Genesis of life-threatening ventricular arrhythmias during the delayed phase of acute myocardial ischemia. Role of cellular electrical coupling and myocardial heterogeneities

de Groot, J.R.

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GENERAL INTRODUCTION

Joris R. de Groot
Background.

Cardiovascular disease is the leading cause of mortality in the industrialized world, with 49826 deceased in the Netherlands in 1998, which accounts for 36% of total mortality in that year. Most of the deaths from cardiovascular disease, 14101 or 28%, resulted from acute myocardial infarction (which makes this disease the single most frequent cause of death) and often occurred suddenly through ventricular fibrillation. The majority of cardiac deaths occurs outside the hospital and studies on cardiac resuscitation confirm that indeed 62% of the arrhythmia observed by paramedics during out of the hospital resuscitations is ventricular fibrillation.

The main cause of coronary artery occlusion resulting in myocardial infarction, is thrombus formation on a ruptured atherosclerotic plaque, causing obliteration of the lumen of the artery and subsequent ischemia in its flow region. The in hospital management of acute myocardial infarction is aimed at rapid reperfusion of the ischemic area through administration of thrombolytic agents, balloon angioplasty or occasionally rescue coronary bypass grafting.

Although during the acute phase of myocardial infarction, ventricular arrhythmias form the main cause of mortality, the incidence of death through pump failure becomes more pronounced in the first days to weeks following myocardial infarction. Patients remain at increased risk of arrhythmogenic death up to a year following after the critical acute phase of myocardial infarction. Attempts to reduce the number of lethal arrhythmias during the subacute and chronic phase of myocardial ischemia have failed. In the CAST trial, a study aimed at reducing mortality by suppressing the number of premature beats that induce lethal arrhythmias, treatment of the study population with flecainide, encainide or moricizine resulted in increased mortality in the treated groups. The SWORD study, investigating the use of d-sotalol, shows similar results. The result of these trials demonstrate that the underlying mechanism of life threatening arrhythmias are not understood to the extent that effective pharmacotherapy can be applied. This lack of understanding is underlined by the fact that the single drug that did show a reduction in arrhythmogenic death (but not total mortality) is amiodarone, a drug that combines about all antiarrhythmic properties. The only pharmacological intervention after myocardial infarction that does provide desirable clinical outcomes, is beta blockade, although the mechanism is probably related to mechanical and metabolic unloading of the heart and reduction in resting heart rate, rather than to a direct antiarrhythmic effect.

The detrimental outcomes of the antiarrhythmic drug trials have led to a different look at antiarrhythmic drug action, based on the electrophysiological mechanism of the arrhythmia, but this approach has not yet been implemented clinically and alternative therapies among which the implantation of defibrillators are now becoming more popular. Although this very expensive treatment is cost-effective in extremely high risk populations, its use is a surrogate treatment for a disease whose mechanisms are inadequately understood. The incidence of arrhythmogenic death stresses the importance of further insight in its mechanisms, which might provide future therapeutic options that are suitable for a larger population.

The mechanism of arrhythmias during ischemia has been investigated in numerous experimental studies, and the majority of our knowledge is derived from studies in young, healthy animals. Surprisingly, although it is known that lethal arrhythmias occur in two distinct phases during the first hour of acute ischemia, of which the second phase comes with the same number or more lethal events in dogs and pigs, the vast majority of these studies is confined to the first (1A) phase, and little is known about the mechanism of the delayed, IB, phase of arrhythmias. For more than a century, but especially the last decades, many investigators have studied the mechanism of ventricular fibrillation in normoxic and ischemic hearts, and it is now generally accepted that reentry underlies the mechanism of ventricular fibrillation in most but not all cases. In the sparse literature available, ischemia-induced cellular uncoupling has been associated with the occurrence of delayed, IB, arrhythmias.
Mechanisms of Reentrant Arrhythmias.

Reentrant excitation refers to the persistence of an activating impulse that re-excites the tissue it previously excited as soon as it is no longer refractory\(^4\). George Ralph Mines already in 1914 defined the criteria that are required for reentry\(^5\): a zone of unidirectional conduction block, so that the (premature) impulse enters the circuit in one direction only, whereafter it completes the circle and the previously inexcitable limb of the circuit is activated retrogradely. Secondly, conduction velocity should be low enough to allow reexcitation once the impulse reaches tissue that was excited previously. The ultimate proof for the presence of reentry is that cutting the reentrant circuit terminates the arrhythmia.

The length of the reentry circuit is determined by the product of the refractory period and the conduction velocity, the so-called wavelength\(^3\), describing the distance that the activation front travels during the refractory period of a certain site. During anatomic reentry, the path length around an obstacle may be longer than the wavelength, resulting in an excitable gap. Indeed, fibrillation ceased in small pieces that were cut from fibrillating atria or ventricles, underlining that a minimal mass of tissue is required for sustaining reentry\(^5\). Although recently this “critical mass hypothesis” was challenged\(^8\), the concept of wavelength still serves as a paradigm for our current understanding of reentry.

Decreased excitability

A variety of factors in normal or diseased myocardium can cause conduction slowing or activation block, the conditions required for reentry. Conduction velocity in the conduction system and in atrial and ventricular myocardium depends mainly on the rapid upstroke of the action potential (V\(_{\text{max}}\))\(^5\), for which the fast sodium current, I\(_{\text{Na}}\), which can be blocked by class I antiarrhythmic drugs, is responsible\(^6\). At decreased transmembrane potentials, I\(_{\text{Na}}\) is closer to its threshold that lies around \(-60\) mV (thus less current is required to induce an action potential)\(^6\), but also a larger fraction of the sodium channels is in inactivated state, whereby less sodium current is available and V\(_{\text{max}}\) decreases\(^6\). Indeed, when during ischemia increased extracellular potassium concentration causes decreased resting transmembrane potential\(^40;42\), conduction velocity is markedly decreased\(^6\) and (additional) pharmacological blockade of the sodium channels during ischemia converts conduction slowing to conduction block\(^63\). Experimental studies as well as computer models show that blocking fast inward sodium current can decrease conduction velocity to about 30% of its initial value\(^44;45\). Pharmacological decrease of electrical cellular coupling can decrease conduction velocity to less than 1 cm/s\(^65;66\).

When I\(_{\text{Na}}\) is fully available, but relatively little “source” current excites a large bulk of tissue, conduction velocity decreases also\(^68;69\). This can occur when a small bundle connects to a large mass of tissue\(^6\), at branching structures\(^70\) and when an isthmus is present\(^68\). The ratio between the “source” current and the “sink” (the safety factor) can be insufficient to excite the larger tissue part, or can be just large enough to allow only slow conduction. Conduction slows when activation propagates from tissue with a normal resting membrane potential into progressively depolarized tissue, referred to as “decremental conduction” by Hoffman and Cranefield\(^71\). In addition, the current generated by the depressed tissue can be insufficient to enable propagation into non-depressed tissue and activation block occurs. In this case, activation can propagate from the non-depressed into the depressed tissue\(^72;73\). Paradoxically, conduction can be restored by uniform cellular uncoupling when longitudinal block occurs and more current can excite the tissue in the transverse direction\(^66;74;75\). Altered wavefront curvature and increased anisotropy also attenuates load mismatch\(^6\).
Dispersion of refractoriness

Shortened refractory periods decrease the wavelength (more wavelets can be present at the same time)\textsuperscript{56}, and lead to an increased vulnerability to reentry\textsuperscript{77}. Heterogeneities in refractory periods can lead to activation block in one direction, while activation continues in other directions. A properly timed extrastimulus could induce reentry in rabbit atria\textsuperscript{77,78}, but in the ventricles differences in refractory periods are usually too small to allow reentry. However, during acute ischemia refractory periods dissociate from action potential duration, referred to as post repolarization refractoriness\textsuperscript{77,80} causing decreased recovery of inactivation of the Na\textsuperscript{+} channels, and heterogeneities large enough to cause reentrant activation can occur\textsuperscript{81}. In simulated ischemia, availability of the fast inward sodium current depends heavily on the resting membrane potential\textsuperscript{82}.

However, once established, reentrant activation remains sustained in large hearts also in the absence of previous inhomogeneities. Multiple wavelets\textsuperscript{56}, or one (rapidly moving) dominant source rotor\textsuperscript{78,83} or "leading circle"\textsuperscript{77,78,84} give rise to many daughter wavelets that activate the ventricle. Dependent on the presence of inexcitable barriers and on local excitability such wavelets either break down in more wavelets or die out\textsuperscript{85-87}. In three dimensional excitable tissue scroll waves around an inexcitable filament form the three dimensional representation of rotors around a phase singularity point\textsuperscript{85,87}.

Tissue architecture

Tissue architecture causes anisotropic conduction velocity in normal myocardium: because intercellular resistance is lower in longitudinal (parallel to the muscle fibers) than transversal (perpendicular to the fibers) direction, conduction velocity is larger in former than in the latter. Changes in tissue architecture such as nonuniform anisotropy resulting from remodeling after ischemia can cause conduction slowing and reentry\textsuperscript{67,88-90}. During the subacute phase of myocardial infarction, changed distribution of gap junctions\textsuperscript{91,92} underlies enhanced anisotropy, albeit that during this phase of ischemia also active membrane properties have changed\textsuperscript{93-97}. During the chronic phase of myocardial infarction however, prolonged activation delays are related to very long pathways of surviving fibers through the infarcted tissue, with normal conduction velocity\textsuperscript{98}. Development of scar tissue and the generation of myocardial fibrosis add to the increased anisotropy and lead to non-homogeneous propagation of the impulse. Hence, changed properties of tissue architecture during acute, subacute and chronic myocardial infarction may cause slow conduction, shorten wavelength and thus favor reentrant arrhythmias.

Acute Myocardial Ischemia.

Definition and Mechanism

Myocardial ischemia is defined as an imbalance between oxygen demand and supply\textsuperscript{99}. Oxygen is required for the generation of high energy phosphates that form the energy source for all cellular processes. A rapid initial decrease in the free energy of ATP hydrolysis\textsuperscript{100-102} results in a transsarcolemmal redistribution of potassium resulting in a biphasic increase in extracellular potassium concentration and subsequent depolarization of the sarcolemma\textsuperscript{45,103,104}. Also, action potential duration decreases, possibly associated with opening of the ATP sensitive potassium channels through intracellular ATP depletion\textsuperscript{105}. For a limited period of time, the myocytes can maintain their metabolic competence through anaerobic glycolysis, which results in lactate production and thus acidosis\textsuperscript{106}. With increasing duration of ischemia, extracellular potassium
concentration rises further and transmembrane potential decreases leading to electrical inexcitability. The combined effects of acidosis and accumulation of metabolites result in decreased efficacy of anaerobic glycolysis, and a further metabolic deterioration. Subsequently, a longer duration of ischemia results in intracellular calcium overload, closure of the gap junctions and rigor. Thereafter, cells will lose their membrane integrity, enzymes will leak out of the cell and the cell dies. Myocardial necrosis defines infarction.

In the intact heart, ischemic damage develops heterogeneously and certain parts of the ischemic zone survive the ischemic burden: subepicardium and subendocardium remain viable through diffusion of oxygen and glucose from surrounding tissues, also during the subacute and chronic phase of myocardial ischemia, although active membrane properties change.

Two periods of ventricular arrhythmias during the acute phase of myocardial ischemia

Within the first 60 minutes of acute ischemia, coined phase 1 by Harris, lethal ventricular arrhythmias occur in two distinct phases, termed 1A and 1B respectively. These are separated by a period relatively free of arrhythmias. Although the precise difference in the mechanism of 1A and 1B arrhythmias is not certain, it is now clear that these separate phases exist in dogs, pigs, sheep, and rats, but that they are less clear in rabbits and cats, although in individual animals the bimodal distribution of arrhythmias might differ.

Early, 1A, ventricular arrhythmias

The first or 1A phase has been studied extensively, and is related to conduction slowing and depressed excitability. The changed energy homeostasis of ischemic tissue leads to acidosis and efflux of potassium, resulting in depolarization of the cell membrane. Extracellular potassium concentration and the degree of depolarization change heterogeneously within the ischemic zone. When cellular electrical coupling is intact, differences in membrane potential between ischemic and normoxic myocytes cause a current that flows from the ischemic toward the normal zone. During the repolarization phase, this so called "injury current" can be large enough to induce a premature depolarization in the non-ischemic tissue that can trigger sustained arrhythmias. The inhomogeneous rise in extracellular potassium concentration causes severe conduction slowing and large areas of functional activation block. Hence, during this phase of acute ischemia, the conditions for reentry defined by Mines are present. Also non reentrant mechanisms have been shown to underlie (the initiation of) early arrhythmias during acute ischemia.

In summary, during the 1A phase of acute ischemia, redistribution of potassium over the sarcolemma causes depolarization, depressed excitability, heterogeneously within the ischemic zone. This in turn creates the conditions required for reentry, that can be triggered by injury current induced premature beats. These changes are fully reversible upon reperfusion of the ischemic zone.

Delayed, 1B, ventricular arrhythmias

Contrary to the ventricular arrhythmias that occur within the first 10 minutes of coronary occlusion, sofar the delayed type (1B) of arrhythmias have hardly been studied. It is plausible to assume that both types of lethal arrhythmias have a different mechanism. In favor of this is the fact that a period of relative arrhythmogenic quiescence separates both phases. Also, prominent subepicardial conduction slowing has been demonstrated during the 1A phase and is largely absent during the 1B phase. The scarce animal studies that have been performed to elucidate the mechanism of the 1B arrhythmias show that this phase is accompanied by more lethal events than is the 1A phase. Exact data on the bimodal distribution of early arrhythmias during acute ischemia...
in man is lacking. Probably, the 1B phase is as lethal in man as in experimental animals. The electrophysiological changes during early ischemia occur more rapidly in diseased human hearts compared to healthy laboratory animals\textsuperscript{40} thereby reducing the period that 1A arrhythmias can occur, and advancing the moment that 1B arrhythmias can arise.

Several phenomena have been associated with the occurrence of 1B arrhythmias, among which the onset of cellular electrical uncoupling and the ultimate rise of extracellular potassium\textsuperscript{20,43,129}. Indeed, heterogeneous cellular uncoupling can create a largely heterogeneous substrate in which long activation pathways are possible\textsuperscript{26,139}. However, although a temporal relation between cellular uncoupling and 1B arrhythmias has been described\textsuperscript{26,134}, studies that forwarded the hypothesis that cellular uncoupling is causally related to 1B arrhythmias were primarily performed in models that do not produce sustained arrhythmias\textsuperscript{53,107,131,133}. In addition, as outlined above, conduction slowing through cellular uncoupling can be far more pronounced than the amount of conduction slowing achieved with blockade of the active membrane properties (i.e. the fast inward sodium current). Both model studies and experiments in cultured cell layers have shown that reduction of intercellular coupling can indeed slow conduction to the level of millimeters per second\textsuperscript{64,67,134}. In addition, microreentry was demonstrated around a few cells, with ultraslow conduction as slow as 0.25 cm/s\textsuperscript{66}. Such observations in vitro do not preclude the mechanism in the intact heart subjected to ischemia. Smith et al. have demonstrated that the spontaneous onset of 1B ventricular fibrillation in open chested pigs is associated with the onset of rise in tissue impedance, which is an indirect measure for cellular coupling\textsuperscript{26}. After a period of ischemic preconditioning, both the time of onset of tissue impedance rise and the occurrence of lethal arrhythmias are postponed, which further strengthens this relation\textsuperscript{54}. However, these studies have not demonstrated the causal electrophysiological mechanism of the arrhythmia. Thus, the association between 1B arrhythmias and the onset of cellular uncoupling seems clear, but the assumption that microreentry, caused by very slow conduction or largely heterogeneous conduction block, is the underlying electrophysiological mechanism is still very speculative.

The failure to demonstrate epicardial activation slowing\textsuperscript{25} during the 1B phase could also be explained when the reentrant circuits are principally restricted to the intramural tissue. Indeed, Patterson et al. demonstrated localized intramural reentry of premature beats during the 1B phase\textsuperscript{135}. Among other temporal associations of phenomena during the 1B phase is the release of endogenous noradrenaline during this phase. Indeed, noradrenaline is released from the adrenergic nerve endings between 15 and 30 minutes of ischemia, both in intact animals and in isolated hearts\textsuperscript{136-139}. Catecholamines exhibit positive chronotropic and inotropic actions that increase myocardial energy expenditure. The electrophysiological effect of catecholamines is mainly related to G-protein related intracellular calcium increase. Both enhanced energy expenditure and intracellular calcium concentration increase can add to arrhythmogenesis. However, preliminary data show that the occurrence and severity of 1B arrhythmias in the open chested pig is not reduced after beta blockade with propranolol (J.R. de Groot, unpublished data). In other studies, different adrenergic antagonists have been shown to be antiarrhythmic, but in most cases this was related to a reduction in blood pressure, heart rate and infarct size as well\textsuperscript{140}, indicating that the entire substrate for arrhythmias had changed. However, many studies did not discriminate between 1A and 1B arrhythmias, and in those that did, adrenergic blockade seemed to result in a larger 1A than 1B arrhythmia reduction\textsuperscript{46,49,141}. In addition, catecholamines enhance the rate of the Na\textsuperscript{+}/K\textsuperscript{+} pump, resulting in a decrease in extracellular potassium concentration\textsuperscript{137}, whereas 1B arrhythmias occur when the second rise in extracellular potassium takes place. Hence, catecholamines may enhance arrhythmias during ischemia, but a causal link to the mechanism of 1B arrhythmias in particular is lacking.

Reduction of contractile force could contribute to the reduction of 1B arrhythmias\textsuperscript{28,142}. Various studies have demonstrated the relation between myocardial stretch and arrhythmias in general\textsuperscript{28,143}. Recently, the group of Kléber showed that pulsatile stretch changes the arrangement of gap junctions in a cultured cell monolayer, which can provide insight in the further understanding of
the role of cellular coupling in the substrate of such arrhythmias\textsuperscript{144}. Although it is virtually impossible to experimentally dissect the arrhythmogenic trigger from the substrate\textsuperscript{145}, there are now reports that mechanical factors play a pivotal role in the induction of IB arrhythmias\textsuperscript{142}, whereas there is evidence that also mechanical loading prior to the development of ventricular fibrillation contributes to the arrhythmogenic substrate\textsuperscript{144,146}.

Another temporal relation has been observed between 1B arrhythmias and the terminal rise in extracellular potassium concentration\textsuperscript{42,103,147}. Increase in [K\textsuperscript{+}]\textsubscript{o} depolarizes the cell membrane, and causes activation block when the membrane potential rises above the threshold for the fast sodium current. Coronel et al. have shown that during the 1A phase, large heterogeneities in potassium concentration within the ischemic zone correlate with the arrhythmogenicity\textsuperscript{40,103}. During later phases of ischemia, the extracellular potassium concentration is expected to rise further and to cause activation block more homogeneously, which is considered antiarrhythmic\textsuperscript{40,103}. Thus, potassium is an unlikely factor to be the sole cause of these arrhythmias, because it seems to continue to rise after the 1B phase.

In summary, no conclusive evidence about the exact mechanism of IB arrhythmias is present to date. The common denominator seems to be the temporal relation between onset of ischemia-induced irreversible changes in the myocardium such as catecholamine release, third rise in potassium and cellular uncoupling and the occurrence of these arrhythmias.

\textbf{Gap Junctions and the role of Cellular Coupling in Arrhythmogenesis.}

\textit{Biophysical properties of gap junction channels}

In intact myocardium, myocytes are connected to each other at the intercalated disks. The intercalated disk is composed of the fasciae adherentes, the maculae adherentes and the gap junction channels, that mediate the metabolic and electrical coupling between myocytes (for review see\textsuperscript{148,149}). Gap junction channels are composed of two hemi channels (connexons) in the two adjacent cells, each built up from six connexins, which form a pore in the cell membrane. Figure 1.1 shows an artist's expression from connexons (shaded grey) floating within the lipid bilayer. Connexons can consist of different types of connexins (heteromeric), and the channels can consist of different connexons (heterotypic). In the heart, connexin43, connexin40 and connexin45 are expressed, of which connexin43 is the most abundant in ventricular myocardium. Gap junction channels are relatively non-selective channels, permeable for various ions\textsuperscript{150-152} and for small molecules, including cellular second messengers as cAMP\textsuperscript{153,154}, cGTP\textsuperscript{154} and IP\textsubscript{3}\textsuperscript{153,155-157}, with a molecular weight of up to 1000 D\textsuperscript{158}. In the normal heart, gap junctional conductance has to be reduced by more than 90% before changes in conduction velocity become apparent, yielding a large safety factor for conduction\textsuperscript{159}. Closure of gap junctions relates to twisting of the connexins\textsuperscript{160}, and subconductance states have been described\textsuperscript{161,162}. During ischemia dephosphorylation and intracellular redistribution of connexin43 protein contribute to cellular uncoupling\textsuperscript{163}. Various compounds among which calcium\textsuperscript{107,108}, protons\textsuperscript{106}, decreased ATP\textsuperscript{164}, cAMP\textsuperscript{165,166}, cGMP\textsuperscript{165}, anaesthetics\textsuperscript{167}, alcohols\textsuperscript{56,74,168,169} and fatty acid metabolites\textsuperscript{170,172} alter gap junctional conductance.
Figure 1.1. Artist's impression of connexons within the lipid bilayer. Connexon is build up from six connexins that form a transmembrane pore. Twisting of the connexons results in closure of the pore\textsuperscript{190}.

Role of gap junctions in impulse propagation

As pointed out above, decrease in gap junctional conductance can lead to conduction slowing to an extent that is much larger than what can be achieved by blocking the active membrane properties alone\textsuperscript{64,65}, and microscopic regions of block can occur\textsuperscript{129,130}. It has been shown that conduction is successful at gap junctional conductance of 5-8 nS\textsuperscript{173}, whereas block occurs at 1.3 nS\textsuperscript{159}. Factors such as enhanced anisotropy and preferential conduction slowing in longitudinal direction, might add to arrhythmogenesis\textsuperscript{67,88}. Non uniform electrical uncoupling increases safety\textsuperscript{74}, especially in situations where a load mismatch is present\textsuperscript{58,74}. Diminished expression of connexin43 in heterozygous knockout mice and altered distribution resulting from various diseases, change the conductive properties of myocardium\textsuperscript{91,138,174}.

The notion of very slow conduction and microscopic conduction block has given rise to the thought that microreentry might underlie the mechanism of delayed ventricular arrhythmias during acute ischemia\textsuperscript{26,33,128}. However, although very slow conduction has been reported\textsuperscript{66,175}, microreentry in the setting of the 1B phase of acute ischemia has never been described.

Another way through which cellular coupling can influence the conductive properties of the heart relates to electrotonic coupling between healthy and diseased cells. An example for this was described by Mendez et al. who showed that action potentials can shorten because of coupling to a strand of non depolarized tissue\textsuperscript{176}. Also, action potentials in normal cells can be electrotonically influenced\textsuperscript{116,177,180}. This effect is reduced during partial uncoupling, when very slow but safe conduction is possible\textsuperscript{66,70,74}.

The influence of electrotonic interactions is expected especially during critical cellular coupling and in regions were a small volume of tissue is coupled to a large volume\textsuperscript{181}.
A

B

C

-90 mV
Intact cellular coupling might mask electrophysiological heterogeneities in normal myocardium, and such heterogeneities might only be revealed when coupling conductance decreases, resulting in an arrhythmogenic substrate\(^\text{182}\).

**Hypotheses.**

During the 1B phase of acute ischemia, several transient changes in functional properties of the myocardium take place, as is outlined above. The notion that a thin rim of tissue survives the ischemic burden, whereas the midmyocardium dies\(^\text{113}\), in combination with the temporal association of the occurrence of ventricular arrhythmias and the start of cellular electrical uncoupling\(^\text{26,53,54}\), allows us to formulate the following hypotheses.

The central hypothesis that we test in this study, is that reentry can occur during residual electrical cellular coupling of the subepicardial and subendocardial layer overlying the ischemic zone and the ischemic midmyocardium. Persistence of coupling to the more depressed ischemic midmyocardial tissue during the 1B phase, causes electrotonic depression of the intrinsically viable subepicardium and subendocardium\(^\text{179}\). Figure 1.2 graphically displays the hypothesis for the mechanism of the substrate for 1B VF. Panel A shows the situation before coronary occlusion: in a viable piece of left ventricle subepicardium and midmyocardium a well coupled and epicardial conduction velocity is fast (arrow). Both subepicardial and midmyocardial cells produce normal action potentials (right). When ischemia causes depression of the midmyocardium (panel B), subepicardium is electrotonically depressed through partially intact cellular coupling. Hence, a slow action potential arises from a decreased take off potential, whereas midmyocardial tissue is inexcitable (straight line). Panel C shows that when cellular uncoupling is complete, subepicardium can restore its initial electrophysiological properties, conduct the activation wavefront normally (arrow) and produce a normal action potential because the interaction with the severely depressed midmyocardium (no action potential) has ceased. Interaction between viable and ischemic tissue is expected to play a much smaller role in the lateral ischemic border where the volumes of the diseased and the normal tissue are better matched and thus the load from diseased tissue is counteracted by the load from the normal tissue. Subsequent progression of uncoupling during the course of ischemia terminates this interaction and results in recovery of subepicardial electrophysiological properties. This hallmarks the end of the period during which 1B ventricular fibrillation can be induced.

The main test implications of the central hypothesis are that 1) VF during the 1B phase can be induced only within a restricted time window when 2) cellular uncoupling is not yet complete. 3) Progression of cellular uncoupling should result in electrophysiological recovery of the subepicardial layer overlying the ischemic zone. The investigation of these test implications is described in chapter 3.

*Figure 1.2. Graphic display of the central hypothesis of this thesis. A. Before coronary occlusion, cellular coupling is intact (open gap junctions (white)) and both subepicardium and midmyocardium are viable and produce normal action potentials (Epi and Mid respectively). Conduction velocity is fast and undisturbed (arrow). B. During ischemia, midmyocardium is depressed cellular uncoupling advances (closed gap junctions, grey), but is still partially intact (open gap junctions, white) and causes depression of the subepicardium. Conduction velocity is hampered. C. After complete uncoupling, subepicardium and midmyocardium are dissociated. Hence, midmyocardium remains severely depressed, but in subepicardium normal electrophysiological properties can restore (normal action potential, normal conduction velocity).*
An extension of the central hypothesis is that the subepicardium is the principal excitable domain during the IB phase. We hypothesized that 1) activation patterns of ventricular fibrillation become confined to the two-dimensional structure of the subepicardium during the IB phase. Also, 2) electrophysiological parameters in the subepicardium are expected to partially recover when uncoupling from the midmyocardium progresses.

We investigated subepicardial action potentials and tested whether during the course of the IB phase the number of breakthrough activations during ventricular fibrillation decreased. Additionally, we studied the life time of phase singularity points which is expected to increase when activation patterns become more stable in the two dimensional plane. The recovery of electrophysiological parameters was studied by measuring the action potential duration, local conduction velocity and dominant frequency of ventricular fibrillation.

We describe these experiments in chapter 4.

The central hypothesis does not discriminate macroreentrant mechanisms, caused by homogeneous conduction slowing and -block from microreentrant mechanisms that could arise when heterogeneous cellular uncoupling causes functional heterogeneity at a microscopic level. Therefore we hypothesized that microreentry is the principal mechanism of ventricular fibrillation during the IB phase which implies that 1) It should be possible to induce VF and maintain the arrhythmia in a relatively small piece of ischemic left ventricle. Hence, 2) the wavelength should be short enough to be compatible with microreentry. For this to happen, conduction velocity should be very low, while refractory periods are only moderately shortened.

To test the implication of this hypothesis we studied inducibility of ventricular fibrillation during the IB phase in isolated pieces of ischemic left ventricular free wall. Also, we measured conduction velocity and refractory periods. The results of this study are described in chapter 5.

It follows from the central hypothesis that the arrhythmogenic substrate is present only when cellular coupling is critical, and uncoupling is not yet complete. The duration of the process of cellular uncoupling in the ischemic heart can be caused by either a slow but homogeneous rise in gap junctional resistance between the myocytes, or by a fast but largely heterogeneous closure of the gap junctions. We hypothesize that intrinsic metabolic differences that are present within individual myocytes account for a heterogeneous tolerance to ischemia in individual cells. Thus, a large variance in cellular uncoupling should be present in a group of isolated myocytes. Such variance is expected to be larger in hypertrophy, where there is a reduced cellular coupling and increased electrophysiological and metabolic heterogeneity. Conversely, in coupled cell pairs, intact coupling is expected to neutralize intercellular differences. Alternatively, a slow but homogeneous process is responsible for the duration of cellular uncoupling. Figure 1.3A shows the time course of cellular uncoupling in normal and hypertrophic myocardium reproduced from a paper by Dekker et al.132. Panel B and C show the hypotheses for the duration of the process of cellular uncoupling: on a cellular level this is either caused by a rapid but largely heterogeneous process (panel B) or by a slow but more synchronized increase in gap junctional resistance (panel C).

We investigated action potentials, rise in cytosolic [Ca^{2+}], and time to rigor (which we use as a parameter for cellular uncoupling, since isolated cells are obviously not coupled) during metabolic inhibition in isolated rabbit myocytes, coupled cell pairs and in myocytes from hypertrophic hearts. In chapter 6 the results of this study are described.

**Figure 1.3A.** Time course of cellular uncoupling in normal (open circles) and hypertrophic (closed circles) myocardium (reproduced from Dekker et al.132, with permission). Time course is either caused by rapid but temporarily heterogeneous uncoupling (panel B) or by a slow but more synchronized increase in gap junctional resistance (panel C).
For an arrhythmia to occur, both a suitable electrophysiological substrate and an initiating trigger should be present at the same time. Within a few minutes of regional ischemia, the flaccid ischemic zone stops contracting. This leads to a force exerted on the ischemic border zone by the normally contracting non-ischemic tissue. When the ischemic zone undergoes contracture, it exerts a force on the border between normal and ischemic tissue. We hypothesize that this increased force on the border zone causes focal ectopic premature beats that form the trigger for 1B ventricular fibrillation. The implication of this hypothesis is that 1) premature beats during the 1B phase should arise preferentially from the ischemic border zone, 2) More premature beats occur in mechanically loaded compared to unloaded hearts, and 3) the occurrence of arrhythmias relates to ventricular contraction. We studied these implications and described the experiments in chapter 7.

Chapter 8 concludes this thesis with a general discussion on the role of cellular electrical coupling and myocardial heterogeneities in arrhythmogenesis and more specifically in the induction and perpetuation of delayed ventricular arrhythmias.

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