium* compared to the endocardium or midmyocardium, at least in the presence of drug-induced I_{Kr} block or activation of the late I_{Na}. In basal, drug-free conditions, although arrhythmia is not inducible at any stimulation site, the transmural dispersion of repolarization was found to be greater upon epicardial pacing. Importantly, in these studies, transmural APD values remained the same upon switching from endocardial to epicardial pacing, and therefore the aforementioned increase in transmural dispersion of repolarization time (a sum of activation latency and APD) was ascribed to a greater conduction delay when the activation proceeds from the epicardium to the endocardium compared to that determined with the opposite (i.e., endocardium to epicardium) sequence of activation. A significant delay in conduction between the epicardial and midmyocardial layers is likely accounted for by increased tissue resistivity in the deep subepicardium in the canine ventricular wall, an effect resulting from sharp transition of cell orientation in this area (Yan *et al.* 1998).

9.1.2 Clinical studies

In cardiac patients, ventricular pacing may be either instituted acutely upon electrophysiological testing, or maintained permanently in order to treat symptomatic

bradycardia caused by sinus node dysfunction or severe AV block. The RV endocardium is commonly used as the preferred stimulation site, because it is easily accessible via transvenous catheterization, and shows no pacemaker lead dislodgements and stable excitation thresholds in the long-term (Sweeney & Prinzen 2006). Epicardial pacing is less common, and reserved only for selected groups of patients in whom it is applied either via coronary sinus, or directly upon thoracotomy. For example, epicardial pacing is often required in pediatric patients, in whom the placement of an RV endocardial lead via the transvenous route is not feasible because of a small body size, or vascular barriers, or congenital defects in cardiac anatomy (Cohen *et al.* 2001; Dodge-Khatami *et al.* 2005). In adults, LV epicardial pacing could be used in patients who have undergone tricuspid valve replacement, or it can be implemented for electrophysiological assessments during cardiac surgery, or post-operatively, using implanted epicardial leads.

Ventricular pacing may be associated with clinically important proarrhythmic effects. For example, permanent unipolar RV endocardial pacing has been shown to occasionally provoke a malignant tachyarrhythmia resulting from interference of the pacemaker rhythm and spontaneous ectopic beats. In this setting, sudden cardiac death was reported to account for 23% of all lethal outcomes noted in pacemaker-implanted patients during a 2-year observation period (Zehender *et al.* 1992). Non-fatal pacing-induced VT was documented in 26% of patients with implanted cardioverter-defibrillator which incorporated a pacemaker feature to treat post-defibrillation bradycardia (Himmrich *et al.* 2003). When electrophysiological testing is performed after cardiac surgery, in about 40% of patients in whom VT is non-inducible upon epicardial stimulation, it could be provoked by endocardial stimulation (Sheppard *et al.* 1993). In a study that employed post-operative assessments of the efficacy of anti-tachycardia surgery, epicardial stimulation alone was found to underestimate

the number of patients at risk for recurrent tachyarrhythmia by 25% (Borggrefe *et al.* 1988). Accordingly, endocardial stimulations were reported to have a superior sensitivity and an improved negative predictive value in proarrhythmic assessments.

In contrast to the aforementioned observations, there is a line of evidence that indicates a greater proarrhythmic potential of epicardial compared to endocardial pacing. This evidence largely stems from studies on resynchronization therapy in heart failure (for review see Ray *et al.* 2007; Turito & El-Sherif 2007), wherein permanent RV endocardial pacing is implemented along with LV epicardial stimulation in order to eliminate interventricular electromechanical dyssynchrony. In this group of patients, LV epicardial stimulations are reportedly associated with a longer QT interval, a greater transmural dispersion of repolarization, and hence a greater chance to develop tachyarrhythmia, compared to either RV endocardial or biventricular stimulations (Medina-Ravell *et al.* 2003; Fish *et al.* 2005; Bai *et al.* 2006; Harada *et al.* 2006).

This notion, nevertheless, remains a matter of debate, as other studies have shown that transmural dispersion of repolarization is *less* during biventricular pacing compared to conventional RV endocardial pacing (Van Huysduynen *et al.* 2005; Santangelo *et al.* 2006). Moreover, VT inducibility by programmed stimulation in patients with ischemic cardiomyopathy was found to be lower during biventricular compared to RV endocardial pacing (Zagrodzky *et al.* 2001; Kowal *et al.* 2004). When applied alone, RV endocardial, RV epicardial, and LV epicardial stimulations were reported to yield concordant results (Dailey *et al.* 1989; Santangelo *et al.* 2006). There is also an indication of the low, or even reduced, occurrence of VT over a long-term follow-up during either a dual-chamber pacing (Higgins *et al.* 2000; Walker *et al.* 2000; Bhatia *et al.* 2007; Tereshchenko *et al.* 2011; Shahrzad *et al.* 2012; Kutyifa *et al.* 2015) or LV epicardial pacing applied alone (Touiza *et al.* 2001; Blanc *et*

al. 2004; Wong *et al.* 2004). Hence, the question about the most optimal and clinically safe pacing algorithm is still open.

9.2 Left ventricular vs. right ventricular pacing

Studies on anesthetized dogs demonstrate that RV stimulations are associated with lower VF threshold and higher inducibility of arrhythmia upon ectopic activation, compared to the spatially matched (either epicardial or endocardial) LV stimulations (Tamargo *et al.* 1975; Horowitz *et al.* 1981; Hamer *et al.* 1984; Hunt & Ross 1990). In human patients with a documented history of spontaneous arrhythmia, a sustained ventricular tachycardia is more often inducible by programmed electrical stimulation applied to the RV compared to the LV endocardium (Lin *et al.* 1987). These findings therefore suggest a greater arrhythmic vulnerability in the RV vs. LV chamber, at least in canine and human hearts. It is noteworthy, however, that in 10-20% of patients, tachyarrhythmia is not inducible upon RV stimulations, but could be provoked by stimulations applied at the LV chamber (Michelson *et al.* 1979; Robertson *et al.* 1981; Kudenchuk *et al.* 1988; Chen *et al.* 1994).

In the perfused guinea-pig heart, the relationships between ventricular pacing site and arrhythmic vulnerability are different from those outlined above. In this model, the electrical stimulation protocols reveal similar arrhythmic susceptibility in the RV vs. LV epicardium during normokalemic perfusion (Osadchii *et al.* 2009). However, when extracellular K^+ concentration is reduced, the VF threshold becomes lower, and inducibility of tachyarrhythmia by rapid pacing or extrasystolic stimulation is increased in the LV compared to the RV chamber. This effect is accounted for by hypokalemia-induced reduction in ERP in the LV but not RV epicardium. In a clinical context, these findings suggest that interventricular differences in arrhythmic susceptibility may be accentuated in the presence of systemic electrolyte abnormalities associated with cardiac disease, such as hypokalemia.

Electrophysiological mechanisms that account for the stimulation site-dependent variations in arrhythmic vulnerability are likely determined by the relationships between the direction of the interventricular repolarization gradient, and inducibility of the conduction block upon ectopic activation. In the whole heart, the arrhythmia is typically provoked when premature activation is initiated from the site with *short* APD, an effect that is attributed to progressive conduction slowing that develops once the evoked action potential invades myocardial regions with a longer refractory period (Kuo *et al.* 1983, 1985; Bode *et al.* 2002). This contributes to unidirectional conduction block, thus precipitating re-entry. Stimulations applied at a site with *long* APD usually fail to induce arrhythmia. Based on this principle, in animal species showing greater APD in the LV chamber (e.g., canines: Verduyn *et al.* 1997a, b; van Opstal *et al.* 2001; Janse *et al.* 2005; Gallacher *et al.* 2007), inducibility of VT may be facilitated upon electrical stimulations applied at the RV site. However, the opposite relations could be found in animal species that exhibit a longer APD in the RV than LV (e.g., guinea-pig: Poelzing & Veeraraghavan 2007; Osadchii *et al.* 2009); in this case, the aforementioned mechanism would facilitate VT induction when using the LV stimulation site.

Chamber-specific difference in arrhythmic vulnerability can be partly accounted for by non-uniformities in ion channel expression. For example, in the guinea-pig heart, a higher I_{K1} density in LV vs. RV is thought to contribute to the reduced central core of the spiral wave, which results in a higher frequency of excitation during ventricular fibrillation, and hence favors stabilization of the rotor, a source of re-entrant activation, in the LV chamber (Samie *et al.* 2001; Warren *et al.* 2003). Apart from the electrical heterogeneities, the arrhythmic substrate can be strongly determined by tissue geometry and architecture. In this regard, a larger overall thickness of the LV vs. RV wall can account for more complex spatiotemporal activation patterns during VF in the LV chamber (Rogers *et al.* 2000). It is also noteworthy

that in patients with coronary artery disease, myocardial infarction and subsequent remodeling more often occur in the left ventricle, meaning that electrical activation initiated from LV stimulation site may have a greater chance of entering the re-entrant circuit and provoking VT.

The aforementioned considerations suggest that stimulation site-dependent variations in arrhythmic vulnerability are determined by a complex interplay of multiple factors. Depending on which mechanism prevails in the specific model chosen for electrophysiological assessments (animal species used, normal or diseased cardiac tissue, type of pharmacological treatment), arrhythmic vulnerability may be greater either in the LV or RV, or show no interventricular difference.

10. Arrhythmogenic responses in hypokalemia

10.1 Clinical impact of hypokalemia in cardiovascular disease

Hypokalemia refers to reduced serum K^+ levels (less than 3.5 mM), and represents the most common electrolyte disorder seen in cardiac patients. Hypokalemia typically develops as a side effect of diuretic therapy prescribed to normalize blood pressure and reduce cardiac loads, or may be caused by neurohormonal derangements in heart failure, including over-activation of the renin-angiotensin-aldosterone system and sympathetic nervous system. Aldosterone increases renal K^+ excretion, whereas noradrenaline increases K^+ uptake from blood plasma by skeletal muscles, an effect that is mediated via activation of Na^+-K^+ ATPase coupled to β_2-adrenergic receptor on myocyte membrane (for review see Clausen 2010). Overall, it follows that hypokalemia may be promoted by abnormal K^+ loss, or re-distribution of K^+ between interstitial and intracellular compartments, or both.

Hypokalemia occurs in about 16% of patients admitted to the hospital (Jensen *et al.* 2015), with the risk being about 12-fold higher in users of thiazide diuretics (Rodenburg *et al.*

2014). When diagnosed, hypokalemia is corrected by oral K^+ supplements, or with K^+-sparing agents such as aldosterone receptor antagonists (Vardeny *et al.* 2014). In the setting of myocardial infarction, the incidence of hypokalemia is markedly reduced upon the administration of β-blockers (Nordrehaug *et al.* 1985; Simpson *et al.* 1987).

Hypokalemia is an important contributing factor to cardiac electrical instability. The ventricular ectopic activity develops in about one-third of hypertensive patients treated with diuretics (Holland *et al.* 1981), and there is a strong inverse correlation between the number of extrasystolic beats on ECG and serum K^+ levels (Medical Research Council Working Party on Mild to Moderate Hypertension 1983). The Multiple Risk Factor Intervention Trial (MRFIT) has shown that each 1 mM reduction in serum K^+ is associated with a 28% increase in the risk of ventricular ectopy (Cohen *et al.* 1987).

Hypokalemia is an important arrhythmogenic factor in patients with acute myocardial infarction, whereby its degree is proportional to the elevated plasma catecholamine levels (Nordrehaug & von der Lippe 1983; Nordrehaug *et al.* 1985; Simpson *et al.* 1987; Clausen *et al.* 1988). In these patients, the occurrence of tachyarrhythmia over 24 hours upon admission to the hospital is inversely related to serum K^+ concentrations, and ventricular fibrillation develops in more than 30% of patients when serum K^+ drops below 3 mM (Nordrehaug & von der Lippe 1983). Although the incidence of arrhythmia is likely to be lower in contemporary clinical practice because of the widespread use of β-blockers, reperfusion therapy, and early invasive management, the casual links between serum K^+ levels and VF in post-infarcted patients remain a matter of important clinical concern (Goyal *et al.* 2012).

Proarrhythmic effects of hypokalemia contribute to poor clinical prognosis. Diuretic therapy has been shown to substantially increase the risk of cardiac arrest in hypertensive patients (Siscovick *et al.* 1994), and eliminate the benefits associated with blood pressure

reduction (Franse *et al.* 2000). Large multicenter clinical trials indicate that hypokalemia is an independent risk factor that increases mortality in a wide spectrum of ambulatory patients with chronic heart failure (Cooper *et al.* 1999; Ahmed *et al.* 2007; Alper *et al.* 2009; Bowling *et al.* 2010). For example, in the SOLVD (Studies on Left Ventricular Dysfunction) trial, the incidence of arrhythmic death was reported to be about 80% higher in patients that received diuretic treatment, compared to controls (Cooper *et al.* 1999). These studies provide a strong argument for the notion that maintaining normal serum K^+ levels is pivotal for improving survival in cardiovascular disease.

10.2 Mechanisms of hypokalemia-induced cardiac arrhythmia

10.2.1 Arrhythmic triggers

Hypokalemia can precipitate triggered arrhythmia by increasing firing rate in ectopic pacemakers. Low extracellular K^+ levels contribute to the reduced background outward K^+ current in Purkinje fibers, which results in steepening the slope of spontaneous diastolic depolarization (Lee & Fozzard 1979). In quiescent sheep Purkinje fibers, hypokalemic perfusion provokes depolarization of the resting membrane potential and subthreshold voltage oscillations, which facilitate the initiation of spontaneous electrical activity (Spiegler & Vassale 1995). In guinea-pig and cat papillary muscle, automaticity could be acquired upon the application of depolarizing constant current in the presence of low K^+-perfusion (Katzung & Morgenstern 1977).

Another source for triggered arrhythmia in hypokalemia is constituted by early and delayed afterdepolarizations. An increased propensity for *early afterdepolarizations* in hypokalemia could be detected both at the cellular and tissue level (Killeen *et al.* 2007b; Madhvani *et al.* 2011; Bapat *et al.* 2012; Pezhouman *et al.* 2015), which is attributed to the excessive prolongation of ventricular APD owing to the inhibition of outward K^+ currents,

such as I_{Kr} (Scamps & Carmeliet 1989; Sanguinetti & Jurkiewicz 1992), I_{K1} (Sanguinetti & Jurkiewicz 1992; Bouchard *et al.* 2004; Killeen *et al.* 2007a; Cordeiro *et al.* 2015), and I_{to} (Killeen *et al.* 2007a). The occurrence of EADs is substantially increased when hypokalemia is applied in combination with other factors that reduce repolarization reserve, such as slow pacing rates or drug-induced I_{Kr} block (Davidenko *et al.* 1989; Sicouri & Antzelevicth 1995). In addition to acute and reversible changes related to decreased K^+ channel conductance, a reduction in the net outward current across myocyte sarcolemma in hypokalemic hearts could be related to decreased cell surface I_{Kr} protein expression caused by I_{Kr} internalization and subsequent endocytic degradation (Guo *et al.* 2009; Massaeli *et al.* 2010). These effects evolve upon long-term hypokalemic exposure, for example, after several weeks on a K^+-deficient diet.

Delayed afterdepolarizations are often promoted when hypokalemic ventricular tissue is subjected to rapid pacing rates (Ohta *et al.* 1987; Gilat *et al.* 1990). DADs are attributed to Ca^{2+} overload, which develops via at least two different mechanisms. Hypokalemia inhibits Na^+-K^+ ATPase on myocyte sarcolemma (Eisner & Lederer 1979; Aronsen *et al.* 2015), which results in increased intracellular Na^+ levels. This in turn activates the reverse mode of the Na^+-Ca^{2+} exchange, leading to increased cytosolic Ca^{2+} concentration. Second, hypokalemia-induced prolongation of APD implies an increased time for Ca^{2+} entry, which indirectly facilitates cytosolic Ca^{2+} accumulation (White & Terrar 1991).

Recent studies demonstrate a close interplay of the mechanisms leading to generation of EADs and DADs in hypokalemic hearts (Pezhouman *et al.* 2015). Hypokalemia-induced inhibition of Na^+-K^+ ATPase and subsequent Ca^{2+} overload, apart from being critical for the genesis of DADs, appear to play a role in the mechanism of EADs. Increased cytosolic Ca^{2+} activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which then phosphorylates

the L-type Ca^{2+} channels and fast Na^{+} channels, thus increasing I_{Ca} and late I_{Na}. This generates a positive feedback loop that further exacerbates intracellular Ca^{2+} and Na^{+} overload, and progressively impairs the repolarization reserve, leading to EAD-dependent arrhythmia. Targeting the CaMKII-mediated effects, therefore, can be considered an emerging antiarrhythmic strategy in hypokalemic patients.

10.2.2 Effective refractory period and ventricular conduction

Although hypokalemia prolongs ventricular APD, it can reduce the effective refractory period, as shown in studies on perfused heart preparations from mouse (Sabir *et al.* 2007), guinea-pig (Osadchii & Olesen 2009; Osadchii *et al.* 2010: study I; Osadchii 2014d: study XI), and rabbit (Wolk *et al.* 1998, 2002; Osadchii 2014d: study XI). This change can be attributed to the increased availability of voltage-dependent Na^{+} channels in a setting of hypokalemia-induced hyperpolarization of the resting membrane potential. In support of the role played by this mechanism, the amplitude of phase 0 and the maximal velocity of action potential upstroke (V_{max}) were shown to be increased in hypokalemic guinea-pig ventricles (Gjini *et al.* 1996; Marschang *et al.* 1998). In the presence of low extracellular K^{+} levels, the concentration of Na^{+} channel blocker that is required to reduce V_{max} is enhanced by about 10-fold, which again indirectly suggests the increased activation of the Na^{+}-carrying system (Singh *et al.* 1971). A reduction of ERP in hypokalemia could also be linked to triangulation of the action potential waveform (Osadchii & Olesen 2009), which imposes changes in membrane voltage profile for Na^{+} channel recovery from inactivation during repolarization.

Hypokalemia is associated with slowed conduction of the action potential. At the whole heart level, this effect translates to prolonged LV-to-RV transepicardial and LV transmural conduction times (Wolk *et al.* 1998, 2002; Osadchii & Olesen 2009; Osadchii 2014d: study XI). Hypokalemia-induced conduction slowing is thought to be caused by hyperpolarization

of the myocyte sarcolemma, which results in an increased excitation threshold and membrane input resistance in cardiac cells (Kishida *et al.* 1979; Ruiz-Ceretti *et al.* 1982). Likewise, Ca^{2+} overload in severe hypokalemia (K^+ less than 1.5 mM) has been shown to promote subcellular alterations in the distribution of connexin-43, which results in impaired cell-to-cell coupling (Tribulova *et al.* 2001), thus providing another mechanism for slowed conduction velocity.

The aforementioned changes markedly increase arrhythmic vulnerability in hypokalemic hearts (Figure 15). Reduced ERP, along with slowed ventricular conduction, contribute to decreased excitation wavelength, and hence facilitate re-entry. An abbreviated ERP also accounts for the reduced size of the central core around which the spiral wave rotates, thus sustaining re-entry and increasing its rate (Uchida *et al.* 1999). In addition, ERP shortening in the presence of prolonged APD significantly increases the time window for LV re-excitation over late repolarization (Sabir *et al.* 2007; Osadchii & Olesen 2009; Osadchii *et al.* 2010: study I), which facilitates arrhythmia.

10.2.3 Spatial repolarization gradients

Hypokalemia-induced APD lengthening is not uniform at distinct ventricular sites. For example, in perfused rabbit hearts (Osadchii 2014d: study XI), monophasic action potential recordings taken from six epicardial sites demonstrate that hypokalemic perfusion is associated with APD prolongation in the LV anterior and posterior wall (site 2 and site 3, respectively, in Figure 16, panel A) and RV anterior and posterior wall (site 2 and site 1, respectively, in Figure 16, panel B), while producing no APD change at the lateral wall in both ventricular chambers (site 1 in panel A and site 3 in panel B). The standard deviation of the mean epicardial APD value, an index of APD dispersion, was significantly increased in hypokalemic hearts. Increased RV-to-LV transepicardial repolarization gradients in hypokalemia have also been determined in perfused guinea-pig hearts (Poelzing &

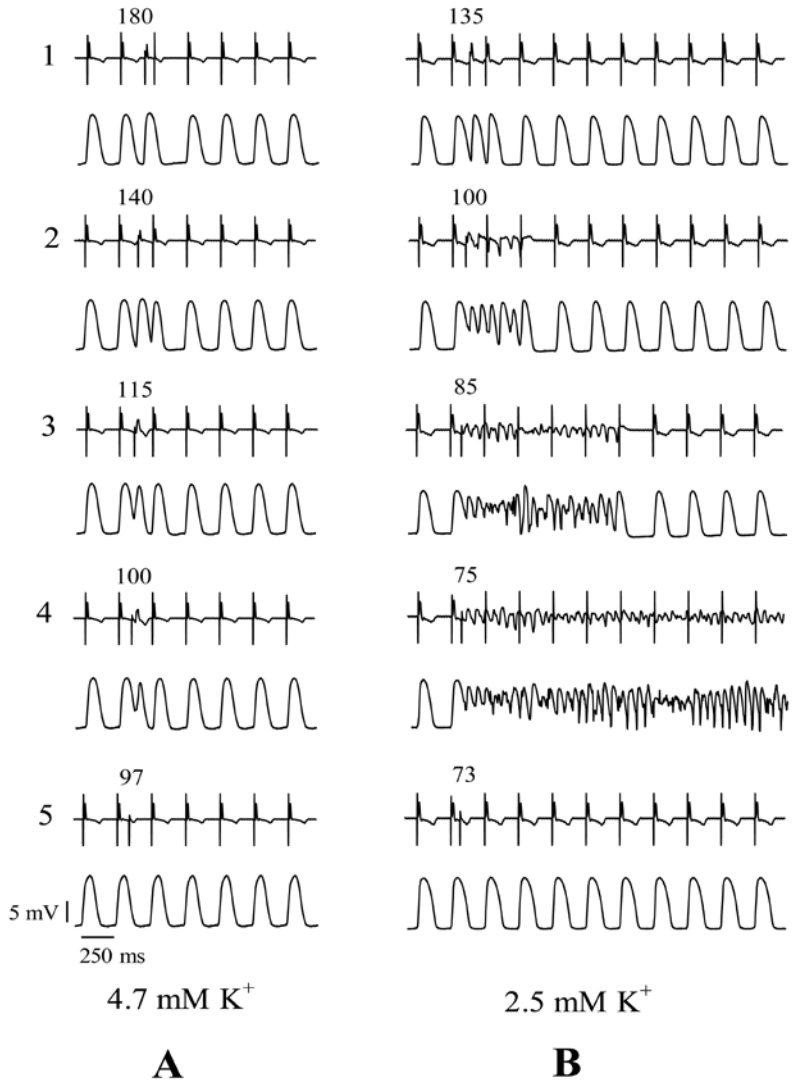

Figure 15. Effects of hypokalemia on the inducibility of tachyarrhythmia by programmed stimulation in perfused guinea-pig hearts

In fragments 1-5 in each panel, the upper trace is the volume-conducted ECG, and the lower trace is the LV epicardial monophasic action potential recording. The vertical deflections on ECG represent the regular pacing stimulus and the premature extrasystolic stimulus artefacts. The numbers above ECG traces indicate the duration (ms) of coupling stimulation intervals. Note that hypokalemic perfusion is associated with reduction of the effective refractory period from 97 to 73 ms (fragment 5, A vs. B), and that extrasystolic stimulation at coupling intervals close to the refractory period (fragments 2-4) elicits ventricular tachyarrhythmia during hypokalemic (B), but not normokalemic (A) perfusion. Adapted from Osadchii, O.E., & Olesen, S.P. 2009. Electrophysiological determinants of hypokalemia-induced arrhythmogenicity in the guinea-pig heart. *Acta Physiol* (Oxf.) 197, 273-287, with permission from publisher.

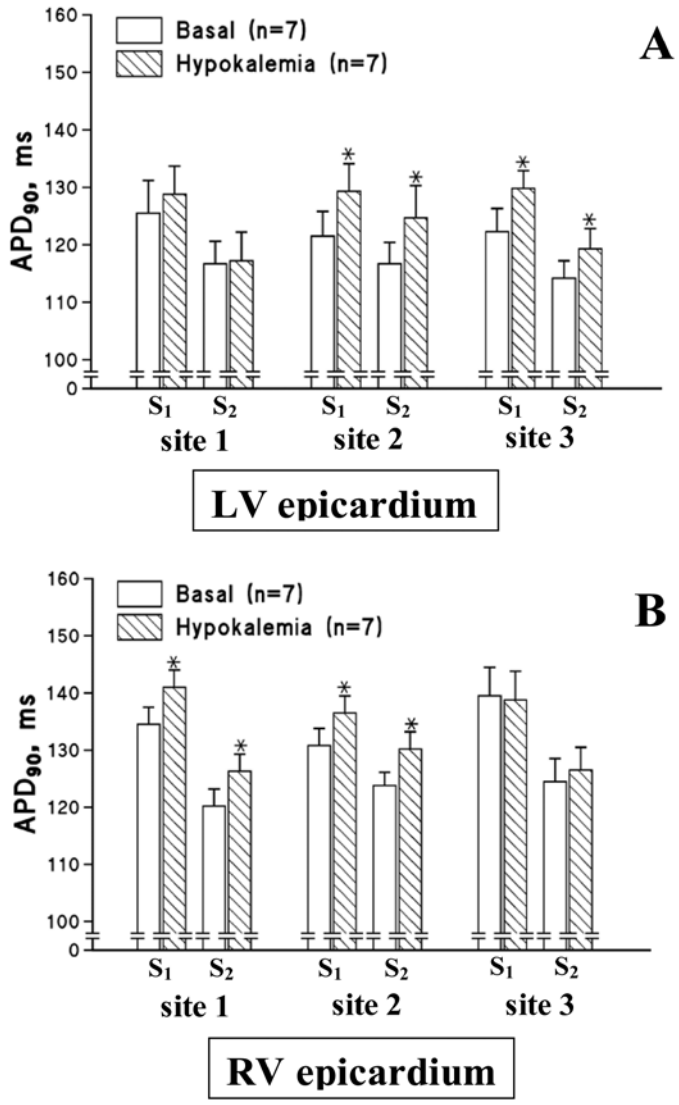

Figure 16. Effects of hypokalemia on the action potential duration at distinct epicardial sites in perfused rabbit hearts

Monophasic action potential duration (APD_{90}) was determined in basal (4.7 mM K^+) and hypokalemic (2.5 mM K^+) conditions at three LV recording sites (site 1 is the lateral LV wall, site 2 is the anterior LV wall, and site 3 is the posterior LV wall) and three RV recording sites (site 1 is the posterior RV wall, site 2 is the anterior RV wall, and site 3 is the lateral RV wall). The assessments were made during steady-state pacing (S_1) and extrasystolic stimulations (S_2) applied at a coupling interval exceeding the effective refractory period by 5 ms. *P<0.05 vs. corresponding basal value. Adapted from study XI (Osadchii, O.E. 2014. Impact of hypokalemia on electromechanical window, excitation wavelength and repolarization gradients in guinea-pig and rabbit hearts. *PLoS* One 9, e105599).

Veeraraghavan 2007; Osadchii & Olesen 2009; Osadchii 2014d: study XI).

On the other hand, the pattern of APD changes provoked by hypokalemia over the *transmural* plane is less clear. In perfused canine ventricular wedge preparations, lowering the extracellular K^+ concentration has been reported to produce a substantially greater increase in APD in M cells compared to either epicardial or endocardial myocytes (Sicouri & Antzelevitch 1995), suggesting an amplified transmural repolarization gradient. In contrast, in guinea-pig, hypokalemia evokes a greater APD lengthening in epicardial vs. endocardial or midmyocardial myocytes (Wan *et al.* 2000), which implies a reduced transmural APD difference. A greater epicardial than endocardial APD prolongation by hypokalemia, and associated reduction in transmural repolarization gradient have been observed in perfused murine hearts (Killeen *et al.* 2007a). Therefore, the contribution of alterations in the transmural APD profile to arrhythmic substrate in hypokalemic hearts remains inconclusive.

10.2.4 Electrical restitution

Hypokalemia has been shown to increase the occurrence of APD alternans during rapid cardiac pacing (Koller *et al.* 2000; Sabir *et al.* 2008; Osadchii *et al.* 2010: study I), which implies that a steepened slope of electrical restitution is likely to be one of the mechanisms that increases the vulnerability to VF in hypokalemic hearts. In support of this notion, both the inducibility of tachyarrhythmia and the maximum APD restitution slope were increased in murine hearts during hypokalemic perfusion (Sabir *et al.* 2008). These findings, however, could not be directly extrapolated to the hearts of larger mammalian species owing to the fast cardiac beating rates and specific features of repolarization in mice. In canine endocardial slices, no change in APD restitution was observed while varying the extracellular K^+ concentration between 2.7 and 4.0 mM (Koller *et al.* 2000).

The impact of hypokalemia on electrical restitution was examined in perfused guinea-

pig hearts by taking monophasic action potential recordings and measuring effective refractory periods at multiple ventricular sites (Osadchii *et al.* 2010: study I). This study has shown that the value of electrical restitution at predicting hypokalemia-induced arrhythmia critically depends on whether APD or ERP is chosen as a variable reflecting the rate-adaptation of ventricular repolarization. The APD restitution assessments by extrasystolic stimulation revealed that hypokalemia reduces the amount of APD shortening in premature beats generated over a range of short (less than 40 ms) diastolic intervals (Figure 17, panel A). As a result, the initial portion of the APD restitution curve was shifted upwards and flattened, thus contributing to reduced maximum slope (Figure 17, panels A, B and C). In contrast, the slope of ERP rate-adaptation was significantly increased in hypokalemic hearts (Figure 17, panels D, E and F). Owing to ERP reduction at fast pacing rates, ventricular capture was maintained at shorter diastolic intervals in hypokalemic compared to normokalemic conditions. Consequently, the initial portion of the restitution curve was extended towards ERP values less than 125 ms (Figure 17, panel D), which contributed to a steepened restitution slope (Figure 17, panels E and F). Contrasting changes in APD vs. ERP restitution kinetics are likely to reflect the different pattern of APD and ERP modifications in hypokalemia, wherein the prolongation of APD may be associated with abbreviated refractoriness. This raises the point that ERP restitution assessments should preferably complement the traditionally used testing of APD rate adaptation, in order to adequately address proarrhythmic changes provoked by diuretic-induced hypokalemia in cardiac patients.

10.2.5 Activation-repolarization coupling

In normal conditions, spatial repolarization heterogeneities are minimized owing to the activation-repolarization coupling (see chapter 5). This mechanism, nevertheless, is impaired in the setting of hypokalemia (Osadchii 2014c: study X), as schematically illustrated in Figure

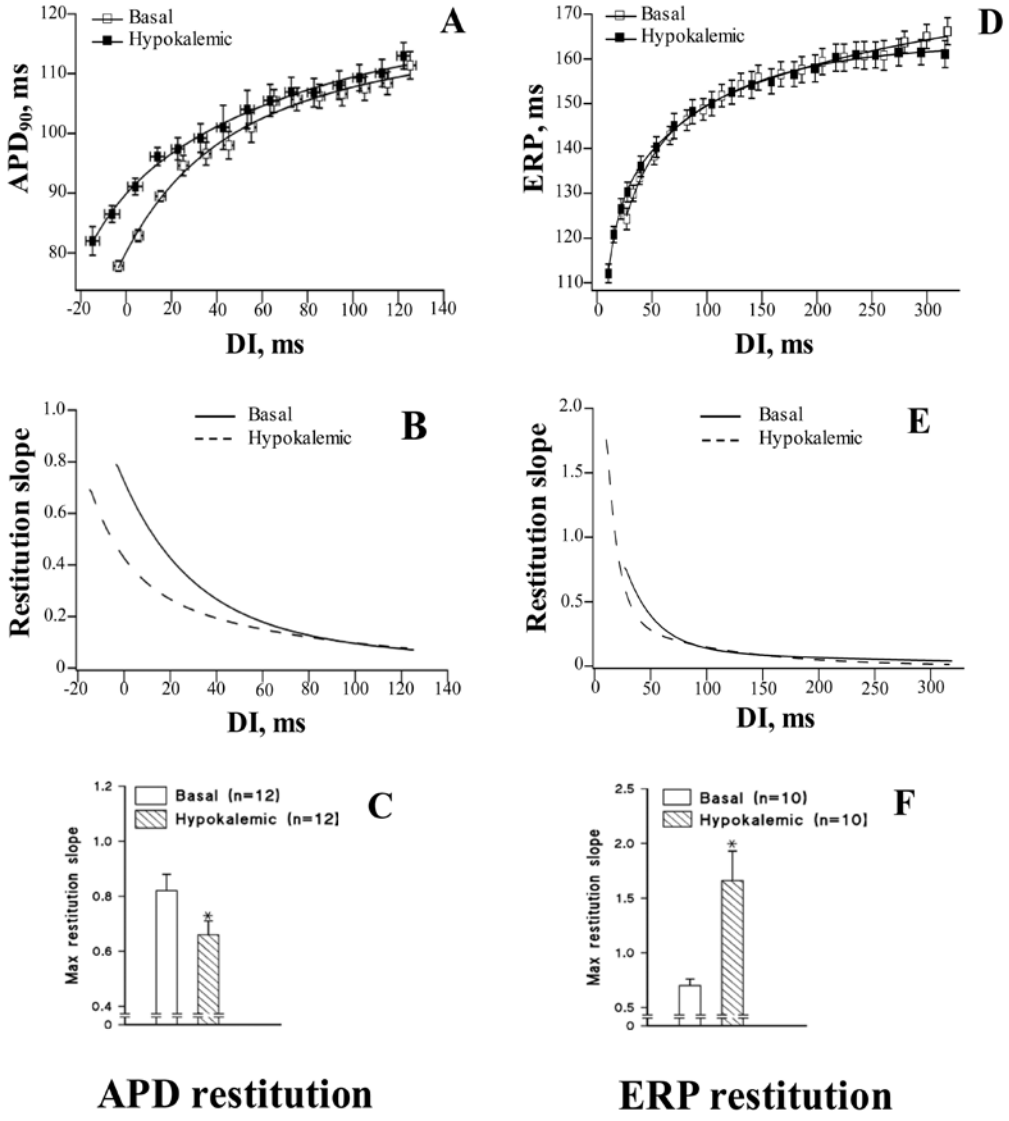

Figure 17. Effects of hypokalemia on the restitution of action potential duration (A, B and C) and effective refractory period (D, E and F) in perfused guinea-pig hearts

Monophasic action potential duration (APD_{90}) (panel A) was assessed at the LV epicardium during premature extrastimulus application at variable S_1-S_2 stimulation intervals. The effective refractory period (ERP) (panel D) was assessed at the LV epicardium over a wide range of regular pacing rates. Both APD_{90} and ERP were plotted as a function of the preceding diastolic interval (DI). The restitution curves shown in panels A and D were differentiated in order to determine changes in the restitution slope over the range of DIs used (panels B and E). Mean values of the maximum restitution slope (panels C and F) were calculated using individual measurements from each experiment. *P<0.05 vs. basal value (in panels C and F). Adapted from study I (Osadchii, O.E. *et al.* 2010. Predictive value of electrical restitution in hypokalemia-induced ventricular arrhythmogenicity. *Am J Physiol Heart Circ Physiol* 298, H210-H220).

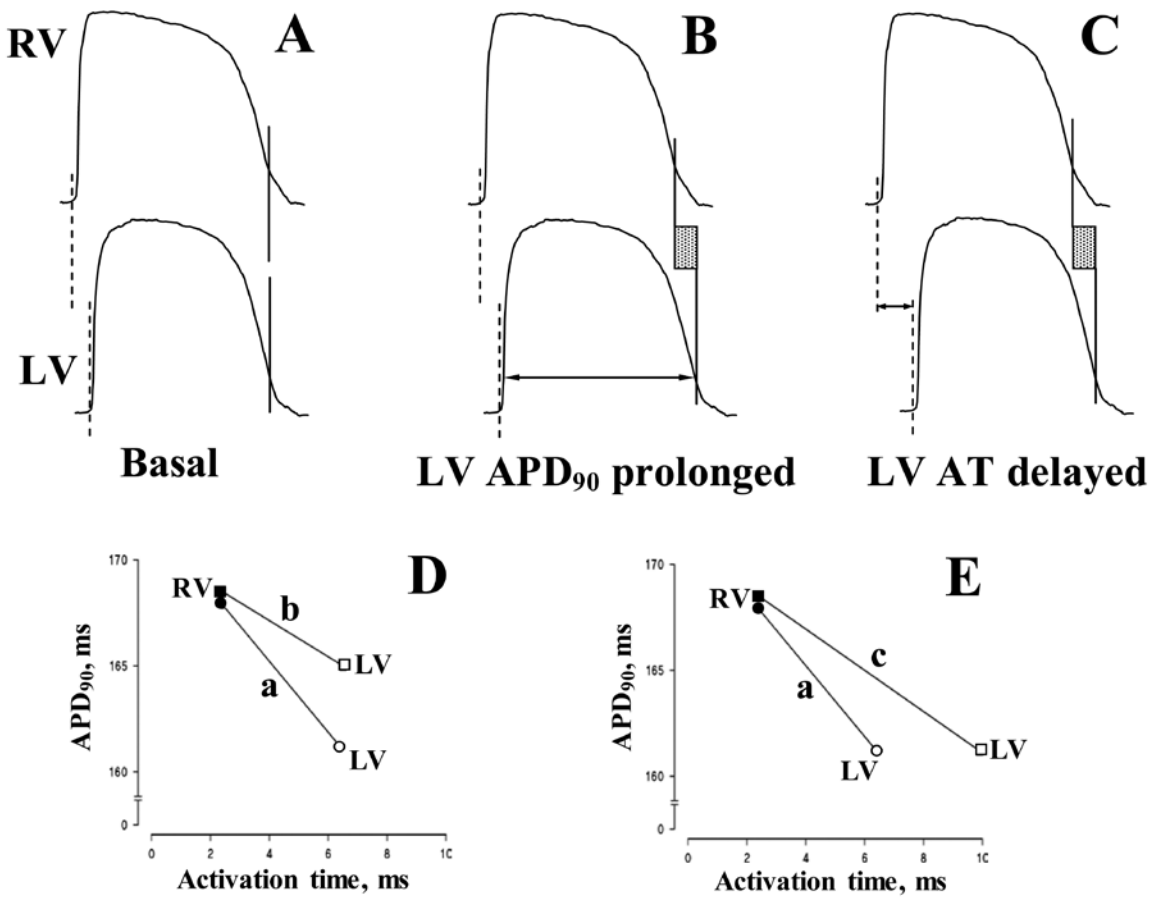

Figure 18. A schematic description of the electrophysiological mechanisms by which hypokalemia may impair the RV-to-LV activation-repolarization coupling in guinea-pig heart.
Monophasic action potential durations (APD_{90}) and local activation times (AT) were determined in the RV and LV epicardium during regular beating. See text for the comments on APD to AT relationships seen in normokalemic and hypokalemic conditions. Adapted from study X (Osadchii,O.E. 2014. Impaired epicardial activation-repolarization coupling contributes to the proarrhythmic effects of hypokalemia and dofetilide in guinea-pig ventricles. *Acta Physiol (Oxf.)* 211, 48-60), with permission from publisher.

18. During normokalemic perfusion of guinea-pig hearts, there is an inverse relationship between APD and activation time over the RV-to-LV transepicardial plane (panel A and line **a** in panel D). In this setting, the intrinsic RV-to-LV difference in APD is compensated for by the appropriate difference in activation time, which allows the moments of final repolarization in the RV and LV chambers to be synchronized (as shown by vertical solid lines in panel A).

Hypokalemia produces two proarrhythmic modifications. First, hypokalemia prolongs APD in LV relative to RV (as shown by double arrow in panel B), which results in an increased interventricular difference in the final repolarization time (as shown by a dotted rectangle). In the graphical form, LV APD lengthening translates to flattening of the linear slope of APD to the activation time relationship (shown by line **b** in panel D), thus indicating attenuated activation-repolarization coupling. Second, hypokalemia increases interventricular conduction delay (as shown by double arrow in panel C), which again translates to increased RV-to-LV dyssynchrony in final repolarization time, in association with a flattened linear slope of the APD to activation time relationship (shown by line **c** in panel E). The aforementioned changes may be further exacerbated in extrasystolic beats (Osadchii 2014c: study X), wherein hypokalemia accentuates spatial non-uniformities in APD as well as conduction slowing provoked by premature ventricular activation.

Clinically, impaired activation-repolarization coupling in hypokalemia, and the loss of interventricular synchrony in repolarization time, are likely to lead to an increased delay in the onset of diastolic relaxation between the RV and LV chambers. This would set abnormal pressure gradients across the ventricular septum, and increase the propensity for stretch-induced arrhythmia (Hardziyenka *et al.* 2009).

11. Arrhythmogenic responses to antiarrhythmic drugs

11.1 Classification of clinically used antiarrhythmic drugs

Antiarrhythmic agents are subdivided into four classes, according to the Vaughan Williams' classification (Vaughan Williams 1984; for review see Singh 1999; Kowey *et al.* 2000; Roden 2006; Shu *et al.* 2009).

Class I agents are Na^+ channel blockers that reduce the maximum velocity of the action potential upstroke and prolong ERP. The former contributes to slowed conduction of both

sinus and ectopic beats, and the latter accounts for the suppression of premature ventricular contractions, and elimination of the excitable gap in the re-entrant circuit. Therefore, prolongation of ERP partly eliminates both the arrhythmic trigger and the vulnerable substrate. In the re-entrant circuit, conduction slowing by class I agents may cause the unidirectional conduction block to convert into the bidirectional block, which would suppress arrhythmia. On the other hand, slowed ventricular conduction also contributes to the reduced excitation wavelength, thus facilitating arrhythmogenesis. These drugs are subdivided into three subgroups according to the fast (class Ib), intermediate (class Ia), and slow (class Ic) kinetics of binding and dissociation from the Na^+ channel.

Class II agents are beta-blockers that eliminate proarrhythmic effects associated with adrenergic over-activation. These agents abolish EADs and DADs induced by catecholamines, and suppress abnormal automaticity resulting from adrenergic-mediated increase in the slope of spontaneous diastolic depolarization in ectopic pacemakers. Beta-blockers are commonly used to prevent sudden cardiac death in clinical conditions associated with sympathetic over-activation, for instance, in heart failure and acute myocardial infarction.

Class III includes agents that prolong ventricular repolarization via the blockade of outward K^+ currents, primarily I_{Kr}. A drug-induced increase in APD accounts for prolonged ERP, which increases excitation wavelength and hence terminates re-entry. In contrast to class I drugs, the class III agents do not cause conduction slowing. Nevertheless, an excessive prolongation of APD may facilitate EADs, and hence favor cardiac arrhythmia, at least in some settings.

Class IV agents are the L-type Ca^{2+} channel blockers that suppress arrhythmogenic effects resulting from increased Ca^{2+} entry, such as EADs, DADs, and ectopic activity

resulting from the increased slope of phase 4 depolarization. As these agents also slow AV nodal conduction, they are used for ventricular rate control in atrial fibrillation. I_{Ca} inhibition, nevertheless, produces a negative inotropic effect, which imposes serious limitations for using these agents to treat arrhythmia in patients with heart failure.

The Vaughan Williams' classification does not encompass the entire armamentarium of available antiarrhythmic therapies. For example, cardiac glycosides, adenosine, and magnesium, although possessing antiarrhythmic effects, are not included in this classification. The arrhythmia could also be corrected by eliminating detrimental structural changes in diseased hearts with non-antiarrhythmic medications, like inhibitors of the renin-angiotensin-aldosterone system, fish oil, or statins. This approach, called *upstream therapy*, is beyond the scope of the Vaughan Williams' classification.

11.2 Antiarrhythmic drug-induced proarrhythmia: lessons from clinical studies

In susceptible patients, antiarrhythmic drugs at therapeutic concentrations may paradoxically aggravate existing cardiac electrical instability or even provoke new symptomatic arrhythmia (Velebit *et al.* 1982; Stanton *et al.* 1989; for review see Roden & Anderson 2006; Frommeyer & Eckardt 2016). The arrhythmogenic responses range from an increased number of ectopic beats, which is rarely perceived by the patient, to malignant VT that can lead to sudden death. While these adverse reactions have been recognized for many years (see e.g. Selzer & Wray 1964), the problem of drug-induced arrhythmia has attracted broad attention relatively recently in connection with the reports from two landmark, large-scale clinical trials, CAST and SWORD. The Cardiac Arrhythmia Suppression Trial (CAST) involved patients with healed myocardial infarction in order to test the hypothesis that the suppression of ventricular ectopy with class Ic Na^+ channel blockers, flecainide and encainide, can decrease the incidence of sudden death (CAST Investigators 1989). Contrary to

expectations, the mortality in the treatment group was found to be 2.5-fold greater compared to patients receiving placebo after a mean follow-up of only 10 months, and the study was discontinued. Another clinical trial, called Survival With Oral D-sotalol (SWORD), was designed to determine whether ERP prolongation by class III antiarrhythmic agent can reduce mortality in patients with LV systolic dysfunction and a prior myocardial infarction (Waldo *et al.* 1996). The SWORD trial, nevertheless, has shown that overall mortality in d-sotalol-treated patients is higher than in the placebo group, with two-thirds of the deaths being interpreted as arrhythmic.

Overall, these trials demonstrate that an empirical approach for arrhythmia treatment is likely to be irrational, as the promising agent that eliminates one arrhythmic mechanism may nevertheless exacerbate others. For example, flecainide may suppress arrhythmia by reducing ectopic activity, but at the same time facilitates arrhythmia via conduction slowing. Sotalol, while producing antiarrhythmic effects owing to prolonged refractoriness, may nevertheless facilitate arrhythmogenic EADs.

Importantly, in some patients, the drug-induced arrhythmia could be more threatening to survival than the arrhythmia being suppressed. The clinical trials on atrial fibrillation, a relatively benign arrhythmia, have shown that Na^+ channel blocker or class III agent therapies aimed at maintaining sinus rhythm may nevertheless induce adverse electrical changes in ventricles, and precipitate life-threatening VT (for review see Falk 1992; Shantsila *et al.* 2007; Camm 2012). The ventricular proarrhythmic responses are recognized as a serious limitation of existing opportunities in pharmacological control of atrial fibrillation.

Drug-induced arrhythmia can be attributed to either *excessive conduction slowing* (e.g., with class Ia or Ic Na^+ channel blocker), or *excessive prolongation of ventricular APD* (e.g., with class III agent), or both (for review see Levine *et al.* 1989; Nattel 1998; Shu *et al.* 2009).

These effects are favored by many confounding factors, including the presence of organic heart disease, myocardial ischemia, contractile failure, elevated adrenergic tone, and hypokalemia. In many patients, however, drug-induced proarrhythmia is poorly predictable and occurs without any premorbid warning signs.

11.3 Proarrhythmia induced by Na^+ channel blockers

11.3.1 Mechanism of action of class I agents at the ion channel level

Na^+ channels may be found in *closed (or rested) state* (during diastole), *open state* (during phase 0 of action potential), or *inactivated state* (during plateau of action potential). I_{Na} blockers exhibit much higher affinity to either open (class Ia and Ic agents) or inactivated (class Ib agents) channels compared to the rested state (*the modulated receptor hypothesis*; for review see Hondeghem 1987). This means that the drug binding affinity substantially varies in the course of a cardiac cycle; during each action potential, the agent binds to Na^+ channel and blocks it, and over the following diastolic interval, it dissociates from the channel, and the block is released. These binding properties translate into important practical implications (for review see Kecskemeti *et al.* 1991; Tamargo *et al.* 1992; Glaaser & Clancy 2006; Roden 2006). First, the drug-induced I_{Na} block is much stronger at fast than normal cardiac activation rates, an effect that is termed the *use-dependency*. The reason for this is the increased time spent by the Na^+ channels in the open/inactivated state during tachycardia, secondary to both the increased number of depolarizations per unit of time, and the shortened diastolic interval for drug unbinding and channel recovery. The use-dependency importantly contributes to antiarrhythmic effects, because it implies that class I agents produce the most prominent conduction slowing and prolongation of refractoriness during the rapid activation rates that occur in tachyarrhythmia.

Second, because the transition from open to inactivated states is promoted upon

depolarization, physiological factors that depolarize myocyte sarcolemma, such as ischemia, hyperkalemia, and acidosis, typically increase the number of inactivated Na^+ channels. As a result, the *inactivated state blockers*, such as class Ib agents, can bind to a greater proportion of Na^+ channels, and therefore produce more powerful blocking effects under these conditions.

Lastly, because the time spent by Na^+ channels in the inactivated state is increased upon lengthening the plateau of action potential, the effect of class Ib blockers is likely to be enhanced in cardiac tissues with prolonged APD, such as Purkinje fibers. Class Ib agents are therefore more effective in suppressing ventricular rather than atrial tachyarrhythmia. These relations also provide a rationale for using inactivated state Na^+ channel blockers in combination with class III agents that delay ventricular repolarization; the latter may accentuate I_{Na} blocking actions of class Ib agents, and hence result in the more pronounced lengthening of the refractory period. In contrast, class Ia and Ic agents are more effective in cardiac tissue with shorter APD, especially in the presence of fast firing rates, which increases proportion of open Na^+ channels. These agents are therefore commonly used to treat supraventricular tachyarrhythmia, such as atrial fibrillation.

Class I includes drugs with variable kinetics of I_{Na} block. Class Ib agents exhibit fast onset/offset kinetics; the steady-state I_{Na} block is achieved in less than five cardiac beats, and the block dissipates in less than 0.5 s during diastole. These agents, therefore, have only a minimal, if any, effect on V_{max}, and do not slow ventricular conduction at normal heart rates, at least in non-ischemic conditions. Class Ia agents exhibit slower onset/offset kinetics; it takes 5 to 20 cardiac beats to achieve a steady-state I_{Na} block, and the block then dissipates in a few seconds. For example, with quinidine, the time constant of I_{Na} recovery is 3-8 s (Varro *et al.* 1985; Hondeghem & Matsubara 1988). The latter means that drug unbinding in diastole,

and hence I_{Na} block, is not fully resolved till generation of the next action potential. These agents can therefore reduce V_{max} and provoke some conduction slowing at normal beating rates, with the effect being accentuated in tachycardia. Class Ic agents exhibit the slowest kinetics of action; it takes more than 20 cardiac beats to achieve a steady-state I_{Na} block, and the drug dissociation from the channel requires more than 10 s. Because this by far exceeds diastolic time over the range of clinically relevant heart rates, the I_{Na} block may persist and cumulate over successive cardiac beats. Class Ic agents, therefore, markedly depress V_{max} and slow ventricular conduction, even at normal heart rates.

The three subgroups of class I agents are also different with regards to their effects on ventricular repolarization. Class Ib agents are pure I_{Na} blockers that may cause a slight APD shortening by reducing the late Na^+ current during the plateau phase. In contrast, class Ia agents typically prolong ventricular APD, as they (in addition to blocking I_{Na}) can inhibit outward K^+ currents. With class Ic agents, such as flecainide, the effects on ventricular repolarization are traditionally thought of as being modest. Nevertheless, there is emerging evidence to demonstrate that a prolongation of ventricular APD, in connection with resulting arrhythmia, should be considered an important attribute of class Ic profile of action (Osadchii 2015).

Clinically, proarrhythmic responses have been largely observed while using class Ia and Ic Na^+ channel blockers, whereas class Ib agents are recognized as safe antiarrhythmics (Frank *et al.* 1991; Shimizu & Antzelevitch 1997; Sims *et al.* 1997; Wyman *et al.* 2004; Lu *et al.* 2010). The following sections will discuss the mechanisms that may potentially contribute to the dissimilar safety profile of I_{Na} blockers.

11.3.2 Ventricular electrophysiological effects induced by class Ia and Ic vs. class Ib agents

11.3.2.1 Transmural gradients in refractoriness

Proarrhythmic effects produced by Na^+ channel blockers are commonly explained by slowing of ventricular conduction, which could be particularly detrimental in post-infarcted, structurally remodeled myocardium (Coromilas *et al.* 1995; Restivo *et al.* 1995). This stimulated the attempts to develop "good" (i.e. lidocaine-like) I_{Na} blockers with no or minimal effects on conduction, as opposed to the "bad" (i.e. flecainide-like) agents that provoke substantial conduction slowing (Lu *et al.* 2010). Nevertheless, the slowed conduction would be unlikely to account for the whole range of arrhythmogenic effects produced by I_{Na} blockers in a clinical setting. Indeed, when used to treat atrial fibrillation, class Ia and Ic agents may often provoke ventricular proarrhythmia in patients with no history of angina or clinical signs of myocardial ischemia, as evidenced by exercise tests or coronary angiography (Prystowsky 1996). The mechanism of drug-induced arrhythmia in normal hearts, free of ischemia and structural remodeling, remains incompletely understood.

In study IV (Osadchii 2012c), the hypothesis was proposed that the difference in safety profile with class Ia and Ic vs. class Ib agents is determined by the reciprocal distribution pattern of I_{Na} and I_{Kr} channels across the ventricular wall. The latter sets a basis for dissimilar changes in transmural ERP dispersion, and hence diverse drug effects on arrhythmic vulnerability. Figure 19 schematically illustrates this principle. In normal hearts, action potential duration, and hence ERP, is longer at the endocardium than the epicardium (panel A). Transmural ERP dispersion could be modified upon drug administration by changingeither Na^+ channel availability, and hence the recurrence of excitability in phase 3 (panel B), or changing I_{Kr} channel availability, and hence APD, at the endocardium vs. the epicardium (panel C). The expression of I_{Na} was shown to be greater at the endocardium than the epicardium (Ashamalla *et al.* 2001; Rosati *et al.* 2006; Gaborit *et al.* 2007; Remme *et al.* 2009; Osadchii *et al.* 2011). Because of the lower I_{Na} reserve, epicardial myocytes are likely

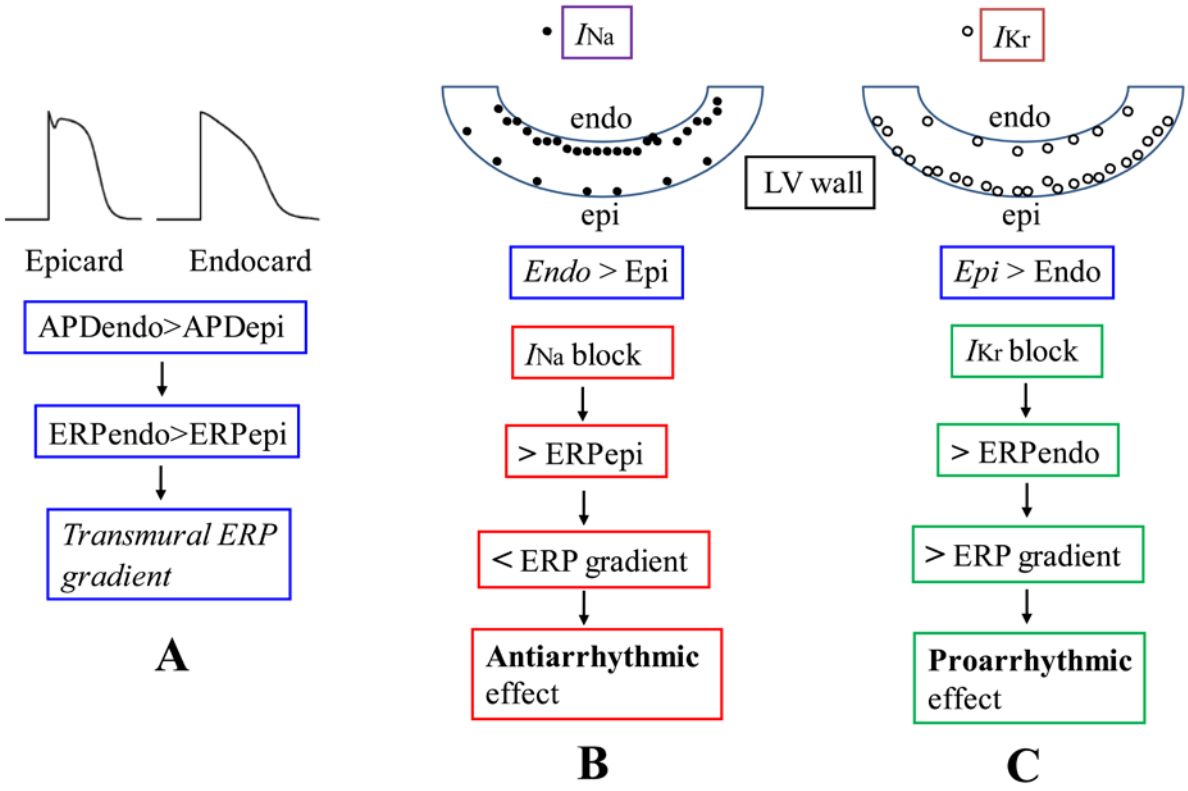

Figure 19. The putative role of transmural distribution of I_{Na} and I_{Kr} in shaping electrophysiological profile of action of class Ia and Ic vs. class Ib agents

APD, action potential duration; ERP, effective refractory period; LV, left ventricular; Endo, endocardium; Epi, epicardium, I_{Na}, the fast Na^+ current; I_{Kr}, the rapid component of the delayed rectifier K^+ current. Filled and open circles within the LV wall (panels B and C) illustrate the epicardial-to-endocardial difference in expression levels of I_{Na} and I_{Kr}. See text for more details. Adapted from study IV (Osadchii, O.E. 2012. Impact of Na^+ channel blockers on transmural dispersion of refractoriness and arrhythmic susceptibility in guinea-pig left ventricle. *Eur J Pharmacol* 691, 173-181), with permission from publisher.

to be more susceptible to ERP lengthening by I_{Na} blocker, than endocardial cells. A greater increase in ERP at the epicardium than the endocardium would contribute to reduced transmural ERP difference, indicating an *antiarrhythmic effect* (panel B). In contrast, I_{Kr} expression levels are greater at the epicardium than the endocardium (Bryant *et al.* 1998; Main *et al.* 1998; Wan *et al.* 2003). Because of the lower I_{Kr} reserve, ventricular endocardium

typically exhibits a greater prolongation of APD, and hence ERP, upon selective I_{Kr} blocker administration (Yan *et al.* 2001b; Kozhevnikov *et al.* 2002). This would contribute to increased transmural ERP dispersion, indicating a *proarrhythmic effect* (panel C). Overall, these arguments raise the possibility that the antiarrhythmic effect of selective I_{Na} blockers, such as class Ib agents, resulting from reduced transmural ERP dispersion, may be eliminated, or even reversed to proarrhythmia, in the presence of concomitant I_{Kr} blockade (e.g. caused by class Ia or Ic agents).

In support of this hypothesis, lidocaine and mexiletine, class Ib agents with selective I_{Na} blocking effects, were found to increase ERP at the epicardium, while producing no effect at the endocardium, in perfused guinea-pig hearts (Osadchii 2012c: study IV). These changes translated to reduced transmural ERP dispersion. No spontaneous or extrasystolic stimulation-induced VT was observed upon the infusion of lidocaine and mexiletine. In contrast, class Ia (quinidine, procainamide) and class Ic (flecainide) agents that possess both I_{Na} and I_{Kr} blocking effects produced a greater ERP prolongation at the endocardium than the epicardium, thus increasing transmural ERP dispersion, and facilitating arrhythmia. Spontaneous episodes of short-lasting monomorphic VT were observed in about 40% of heart preparations during the infusion of class Ia or Ic Na^+ channel blockers. Similar changes in both transmural ERP dispersion and the occurrence of spontaneous VT were observed upon the administration of dofetilide, a selective I_{Kr} blocker, thus lending support to the notion that arrhythmogenic responses to class Ia and Ic agents could be partially accounted for by the presence of I_{Kr}-blocking properties in their electrophysiological profile.

11.3.2.2 Ventricular action potential duration and conduction times upon premature ectopic activation

The assessment of I_{Na} blocker effects on APD and conduction of premature ectopic

beats can be critical for understanding the mechanisms contributing to the distinct safety profile of class Ib vs. class Ia and Ic agents. The rationale for this contention is two-fold. First, fatal VT is often initiated by premature ectopic activation superimposed on the T wave of the preceding sinus beat (the "R-on-T" phenomenon) (Lewis *et al.* 1983; Panidis & Morganroth 1983; Kempf & Josephson 1984; Milner *et al.* 1985; Locati *et al.* 1995). In survivors of acute myocardial infarction, the presence of frequent closely coupled extrasystolic beats on ECG has been shown to independently predict the increased risk of sudden arrhythmic death (Ruberman *et al.* 1981; Mukharji *et al.* 1984; Kostis *et al.* 1987). Second, the increased propensity to develop VT following ectopic activation is thought to be accounted for by amplified regional non-uniformities in ventricular conduction and APD (Kuo *et al.* 1983, 1985; Laurita *et al.* 1996, 1998; Osadchii 2014b: study IX). When premature stimulus is applied immediately upon the recurrence of excitability in phase 3, the induced extrasystolic beat propagates with an increased conduction delay owing to the incomplete recovery of Na^+ channels from inactivation. The amount of conduction slowing can be variable in different myocardial regions, partly owing to the spatial heterogeneities in Na^+ channel expression. The premature activation is also associated with APD shortening (see section 6.2), which is non-uniform at different ventricular sites owing to heterogeneities in the distribution of repolarizing K^+ currents. The amplified dispersion of local conduction times and APD contributes to markedly increased vulnerability to re-entry following ectopic activation.

These considerations imply that the safety profile of a Na^+ channel blocker can be importantly determined by the ability to either reduce or further exaggerate proarrhythmic changes associated with ectopic activation. In contrast to class Ib agents, class Ia and Ic drugs possess I_{Kr}-blocking effects, which translates to APD prolongation, possibly leading to increased spatial repolarization gradients. Furthermore, in contrast to class Ib agents, class Ia

and Ic drugs exhibit much slower Na^+ channel unbinding rates, and hence provoke substantial conduction slowing; this would likely exacerbate conduction abnormalities imposed by ectopic activation. In study IX (Osadchii 2014b), these possibilities were addressed by measuring local activation latencies and APDs in six LV and RV epicardial recording sites in perfused guinea-pig hearts, both during regular pacing and extrasystolic stimulations applied immediately upon the termination of ERP. Both flecainide and quinidine were found to produce non-uniform conduction slowing throughout the ventricular epicardium in extrasystolic beats, thus further accentuating an increase in dispersion of the conduction time imposed by premature activation. Likewise, both flecainide and quinidine were found to prolong repolarization in extrasystolic beats, as compared to values determined prior to drug infusion. The increase in APD was spatially non-uniform, being greater at LV than RV sites, contributing to increased transepicardial APD dispersion. In contrast, lidocaine and mexiletine, class Ib agents, had no significant effects on either conduction time or APD dispersion in extrasystolic beats.

These results support the notion that the proarrhythmic potential of class Ia and Ic Na^+ channel blockers in a clinical setting may be partly attributed to the aggravation of electrical derangements associated with premature ectopic activation. Hence, the risk for drug-induced arrhythmia can be particularly high in patients that exhibit frequent ventricular ectopic beats.

11.3.3 Cardiac arrhythmia and repolarization abnormalities provoked by individual Na^+ channel blockers

11.3.3.1 Quinidine

Quinidine therapy is recognized as one of the most common causes of acquired long QT syndrome; this agent is estimated to prolong QT interval and provoke torsade de pointes in up to 5% of treated patients (Bauman *et al.* 1984; Roden *et al.* 1986). The clinical risk

factors for quinidine-induced arrhythmia include hypokalemia and bradycardia resulting from the high-grade AV block. The arrhythmia is often precipitated by abrupt changes in cardiac cycle length, which could be exemplified by a sudden heart rate slowing upon conversion of atrial fibrillation to sinus rhythm. In connection with these relations, quinidine therapy in atrial fibrillation is often associated with increased mortality rates, especially in patients with organic heart disease (Coplen *et al.* 1990; Flaker *et al.* 1992).

Quinidine has a complex electrophysiological profile. Apart from reducing I_{Na}, it inhibits several repolarizing K^+ currents, including the delayed rectifier (both I_{Kr} and I_{Ks}) (Roden *et al.* 1988; Balser *et al.* 1991; Yang *et al.* 2001; Paul *et al.* 2002; Iost *et al.* 2003; Wu *et al.* 2008), the inward rectifier (I_{K1}) (Salata & Wasserstrom 1988), and I_{to} (Imaizumi & Giles 1987; Slawsky & Castle 1994; Iost *et al.* 2003). Quinidine also decreases inward currents that contribute to the plateau of action potential, such as L-type I_{Ca} (Nawrath 1981; Hiraoka *et al.* 1986; Salata & Wasserstrom 1988), and the late I_{Na} (Salata & Wasserstrom 1988; Wu *et al.* 2008). Collectively, these changes translate to APD prolongation, with the effect being greater at slower than at faster heart rates. Quinidine also modifies the configuration of ventricular action potential; as quinidine shortens the plateau phase, but delays the late repolarization, it contributes to triangular action potential waveform (Hondeghem & Hoffmann 2003; Guerard *et al.* 2008; Wu *et al.* 2008; Osadchii 2013: study VII). As a result, quinidine significantly increases the time spent over the voltage window for I_{Ca} recovery from inactivation, thus facilitating the occurrence of EADs. The EADs, predominantly originating in Purkinje fibers, are thought to serve as a *trigger* for torsade de pointes in quinidine-treated hearts (Roden & Hoffman 1985; Davidenko *et al.* 1989; El-Sherif *et al.* 1989).

Much less is known about the mechanisms by which quinidine therapy may contribute to the vulnerable *arrhythmogenic substrate*. Indeed, quinidine effects on spatial repolarization

gradients are reportedly inconclusive. Although quinidine was shown to increase the dispersion of ventricular repolarization in perfused rabbit heart (Zabel *et al.* 1997; Wu *et al.* 2008), other studies reported no effect (Dhein *et al.* 1993) or reduced repolarization gradients (Milberg *et al.* 2007a) in the same animal model. Quinidine was found to reduce the transmural dispersion of refractoriness in a mouse heart (Martin *et al.* 2011), and had no effect on APD dispersion in canine RV (Brugada *et al.* 1987); these changes suggest an antiarrhythmic (rather than proarrhythmic) profile of action.

The role played by repolarization abnormalities in the mechanism of VT produced by quinidine was further examined in studies on perfused guinea-pig hearts (Osadchii 2013: study VII). During dynamic pacing, quinidine produced a reverse use-dependent increase in APD, with the effect being significantly greater at long compared to short diastolic intervals. As a result, both the amplitude (i.e., the maximum to minimum APD change) and maximum slope of the restitution curve were increased by quinidine (Figure 20, panels A, B and C), indicating a proarrhythmic tendency. Throughout the ventricular epicardium, APD lengthening by quinidine was found to be non-uniform (Figure 20, panel D), with the greatest effect produced at the LV posterior wall (LV epi site 3), and the minimum effect produced at the RV lateral wall (RV epi site 2). Epicardial APD dispersion was significantly increased in quinidine-treated hearts (Figure 20, panel E). At slow cardiac pacing rates, quinidine elicited a greater increase in APD compared to refractory period, thereby markedly extending the vulnerable window (i.e., the APD_{90}-to-ERP difference) for LV re-excitation by premature ectopic activation during late repolarization. This change was accounted for by modifications in the time course of ventricular repolarization; quinidine was found to produce a greater APD prolongation at 90% than 30% repolarization time points, thus increasing triangulation of the action potential waveform. Over the transmural plane, quinidine elicited a greater

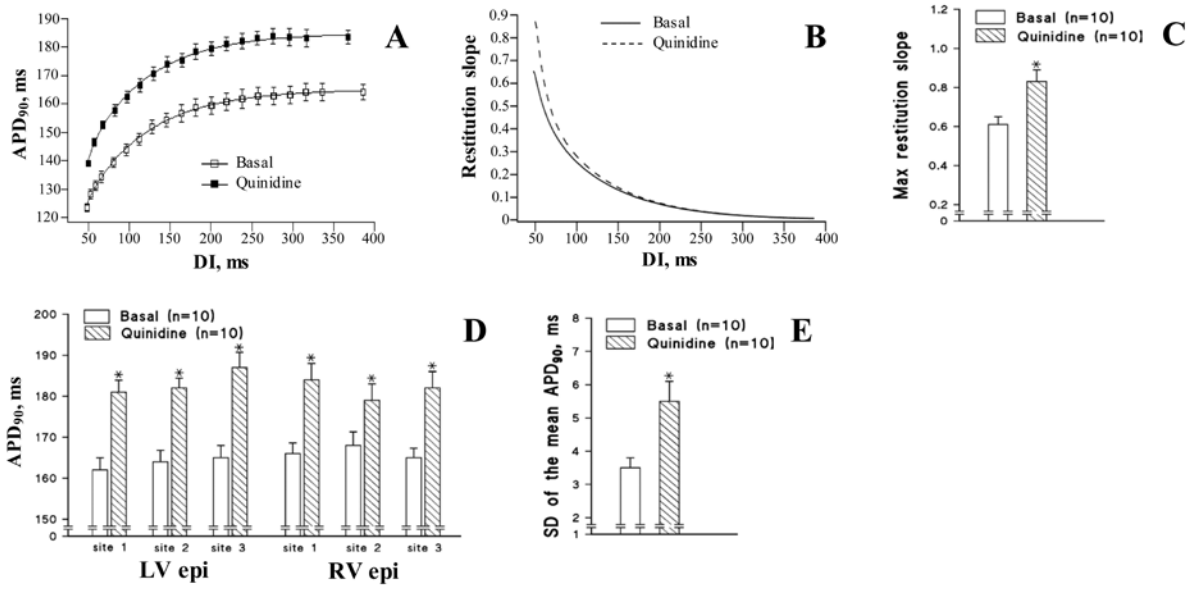

Figure 20. Effects of quinidine on electrical restitution (A, B and C) and spatial dispersion of action potential duration (D and E) in perfused guinea-pig hearts

Mean left ventricular (LV) epicardial (epi) monophasic action potential duration (APD_{90}) was determined over a wide range of progressively reducing pacing cycle lengths (S_1-S_1 from 550 ms to 160 ms), and then plotted as a function of the preceding diastolic interval (DI) in panel A. Panel B shows changes in APD_{90} restitution slope upon dynamic pacing, and panel C shows the maximum (Max) restitution slope values determined before and after quinidine infusion. Panel D shows quinidine effects on APD_{90} measured in six individual recording sites throughout the ventricular epicardium at S_1-S_1 pacing interval of 550 ms (LV: site 1 is the lateral LV wall, site 2 is the anterior LV wall, and site 3 is the posterior LV wall; RV: site 1 is the posterior RV wall, site 2 is the anterior RV wall, and site 3 is the lateral RV wall). The standard deviation (SD) of the mean epicardial APD_{90} value determined at baseline and upon quinidine infusion is shown in panel E. *p<0.05 vs basal value (in panels C, D and E). Adapted from study VII (Osadchii, O.E. 2013. Quinidine elicits proarrhythmic changes in ventricular repolarization and refractoriness in guinea-pig. *Can J Physiol Pharmacol* 91, 306-315), with permission from publisher.

prolongation of endocardial than epicardial ERP, which translated to the amplified dispersion of refractoriness. Taken together, these findings strongly suggest that multiple derangements in ventricular repolarization and refractoriness can importantly contribute to the

arrhythmogenic substrate during quinidine treatment.

11.3.3.2 Procainamide

Procainamide is class Ia Na^+ channel blocker with an electrophysiological profile similar to quinidine. It is widely used to control hemodynamically stable VT and various supraventricular arrhythmias, including atrial flutter and fibrillation. The important pharmacokinetic feature of this agent is that it is extensively metabolized via acetylation in the liver, thus producing an active metabolite, N-acetylprocainamide. The metabolite contributes about 70% of the antiarrhythmic activity of the parent drug.

The therapeutic effects of procainamide are often offset by adverse electrical changes which either reduce its clinical efficacy or even translate to drug-induced arrhythmia. In 50-70% of patients with organic heart disease, intravenous procainamide infusion fails to prevent the induction of VT by programmed stimulation, or even facilitates it (Engel *et al.* 1980; Kang *et al.* 1982; Gallagher *et al.* 1986; Furukawa *et al.* 1989; Stevenson *et al.* 1990; Buxton *et al.* 1991; Sager *et al.* 1992). This refers to the increased number of trials with inducible arrhythmia, to the conversion of non-sustained to sustained VT, or to arrhythmia induction with a fewer extrastimuli. These findings lend support for the suggestion that procainamide can unmask potential re-entry circuits (Stevenson *et al.* 1990), thus providing a rationale for using intravenous procainamide as a provocative challenge that increases the sensitivity of electrophysiological testing in detecting arrhythmic vulnerability (Schreibman *et al.* 2004).

Procainamide was found to occasionally precipitate monomorphic VT when administered to restore sinus rhythm in atrial fibrillation (Fenster *et al.* 1983), or to suppress ectopic activity (Bhandari *et al.* 1986). There is also increasing evidence to show that procainamide, or its active metabolite, N-acetylprocainamide, can prolong QT interval and increase the risk for torsade de pointes (Sclarovsky *et al.* 1979; Strasberg *et al.* 1981;

Olshansky *et al.* 1982; Chow *et al.* 1984; Tzivoni *et al.* 1984; Herpe & Thompson 1985; Stevenson *et al.* 1985; Stratmann *et al.* 1985; Nguyen *et al.* 1986; Habbab & El-Sherif 1990; Prystowsky 1996). Patients characterized as rapid acetylators may have higher than expected plasma levels of N-acetylprocainamide, and hence exhibit an increased propensity for drug-induced arrhythmia. It is therefore recommended that similar to quinidine or class III agents, procainamide therapy should preferably be initiated in the hospital, wherein QT interval monitoring is performed, and the drug dose is appropriately titrated in order to eliminate the chance of developing arrhythmia.

The arrhythmogenic responses to procainamide have been ascribed to drug-induced conduction slowing, an effect that contributes to the increased excitable gap within the re-entrant circuit, and hence sustains arrhythmia (Furukawa *et al.* 1989). Nevertheless, the same mechanism may be employed to explain the antiarrhythmic effect of this agent. For example, it was pointed out that procainamide-induced conduction slowing in the depolarized portion of the re-entrant pathway can potentially result in conversion of the unidirectional conduction block to a bidirectional one, thus terminating VT (Giardina & Bigger 1973). These changes are likely to account, at least in part, for the inter-individual variability of cardiac responses (antiarrhythmic, proarrhythmic, or neutral) to procainamide seen in a clinical setting. In this context, careful consideration should be given to physiological factors that critically determine the amount of procainamide-induced conduction slowing, such as myocardial ischemia, fast heart rates, high extracellular K^+ levels, and the concomitant administration of other antiarrhythmic drugs (Myerburg *et al.* 1982; Cascio *et al.* 1987; Steinberg *et al.* 1992).

Another mechanism for procainamide-induced arrhythmia could be related to the non-uniform prolongation of refractory period, an effect shared with other class Ia (quinidine) and class Ic (flecainide) Na^+ channel blockers (see section 11.3.2.1 for discussion). In the perfused

guinea-pig heart, procainamide was found to increase the dispersion of refractoriness in the LV wall (Osadchii 2012c: study IV). In infarcted hearts, procainamide can impair spatial gradients in ERP by producing non-uniform changes in refractoriness in ischemic vs. normal myocardial regions (Michelson *et al.* 1981b).

Finally, procainamide may promote arrhythmia by inducing multiple repolarization abnormalities. Procainamide has been shown to inhibit I_{Kr} in rabbit ventricular myocytes (Iost *et al.* 2003) and human embryonic kidney cells expressing the HERG channel (Ridley *et al.* 2003), an effect translating to the dose-dependent prolongation of ventricular APD in human patients (Platia *et al.* 1988; Lee *et al.* 1992) and various animal models (Myerburg *et al.* 1982; Kirchhof *et al.* 1998). This change can also contribute to excessive QT lengthening and increased torsadogenic risks during procainamide treatment, as mentioned above. In study VIII (Osadchii 2014a), procainamide-induced APD lengthening was associated with a steepened electrical restitution slope upon dynamic pacing in perfused guinea-pig hearts (Figure 21, panels A, B and C), thus indicating a proarrhythmic tendency. Furthermore, the effect of procainamide on APD was found to be non-uniform across the ventricular epicardium, being greater in the LV than the RV chamber (Figure 21, panel D). This translated to increased spatial APD dispersion (Figure 21, panel E) and impaired RV-to-LV transepicardial activation-repolarization coupling (Figure 22, panels A and B). Notably, lidocaine, a class Ib I_{Na} blocker with a clinically safe profile, had no effect on electricalrestitution, spatial APD dispersion, and activation-repolarization coupling (Osadchii 2014a: study VIII).

11.3.3.3 Flecainide

The overall incidence of arrhythmic events upon flecainide therapy is about 7%, of which three quarters take the form of sustained VT (Morganroth *et al.* 1984, 1986; Fish *et al.*

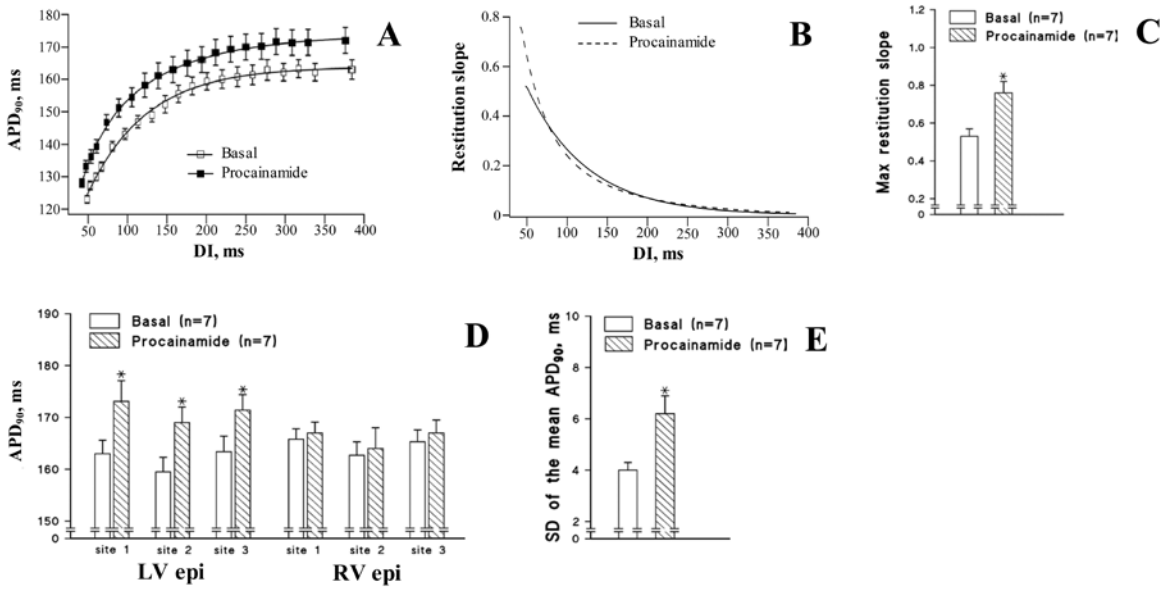

Figure 21. Effects of procainamide on electrical restitution (A, B and C) and spatial dispersion of action potential duration (D and E) in perfused guinea-pig hearts

The data are presented as explained in the legend for Figure 20. Adapted from study VIII (Osadchii, O.E. 2014. Procainamide and lidocaine produce dissimilar changes in ventricular repolarization and arrhythmogenicity in guinea-pig. *Fundam Clin Pharmacol* 28, 382-393), with permission from publisher.

1991). Because the Cardiac Arrhythmia Suppression Trial demonstrated a high risk for flecainide-induced proarrhythmia (CAST Investigators 1989), this agent is no longer prescribed to control arrhythmia in patients with organic heart disease and/or systolic dysfunction. Flecainide, nevertheless, remains one of the first line treatments for symptomatic atrial fibrillation in patients with structurally normal hearts (for review see Apostolakis *et al.* 2013). This practice, however, is increasingly challenged by clinical observations indicating that flecainide can induce VT, often in the form of torsade de pointes, in patients without coronary artery disease or severe heart failure (Muhiddin *et al.* 1982; Nathan *et al.* 1984; Falk 1989; Sihm *et al.* 1990; Prystowsky 1996; Ohki *et al.* 2001; Thevenin *et al.* 2003; Asensio *et al.* 2007; Hayes *et al.* 2013; Kim *et al.* 2013; Oguayo *et al.* 2014; Nasser *et al.* 2015). The

Pediatric Electrophysiology Group reported on the relatively high incidence of flecainide-induced proarrhythmia in young cardiac patients, presumably in the absence of advanced coronary atherosclerosis which is commonly seen in the elderly (Fish *et al.* 1991). Importantly, in a recent clinical trial in Sweden, flecainide treatment in patients with atrial fibrillation and no structural heart disease was found to be associated with a fourfold increased risk of cardiovascular mortality compared to the general population (Almroth *et al.* 2011). As the mechanism of VT is thought to involve repolarization abnormalities that set a stage for re-entry, the aforementioned studies imply that flecainide, in addition to its traditional I_{Na} blocking effects, could also adversely affect ventricular repolarization. There are several lines of evidence in support of this notion.

Firstly, patch-clamp recordings in single cardiac cells suggest that flecainide can significantly reduce repolarizing K^+ currents. Over a range of micromolar concentrations corresponding to its therapeutic plasma levels, flecainide has been found to produce up to 50% reduction in I_{Kr} in hERG-expressing cell lines (Paul *et al.* 2002; Du *et al.* 2011; Melgari *et al.* 2015), and ventricular myocytes from guinea-pig (Wang *et al.* 1996) and cat (Follmer & Colatsky 1990; Follmer *et al.* 1992). This change is likely to contribute to the prolongation of ventricular APD seen in different animal models (Borchard & Boisten 1982; Ikeda *et al.* 1985; Varro *et al.* 1986; Yabek *et al.* 1987; Osadchii 2012e: study VI), and human stem cell-derived cardiac myocytes (Gibson *et al.* 2014). In human patients, flecainide increases the RV monophasic action potential duration (Olsson *et al.* 1981), and prolongs QT interval (Katritsis *et al.* 1995; Sarubbi *et al.* 1998; Lim *et al.* 2010). Arrhythmogenic responses to flecainide are facilitated in the presence of well-known torsadogenic risk factors, such as bradycardia (Kim *et al.* 2013), hypokalemia (Ohki *et al.* 2001), and previously unsuspected long QT syndrome gene mutations (Lehtonen *et al.* 2007), which is in support of the role played by reduced

repolarization reserve in adverse effects associated with flecainide therapy.

Another contributor to flecainide-induced arrhythmia in structurally normal heart appears to be the increased spatial repolarization gradients. Flecainide-induced I_{Na} block results in a decreased amplitude of the action potential upstroke, which has an effect on the subsequent activation of I_{to}, the transient outward K^+ current. This translates to altered action potential morphology (an accentuated notch) and duration, with both modifications being more prominent at the epicardium than the endocardium owing to a greater epicardial I_{to} expression (Krishnan & Antzelevitch 1991, 1993). Non-uniform APD changes in the epicardium vs. the endocardium upon flecainide infusion then contribute to the increased transmural dispersion of repolarization, and promote re-entry (termed "phase 2 re-entry") in the canine heart (Krishnan & Antzelevitch 1991, 1993). Flecainide has also been shown to amplify transmural gradients in refractoriness in mouse (Martin *et al.* 2011) and guinea-pig heart (Osadchii *et al.* 2011; Osadchii 2012c: study IV), which can be accounted for by epicardial-to-endocardial non-uniformities in the distribution of I_{Na}. Alternatively, flecainide-induced arrhythmia can be attributed to increased repolarization heterogeneities at the level of the Purkinje fiber-ventricular muscle junction. Indeed, although flecainide delays repolarization in ventricular cells, it shortens APD in Purkinje fibers (Ikeda *et al.* 1985; Varro *et al.* 1986; Yabek *et al.* 1987), presumably via reduction of the late Na^+ current.

Spatial non-uniformities in the expression of I_{Kr} channels may predispose to dissimilar APD lengthening by flecainide at distinct epicardial sites. In study VI (Osadchii 2012e), flecainide has been shown to produce a more prominent increase in APD in the LV than the RV chamber, thus causing a reversal of the normal interventricular repolarization gradient in the guinea-pig heart. This change, in turn, translated to impaired epicardial activation-repolarization coupling (Figure 22, panels C and D) and amplified spatial dispersion of

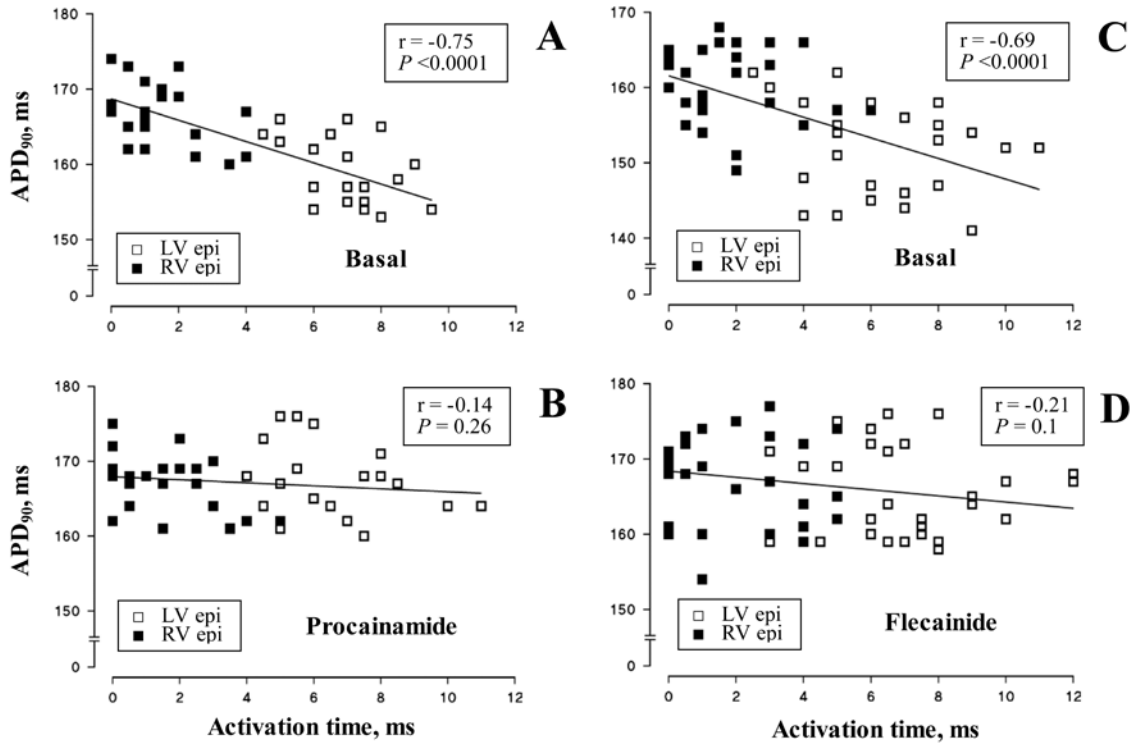

Figure 22. Effects of procainamide (A and B) and flecainide (C and D) on the activation-repolarization coupling in perfused guinea-pig hearts

Monophasic action potential durations (APD_{90}) and the corresponding local activation times were determined in three left ventricular (LV) and three right ventricular epicardial recording sites in spontaneously beating heart preparations before and after drug infusions (procainamide: n=7, 42 data points in total; flecainide: n=10, 60 data points in total). At baseline (panels A and C), an analysis of all data points by a least squares linear fit yielded the inverse correlation between APD_{90} and activation time values. The RV-to-LV epicardial activation-repolarization coupling was lost after drug infusions (panels B and D). Adapted from study VIII (Osadchii, O.E. 2014. Procainamide and lidocaine produce dissimilar changes in ventricular repolarization and arrhythmogenicity in guinea-pig. *Fundam Clin Pharmacol* 28, 382-393) and study VI (Osadchii, O.E. 2012. Flecainide-induced proarrhythmia is attributed to abnormal changes in repolarization and refractoriness in perfused guinea-pig heart. *J Cardiovasc Pharmacol* 60, 456-466), with permission from publishers.

repolarization time, which contributed to the increased occurrence of VT.

In a clinical setting, arrhythmogenic responses to flecainide are often precipitated upon exercise-induced heart rate acceleration (Anastasiou-Nana *et al.* 1987; Falk 1989), which

raises the possibility that an inadequate rate adaptation of ventricular repolarization may contribute to arrhythmic substrate. In normal conditions, an abrupt acceleration in heart rate is associated with immediate APD (and QT interval) reduction over the next few cardiac cycles, which is followed by a slow phase of monotonic APD shortening until a steady-state is reached after a few hundred beats (Franz *et al.* 1988; Seethala *et al.* 2011). The rate-dependent APD reduction ensures a sufficiently long diastolic interval, thus eliminating the possibility of superposition of the next ventricular depolarization on the T wave of a preceding beat (the "R-on-T phenomenon"), which would otherwise serve as an arrhythmic trigger. An attenuated shortening of APD during tachycardia is therefore maladaptive (Gill *et al.* 1993; Pueyo *et al.* 2010). With flecainide, a protracted adaptation of repolarization to sudden cardiac acceleration was observed in perfused guinea-pig hearts (Osadchii 2016). Flecainide was found to reduce the amount of QT interval and ventricular APD shortening upon an abrupt increase in activation rate, and to prolong the fast and slow time constants of the rate adaptation (Figure 23). Therefore, it appears that a drug-induced decrease in I_{Kr} in cardiac cells (Follmer & Colatsky 1990; Follmer *et al.* 1992; Wang *et al.* 1996) may translate to the impaired capacity to develop appropriate APD reduction upon sudden variations in cardiac cycle length, for example, during exercise, or the spontaneous onset of atrial fibrillation.

11.4 Proarrhythmia induced by class III antiarrhythmic agents

11.4.1 Electrophysiological basis for class III agent effects on ventricular repolarization

Class III agents are methanesulfonanilide compounds that produce *open-state* block of I_{Kr} channels (for review see Tamargo *et al.* 2004). The blocking effect is amplified in depolarized cardiac tissue, i.e. at membrane potentials of -60 to -40 mV that yield the maximal outward current via I_{Kr} channels. The drug binding site is located inside the pore of

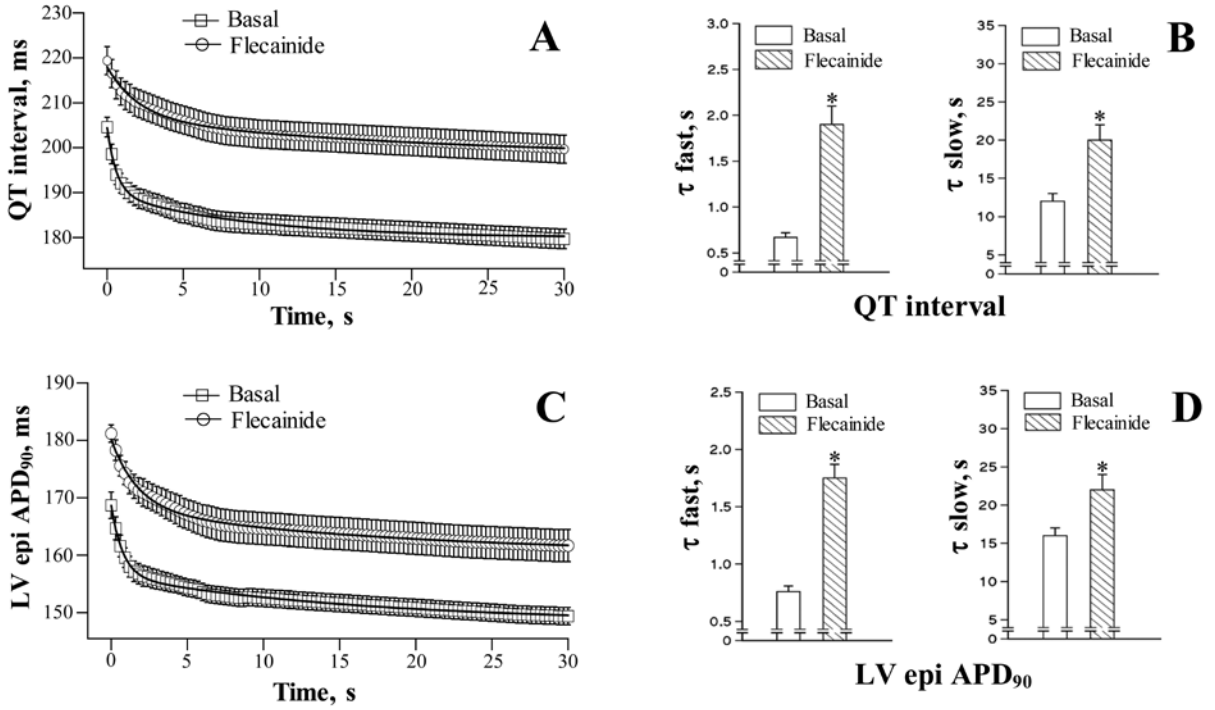

Figure 23. Effects of flecainide on adaptation of the QT interval and ventricular action potential duration to an abrupt acceleration of cardiac beating rate in perfused guinea-pig hearts

A train of 100 regular pulses at pacing interval of 300 ms (total stimulation time is 30 s) was applied in spontaneously beating, AV-blocked heart preparations with a running cycle length of about 600 ms. The QT interval (panel A), and LV epicardial (epi) monophasic action potential duration (APD_{90}) (panel C) were measured in each cardiac beat and plotted vs. time from the beginning of stimulation. The plots obtained at baseline (squares) and upon flecainide infusion (circles) were fitted by a double-exponential function. The fast and slow time constants (τ) of the rate-dependent shortening of QT interval (panel B) and APD_{90} (panel D) were determined from the exponential fits. Note that the rate adaptation plots were shifted upwards upon flecainide infusion (panels A and C), indicating drug-induced prolongation of ventricular repolarization. Also note a slowed kinetics of the adaptation of ventricular repolarization to an abrupt increase in cardiac beating rate, as evidenced by increased τ values (panels B and D). *P<0.05 vs. corresponding basal value (in panels B and D). Adapted from Osadchii, O.E. 2016. Flecainide attenuates rate adaptation of ventricular repolarization in guinea-pig heart. *Scand Cardiovasc J* 50, 28-35, with permission from Taylor & Francis Ltd (www.tandfonline.com).

the I_{Kr} channel, and the blockers typically gain access to it upon channel opening from the intracellular side of the membrane, after crossing the lipid bilayer. Once the activation gate is

closed, the drug can be trapped inside the channel, and the block is not released until the channel re-opens. The I_{Kr} blockers therefore slowly dissociate from the binding site. These agents are commonly characterized as the fast onset/slow offset I_{Kr} blockers, as the time constant for onset of the block (ranging from 1 to 6 s for different agents) is by far shorter than the time constant for channel recovery (several minutes) (for review see Nair & Grant 1997). As such, class III agents may develop cumulative I_{Kr} blocking effects, thus increasing the risk of excessive APD lengthening. The APD is typically prolonged by class III agents in a reverse use-dependent manner (see section 7.3), which may facilitate the EAD-dependent arrhythmia at slow cardiac activation rates.

The I_{Kr} amplitude is counterintuitively reduced upon decreasing extracellular K^+ concentration, despite the associated increase in electrochemical driving force for K^+ efflux. This change is accounted for by the reduced I_{Kr} channel conductance, an effect that causes prolonged ventricular APD. Hypokalemia may therefore synergistically interfere with drug-induced I_{Kr} block, meaning that the proarrhythmic potential of class III agents can be significantly increased in patients treated with diuretics. In contrast, elevated extracellular K^+ levels contribute to the increased I_{Kr}, indicating that the APD-prolonging effect of class III agents and resultant antiarrhythmic action are likely to be attenuated in clinical conditions associated with K^+ accumulation in intercellular clefts, such as myocardial ischemia, or exercise-induced tachycardia. Adrenergic stimulation can reduce class III-induced APD prolongation both by accelerating heart rate, and via the direct activation of $I_{Ks.}$

Apart from specific I_{Kr} blockers (e.g., dofetilide), class III also includes agents that block both I_{Kr} and I_{Ks} (ambasilide, azimilide), block I_{Kr} and I_{to} (tedisamil), or produce multiple ion channel blockade (amiodarone, dronedarone). Ventricular proarrhythmic responses are to much less common when using agents with a complex electrophysiological profile compared

to the specific I_{Kr} blockers (for review see Frommeyer & Eckardt 2016).

11.4.2 Cardiac arrhythmia and repolarization abnormalities provoked by dofetilide

Dofetilide is a highly selective I_{Kr} blocker that prolongs cardiac APD and ERP, but in contrast to class I agents, has no effect on conduction velocity. Another advantage of dofetilide is that it does not reduce cardiac contractility and systemic blood pressure, which are quite common side effects of other available antiarrhythmics. Selective prolongation of refractoriness results in a reduction of the excitable gap in the re-entrant circuit, thus favoring the termination of tachyarrhythmia. Intravenous or oral dofetilide is therefore useful in converting sustained atrial fibrillation or flutter to sinus rhythm, and in preventing arrhythmia recurrence over the long-term follow-up (Falk *et al.* 1997; Torp-Pedersen *et al.* 1999; Singh *et al.* 2000).

Dofetilide, nevertheless, may provoke arrhythmogenic responses in susceptible patients. The DIAMOND clinical trial (Danish Investigations of Arrhythmia and Mortality on Dofetilide) has shown that in patients with profound systolic dysfunction due to either congestive heart failure (Torp-Pedersen *et al.* 1999) or recent myocardial infarction (Kober *et al.* 2000), dofetilide therapy is associated with a greater incidence of torsade de pointes compared to the placebo group. The major risk factors for dofetilide-induced arrhythmia are female gender, recent myocardial infarction, severe heart failure, and prolonged basal QT value (Pedersen *et al.* 2007). About 76% of arrhythmic episodes occur within three days after the initiation of therapy (Torp-Pedersen *et al.* 1999), indicating that clinical outcomes can be significantly improved by initiating treatment in the inpatient setting for close electrocardiographic monitoring of the QT interval response and renal function, in order to adjust the dose of dofetilide.

The mechanisms contributing to dofetilide-induced arrhythmia remain incompletely

understood. The arrhythmic responses provoked by I_{Kr} blockade are commonly attributed to the excessive prolongation of APD and therefore an increased propensity for EADs. However, the clinical value of this mechanism could be questioned because these proarrhythmic changes are likely to occur only at non-physiological slow beating rates (e.g. those observed in complete AV block), whereas at faster beating rates, APD prolongation by dofetilide is significantly attenuated (the reverse rate-dependent effect). Cardiac patients often exhibit a tendency toward increased rather than slowed resting heart rates due to the sustained sympathetic activation which occurs in heart failure, thus providing no favorable grounds for inducing EADs. Notably, no evidence of EADs was obtained upon monophasic action potential recordings in patients with ischemic heart disease that received intravenous dofetilide (Sedgwick *et al.* 1992; Yuan *et al.* 1994).

There is a possibility that the development of torsade de pointes in dofetilide-treated patients is precipitated by an abrupt slowing of ventricular activation rate after the conversion of atrial fibrillation to sinus rhythm, which may enhance sensitivity to QT interval prolongation by I_{Kr} blocker (Choy *et al.* 1999). However, clinical studies revealed no reverse use-dependency of dofetilide effects over the range of heart rates (75-150 beats/min) close to those determined in these patients (Sedgwick *et al.* 1992; Yuan *et al.* 1994; Bashir *et al.* 1995), thus challenging the role played by this mechanism.

Proarrhythmic modifications of ventricular repolarization by dofetilide were analysed in experimental studies. Consistent with spatial heterogeneities in the distribution of I_{Kr}, dofetilide was found to produce non-uniform lengthening of APD and/or the refractory period in canine (Van Opstal *et al.* 2001; Bauer *et al.* 2002; Kozhevnikov *et al.* 2002; Wu *et al.* 2005; Meijborg *et al.* 2015), rabbit (Wu *et al.* 2005; Dhein *et al.* 2008; Qi *et al.* 2015), and guinea-pig hearts (Osadchii 2012c; 2012d, 2014c: studies IV, V, and X, respectively). Over

the transepicardial plane, dofetilide evokes a greater APD lengthening in LV than RV chamber, an effect that contributes to attenuated RV-to-LV activation-repolarization coupling, as evidenced by markedly reduced slope of the local APD-to-activation time linear relationships (Figure 24). This change results in increased epicardial dispersion of repolarization time in dofetilide-treated heart preparations, both during regular pacing and in extrasystolic beats (Osadchii 2014c: study X). Across the LV wall, dofetilide provokes a greater prolongation of refractory periods at the endocardium compared to the epicardium, thus increasing the transmural dispersion of refractoriness (Bauer *et al.* 2002; Osadchii 2012c, 2012d: studies IV and V, respectively). Two other important attributes of dofetilide-induced arrhythmogenicity appear to be the steepened electrical restitution, and abnormal temporal relations between ventricular APD and refractory period. Because dofetilide evokes a greater APD prolongation at long compared to short diastolic intervals, it increases the amplitude of maximum-to-minimum APD change upon dynamic pacing, which steepens the slope of the restitution curve (Figure 25, panels A, B and C). Monophasic action potential recordings at multiple epicardial sites revealed spatially non-uniform dofetilide effects on electrical restitution in perfused guinea-pig hearts, with steepening of the maximum restitution slope being greater in the LV than the RV chamber (Osadchii 2012d: study V). As a result, dofetilide was found to increase the spatial variability in the distribution of maximum restitution slope value throughout the epicardial surface. The contribution of this effect to proarrhythmia is underscored by computational and clinical studies demonstrating that amplified spatial heterogeneities in electrical restitution can promote a wavebreak and initiate VF independently from the absolute value of the restitution slope measured at individual recording sites (Nash *et al.* 2006; Pak *et al.* 2004).

Dofetilide-induced I_{Kr} block results in a reduced slope of the phase 3 repolarization,

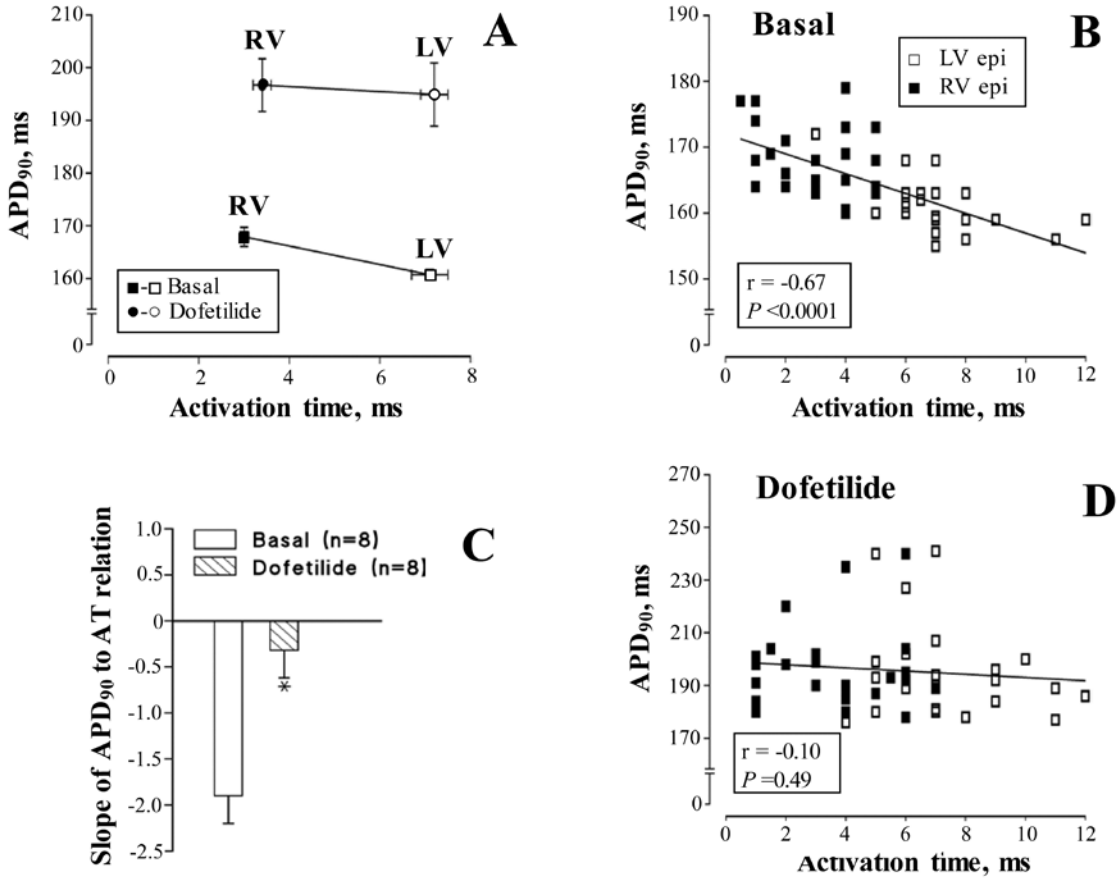

Figure 24. Effects of dofetilide on the epicardial activation-repolarization coupling in perfused guinea-pig hearts

Monophasic action potential durations (APD_{90}) and the corresponding local activation times (AT) were determined in three left ventricular (LV) and three right ventricular epicardial recording sites in spontaneously beating heart preparations before and after infusion of dofetilide. Panel A shows mean RV and LV APD_{90} values plotted vs. mean activation times. Panels B and D show linear regression relations obtained by plotting individual APD_{90} vs. activation time values (48 data points in total from 8 perfused heart preparations). The mean linear regression slope values are shown in panel C. Note that dofetilide prolongs APD_{90}, with the effect being greater in LV than RV (panel A). Also note that the inverse correlation between APD_{90} and activation time (i.e., the activation-repolarization coupling) seen in basal conditions (panel B) was lost after infusion of dofetilide (panel D). *P<0.05 vs. basal value (in panel C). Adapted from study X (Osadchii, O.E. 2014. Impaired epicardial activation-repolarization coupling contributes to the proarrhythmic effects of hypokalemia and dofetilide in guinea-pig ventricles. *Acta Physiol (Oxf.)* 211, 48-60), with permission from publisher.

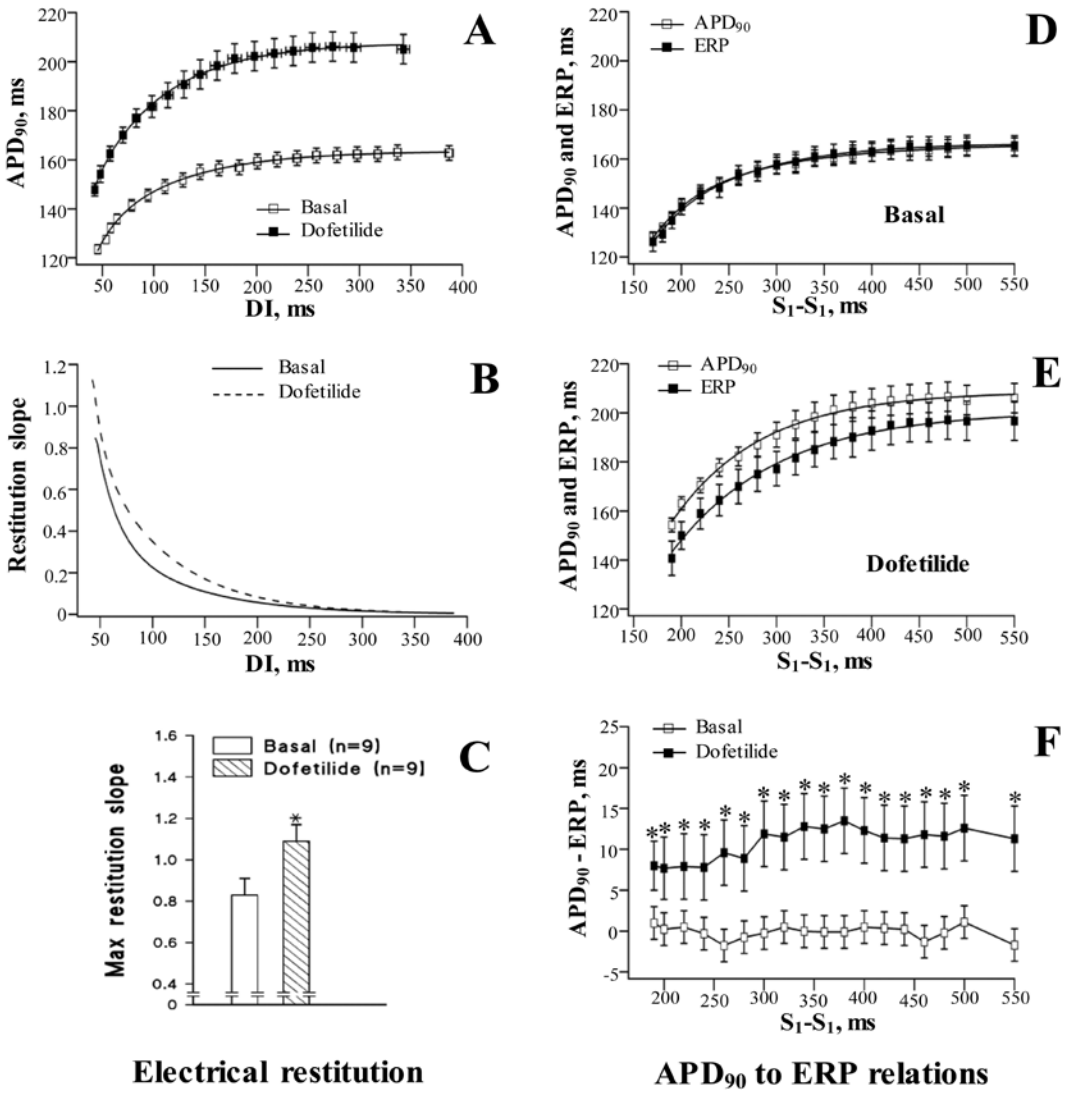

Figure 25. Effects of dofetilide on electrical restitution (A, B and C) and temporal relations between the action potential duration and effective refractory period (D, E and F) in perfused guinea-pig hearts Monophasic action potential duration (APD_{90}) determined at the LV epicardium over a wide range of progressively reducing pacing cycle lengths (S_1-S_1 from 550 ms to 160 ms) was plotted as a function of the preceding diastolic interval (DI) in panel A. Panel B shows changes in APD_{90} restitution slope upon dynamic pacing, and panel C shows the maximum (Max) restitution slope values determined before and after dofetilide infusion. In panels D and E, basal and dofetilide-induced APD_{90} and effective refractory period (ERP) values are plotted in the same coordinates. Panel F shows dofetilide effect on the difference between APD_{90} and ERP (i.e., the critical interval for LV re-excitation). *P<0.05 vs. corresponding basal value (in panels C and F). Adapted from study V (Osadchii, O.E. 2012. Dofetilide promotes repolarization abnormalities in perfused guinea-pig heart. *Cardiovasc Drugs Ther* 26, 489-500), with permission from publisher.

thus contributing to more triangular action potential waveshape. Quantitatively, this change is evidenced by a greater APD lengthening by dofetilide at 90% vs. 30% repolarization time points (Lu *et al.* 2002; Dujardin *et al.* 2008; Osadchii 2012d: study V). Secondary to modification of the action potential voltage profile, dofetilide can prolong APD to a greater extent than ERP, thus widening the vulnerable window (i.e., the APD_{90}-to-ERP difference) for LV re-excitation over late repolarization (Figure 25, panels D, E and F).

The increased propensity for torsade de pointes upon dofetilide treatment could be partially accounted for by the increased beat-to-beat APD (and QT interval) variability (Thomsen *et al.* 2006b; Dujardin *et al.* 2008; Oros *et al.* 2010; Champeroux *et al.* 2015; Szentandrassy *et al.* 2015), which fully complies with the TRIaD concept (see section 7.4 for discussion)

12. Summary and conclusions

12.1 Methodological aspects of ventricular repolarization assessments (studies II and III)

The outcomes of electrical restitution assessments depend on whether the standard (S_1-S_2) or dynamic (S_1-S_1) pacing protocol is used to examine the APD rate adaptation. In perfused guinea-pig hearts, the maximum restitution slope, a commonly used proarrhythmic determinant, is greater upon standard extrasystolic stimulations compared to dynamic steady-state pacing, both at the epicardium and endocardium (Osadchii 2012b: study III). This difference has been attributed to both the impact of previous pacing history, and the ability to preserve ventricular capture at shorter diastolic intervals during extrasystolic stimulations compared to dynamic pacing. The latter is explained by shorter ERP than APD_{90} relationships in ventricular myocytes, which allows the early premature pulse to encroach the repolarization phase of the previous regular beat. An increase in ERP-to-APD_{90} ratio produced by Na^+ channel blocker both eliminates early premature beats generated at critically short diastolic

intervals, and flattens the standard restitution curve, while producing no change in dynamic restitution. Furthermore, in hypokalemic hearts, increased arrhythmogenicity is paralleled by the increased maximum slope of the dynamic, but not standard APD restitution curve. These findings challenge the value of electrical restitution assessments based on extrasystolic stimulation alone, as commonly performed in human patients with cardiac arrhythmia.

Arrhythmic susceptibility is partially determined by the location of ventricular pacing site. In the guinea-pig heart, ventricular fibrillation threshold is lower, and the inducibility of tachyarrhythmia by extrasystolic stimulation is higher at the endocardium than the epicardium (Osadchii 2012a: study II). This difference is accounted for by at least two contributory mechanisms. First, the direction of transmural activation sequence can modulate ventricular repolarization via electrotonic effects. This modulation amplifies the intrinsic endocardial-to-epicardial APD difference during endocardial stimulation, while minimizing it upon epicardial pacing. Activation sequence-dependent electrotonic effects also result in the increased steepness of electrical restitution during endocardial vs. epicardial pacing. Second, a greater expression of Na^+ channels at the endocardium than the epicardium may contribute to the recurrence of excitability at earlier repolarization time points in endocardial cells, which increases the vulnerable window for LV re-excitation by premature ectopic activation. Likewise, electrical responses elicited at earlier repolarization time points propagate with increased conduction delay, thus favoring re-entry. Collectively, these findings provide insight into the electrophysiological mechanisms of cardiac pacing-induced arrhythmic responses that could be observed in a clinical setting.

12.2 Arrhythmogenic responses in hypokalemia (studies I, X, and XI)

Hypokalemia provokes non-uniform APD lengthening at distinct epicardial regions, thus increasing spatial repolarization gradients (Osadchii 2014d: study XI). Hypokalemia also

contributes to slowed conduction and shortened refractory period; these changes result in reduced excitation wavelength, which sets a stage for re-entry. Prolongation of APD in association with reduced ERP results in widening of the vulnerable window for LV re-excitation over late repolarization. Importantly, the RV-to-LV transepicardial activation-repolarization coupling, a mechanism that coordinates recovery times in two ventricular chambers under normal conditions, is impaired upon hypokalemic perfusion (Osadchii 2014c: study X). Another contributor to arrhythmic substrate in hypokalemic hearts is the steepened electrical restitution. Hypokalemia has been shown to increase the slope of the ERP restitution curve, and facilitate persistent beat-to-beat oscillations in APD (i.e., APD alternans), thus increasing the propensity for ventricular fibrillation (Osadchii 2010: study I). Proarrhythmic responses in hypokalemia, nevertheless, could not be accounted for by a negative electromechanical window, or increased transmural repolarization gradients.

12.3 Arrhythmogenic responses to antiarrhythmic drugs (studies IV-X)

Class Ia (quinidine, procainamide) and class Ic (flecainide) I_{Na} blockers, as well as the class III agent dofetilide (specific I_{Kr} blocker) produce multiple proarrhythmic modifications in ventricular repolarization and refractoriness. In the guinea-pig heart, these agents prolong APD in a spatially non-uniform manner, with the effect being greater at LV vs. the RV epicardium. This change leads to impaired RV-to-LV transepicardial activation-repolarization coupling, and increased spatial variability in the distribution of repolarization time. With dofetilide, quinidine, and procainamide, but not flecainide, APD lengthening is more pronounced at long compared to short diastolic intervals (the reverse use-dependent effect), which translates to the increased steepness of the electrical restitution curve. At a given LV recording site, quinidine and dofetilide have been found to prolong APD_{90} to a greater extent than ERP, thus increasing the vulnerable window for LV re-excitation over late repolarization

(Osadchii 2012d, 2013: studies V and VII, respectively). This effect was likely attributed to the increased triangulation of ventricular action potential waveshape, as evidenced by a greater APD prolongation by both agents at 90% vs. 30% repolarization time points. Over the transmural plane, quinidine, procainamide, flecainide, and dofetilide produce a greater ERP lengthening at the endocardium than the epicardium, which results in the increased transmural dispersion of refractoriness (Osadchii 2012c: study IV). In contrast, class Ib Na^+ channel blockers such as lidocaine and mexiletine, the agents with a clinically safe antiarrhythmic profile, were found to reduce the endocardial-to-epicardial ERP difference. Finally, during extrasystolic stimulation, flecainide and quinidine have been shown to accentuate spatial heterogeneities in ventricular conduction and repolarization associated with premature activation, thus facilitating arrhythmia (Osadchii 2014b: study IX). In contrast, lidocaine and mexiletine had no effect on electrical derangements produced by extrasystolic stimulation. Taken together, the multiple repolarization abnormalities outlined above may provide an electrophysiological basis for ventricular tachyarrhythmia upon the initiation of therapy with Na^+ channel blockers or class III agents.

Dansk resume

I hjertepatienter er livstruende takyarytmi ofte forårsage af unormale forandringer i ventriklens repolarisering og refraktærperiode. Repolariserings-abnormiteter udvikler sig typisk som følge af nedsat funktion af K^+ ionkanaler i hjertemyocytterne, hvilket kan være forårsaget af genetiske defekter eller skyldes forskellige erhvervede patofysiologiske tilstande, herunder elektrisk remodeling som følge af hjertesygdom, ionkanal ændringer som følge af brug af læge-midler, samt systemiske elektrolyt forstyrrelser som ses ved hjertesvigt, såsom hypokaliæmi. Elektrisk ustabilitet i hjertet som følge af unormal repolarisering er afhængig af det komplekse samspil mellem en arytmisk trigger og et sårbart arytmisk substrat. Her spiller hjertets forlængede aktionspotentiale, forkert intracellulær Ca^{2+} håndtering, samt den sænkede ledningshastighed i ventriklerne en vigtig rolle. Denne afhandling beskriver den elektriske aktivitet i ventrikelmyocytter under normale forhold og ved hjertesygdom. Den beskriver også de klassiske elektrofysiologiske mekanismer ved hjertearytmi, og giver en opdatering af de repolariserings-relaterede surrogat parametre, som i øjeblikket anvendes til vurdering af den arytmiske tilbøjelighed, herunder den spatialle dispersion af repolariseringen, aktiverings-repolariserings koblingen, den elektriske restitution, TRIaD (aktionspotentiales triangulering, reverse use dependency, ustabilitet, og dispersion), samt det elektromekaniske vindue. Efterfølge diskuteres de mekanismer, der afgør den arytmiske sårbarheds afhængighed af stedet for den ventrikulære pacing. Endelig præciseres det elektrofysiologiske grundlag for hjertearytmi som følge af hypokaliæmi, og der gives et indblik i den kliniske og patofysiologiske betydning af lægemiddel-induceret arytmi, med særligt fokus på klasse Ia (quinidin, procainamid) og Ic (flecainid) Na^+ kanal blokkere, samt klasse III antiarytmiske midler, som blokerer I_{Kr} kanaler (dofetilid).

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