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

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ROLE OF SUBEPICARDIAL HETEROGENEITIES IN THE GENESIS OF LATE VENTRICULAR ARRHYTHMIAS DURING ACUTE MYOCARDIAL ISCHEMIA.

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Summary.

Background. Delayed (1B) ventricular arrhythmias during acute myocardial ischemia have been associated with ischemia-induced cellular uncoupling. Subepicardium overlying the ischemic zone remains viable whereas the ischemic midmyocardium becomes inexcitable. We hypothesize that during the course of the 1B phase, action potential duration decreases and subsequently recovers to pre ischemic values, and that activation patterns become confined to the two dimensional domain.

Methods. Isolated porcine hearts (n=9) were Langendorff perfused with a blood-Tyrode’s mixture and loaded with the voltage sensitive dye di-4-ANEPPS. Ischemia was produced by occluding the left anterior descending artery for 60 minutes. We recorded fluorescence from a 2x2 cm area at high resolution (64x64 pixels). We studied the effect of premature stimulation (n=4) and direct current induced ventricular fibrillation (VF, n=5). We measured action potential duration at 70% repolarization (APD70), dominant frequency of VF and quantified the number of breakthrough activations and the lifetime of phase singularity points.

Results. APD was 191±20 ms before occlusion and decreased by 74% after 60 minutes. Meanwhile, spatial dispersion of APD, expressed as variance, increased from 123 to 825 ms². Dominant frequency of VF decreased from 7.7±1.3 Hz before occlusion to 6.8±1.4 Hz (15 min, p<0.001) but recovered to 7.8±1.7 Hz after 60 minutes. The number of breakthrough activations decreased from 6.2±4.4 Hz to 0.7±1.2 Hz after 60 minutes (p<0.01). Lifetime of phase singularity points increased from 48±52 to 193±103 ms (p<0.05).

Conclusions. During the delayed phase of acute ischemia, dispersion in APD increases, indicating local recovery and deterioration. Dominant frequency of VF decreases but fully recovers after 60 minutes. Meanwhile, the number of breakthrough activation decreases and lifetime of phase singularity points increases. The results suggest that transition of activation from the three- to the two dimensional domain results in more organized and stable conduction, which could terminate the arrhythmogenic substrate.
Introduction.

Ventricular arrhythmias during acute myocardial ischemia are associated with high mortality. These arrhythmias arise in two distinct phases during the first 60 minutes of ischemia (termed early and delayed or 1A and 1B respectively), that have a different electrophysiological mechanism. A thin subepicardial and subendocardial rim of myocardium survives during acute ischemia, whereas the midmyocardium becomes inexcitable. Previous reports have demonstrated the relation between cellular electrical coupling and the occurrence of the second, delayed phase of ventricular arrhythmias. We have recently demonstrated that critical coupling between the irreversibly damaged midmyocardium and the intrinsically viable subepicardium overlying the ischemic zone exerts a temporary electrophysiological depressant effect on the latter. Progress of ischemia induced cellular uncoupling thus resulted in partial and localized recovery of electrophysiological parameters of the viable subepicardium, leading to increased heterogeneities. Subepicardium was indeed proven partially viable during the subacute and chronic phase of ischemia, although remodeling of membrane currents and gap junction redistribution had occurred. As a result of the two-dimensional structure of the surviving layers, activation patterns during ventricular fibrillation during the delayed phase of ischemia are expected to become more organized and stable, and to show a decreased number of epicardial breakthrough patterns.

The aim of this study was to investigate the contribution of electrophysiological heterogeneities in the ischemic subepicardium to the arrhythmogenic substrate. To gain insight in the two dimensionality of activation, we therefore quantified the number of epicardial breakthrough activations and the life time of phase singularity points using phase mapping during direct current induced ventricular fibrillation in the blood perfused, regionally ischemic porcine heart. We measured dominant frequency of ventricular fibrillation within the ischemic zone during 60 minutes of ischemia and studied the number of domains of the same dominant frequency. In addition, we studied the time course of changes in action potential duration and conduction velocity during ventricular pacing and premature stimulation. In particular we examined whether local heterogeneities can be held responsible for the arrhythmogenic substrate.

Methods.

Isolated heart preparation

Pigs of either sex (n=12) were anesthetized and hearts were isolated as described in chapter 2. The aorta was cannulated and the heart was connected to a Langendorff perfusion setup, as described previously. The left anterior descending artery was prepared free distal to the first diagonal branch, and a ligature was placed underneath. An experimental occlusion of 30 seconds duration defined the location of the ischemic border zone. A bipolar hook electrode was inserted in the non-ischemic left ventricular free wall, approximately 5 mm from the ischemic border. A biventricular electrogram for rhythm monitoring was recorded from two hook electrodes that were inserted in the left ventricular free wall and in the right ventricular outflow tract respectively. The ischemic zone was kept moist through a drip superfusion with warmed oxygenated Tyrode's solution. Ischemia was produced by occluding the artery.
Optical measurements and analysis

Hearts were electro-mechanically uncoupled with diacyl monoxime (15 mM) and loaded with the voltage sensitive dye di-4-ANEPPS (20 uM). The optical setup consisted of a video imaging system as described previously. Details of the procedure are described in chapter 2.

Spatial dispersion in APD was expressed as the statistical variance (SD²) of APD within the ischemic zone. Conduction velocity was consecutively measured from sequential activation maps in the direction where the spacing of isochrones was largest before occlusion. Slow conduction was defined as conduction velocity less than 5 cm/s.

Phase maps were produced as described previously. In short, the fluorescence of each pixel was plotted against the fluorescence of that same pixel offset by a time interval. The angle of the coordinate of the fluorescence at both time points around the mean fluorescence describes the phase, θ(t), with values between -π and π. A new field 0(x,y,t) was constructed using all pixels. Phase singularities were detected as described previously.

Experimental protocols

Hearts were paced continuously at basic cycle length of 450 ms. Before coronary occlusion a control recording was made. Protocol 1 (n=5): a train of 8 basic beats was followed by a short coupled premature stimulus, applied from the pacing electrode in the non-ischemic zone. During the first 60 minutes of ischemia, movies of 10 seconds duration were made from ventricular pacing and premature stimulation every 5 minutes.

Protocol 2 (n=4): recordings of ventricular pacing were made every 2 minutes. Before coronary occlusion and after 15, 30, 45 and 60 minutes of ischemia, ventricular fibrillation was induced by bringing the two poles of a 9V battery in contact with the epicardium that was outside the view of recording. A 10 second recording was made after ventricular fibrillation had established for at least 2 minutes. Hearts were defibrillated with a DC shock.

To be able to compare the time course of ischemia in this DAM loaded preparation with previously published studies, we measured tissue impedance within the ischemic zone with the four electrode technique (protocol 3, n=3). Ten multi electrodes consisting of four platinum pins (length 5 mm, diameter 0.7 mm, interelectrode distance 2.5 mm) were inserted within the border and central ischemic zone. The ischemic border was defined as the first 1 cm within the cyanotic border of the ischemic zone. Recordings were made automatically every 30 seconds. Upon offline analysis, the percentage rise in tissue impedance was calculated with respect to the plateau value after 2 hours of ischemia.

Statistics

Data are presented as mean±SD. Differences between groups were tested with a two sided t-test, or, when data were not normally distributed with a Mann Whitney as a non-parametric alternative. Multiple comparisons were tested with ANOVA. A p<0.05 was considered significant.

Figure 4.1A: Representative example of action potentials within the ischemic subepicardium during 60 minutes of ischemia. Amplitude denoted in arbitrary units of fluorescence (AUF). B. Course of mean action potential duration at 70% repolarization in the same experiment at panel A. Note the initial increase in APD70. C. Mean statistical variance in action potential duration within the ischemic zone. Variance increases with progress of ischemia.
A

Duration of ischemia (min)

0'  15'  30'  45'  60'

50 AUF

200 ms

B

APD70 (ms)

Duration of ischemia (min)

0  15  30  45  60

C

Variance in APD70 (ms²)

Duration of ischemia (min)

0  15  30  45  60

61
Figure 4.2A. Example of development of slowing of conduction during basic pacing within the ischemic zone. A. activation maps recorded before ischemia and at 36 and 60 minutes of ischemia respectively. B. Enhanced decrease in conduction velocity upon premature stimulation. Basic beat and premature beat (coupling interval 150 ms) after 40 minutes of ischemia.

Results.

Subepicardial electrophysiological characteristics during 60 minutes of regional ischemia.

Before coronary occlusion, mean APD70 was 194± 20 ms. An immediate, small but significant increase in action potential duration (from 199±22 to 213±25 ms, p<0.0001) was observed in 5 out of 9 experiments. During the course of the IB phase, action potentials monotonically shortened to an average 74% of control after 60 minutes of ischemia. Figure 4.1A shows an example of subsequent action potentials from the same site within the ischemic zone. Panel B shows the time course of APD70 of this experiment: APD decreased from 191±7 before to 143±27 ms after 60 minutes of ischemia (p<0.0001). Panel C shows the mean time course of variance of action potential duration within the ischemic subepicardium in all experiments. Dispersion of action potential duration increased: mean statistical variance in action potential duration within the recorded ischemic zone increased on average 13 fold compared to control in individual experiments, mean variance increased from 123 to 717 and 825 ms² after 0, 30 (p<0.0001 vs control) and 60 minutes (p<0.0001 vs 30 minutes).

Conduction velocity in the dominant direction decreased from 99±31 to 54±32 cm/s (p<0.05) between 0 and 60 minutes of ischemia. During basic pacing, zones of slow conduction or conduction block developed within the ischemic zone in 7 out of 9 experiments starting from 38±11 minutes. Zones of block and conduction slowing were observed during the premature beat starting after 20±26 minutes of ischemia (p=NS vs basic stimulation). Figure 4.2A shows examples of activation maps from a typical experiment after 0, 36 and 60 minutes of ischemia, in which the decrease of conduction velocity and the development of zones of slow conduction and block can be seen. Note that the majority of the ischemic subepicardium is still excitable after 60 minutes of ischemia. Panel B shows an example of enhanced conduction slowing upon premature stimulation (coupling interval 150 ms) in another experiment than displayed in panel A.

Rise in tissue impedance during ischemia.

In 3 hearts, the course and dispersion of rise in tissue impedance in the presence of DAM was investigated. In our previous study we showed that ventricular arrhythmias are related to only a moderate increase in tissue impedance which started after 14 minutes of ischemia, and that the maximal rate of rise was after 37 minutes. The addition of DAM to the perfusion fluid before occlusion did not change tissue impedance (-0.3%, p=NS). In quiescent heart, it is expected that the onset of rise in tissue impedance is delayed compared to the contracting heart. Indeed, onset of rise of tissue impedance occurred after 41±9 minutes of ischemia. Impedance increased from 380±89°.cm by only 14±19% (p<0.05 vs control) between 0 and 30 minutes of ischemia. After 60 minutes, impedance was 41±16% of its final (after 2 hours) value (p<0.05 vs 30 minutes). No differences in relative rise of tissue impedance between the ischemic border and central zone were found, thus
Figure 4.4A (previous page) Biventricular electrogram of direct current induced ventricular fibrillation. B. Representative action potentials of the same episode of ventricular fibrillation as panel A. C. Power spectrum of 10 seconds of ventricular fibrillation from the same pixel as B. D. Dominant frequency map of the same episode. E. Course of number of domains (>50 pixels) with one distinct dominant frequency during 60 minutes of ischemia. F. Change in dominant frequency in the entire ischemic zone during 60 minutes of ischemia in 5 experiments in which ventricular fibrillation was induced. 

these data were pooled. Hence, the development of cellular uncoupling is delayed by approximately 25 minutes in DAM loaded hearts compared to contracting hearts.

Spontaneously occurring ventricular arrhythmias

Spontaneously occurring ventricular arrhythmias were relatively sparse during the ischemia protocol. In 3/5 experiments, spontaneous premature depolarizations followed premature stimulation protocol in only 7% of the movies. Couplets occurred in 2%. Nonsustained ventricular tachycardia and ventricular fibrillation each only occurred once underlining the role of mechanical activity in the triggering mechanism (see chapter 7). Figure 4.3A and B show the biventricular electrogram and a representative fluorescence signal from the ischemic zone during spontaneous occurrence of ventricular fibrillation after 40 minutes of ischemia. Figure 4.3C-H shows activation maps of the last paced beat and the first 5 beats of this occasion of ventricular fibrillation. The focal occurrence of the first beat of VF is followed by subsequent activations of the recording field from outside the recording area (beat C-H). Note that no apparent regions of conduction block are present, and that the activation waves are planar.

Characteristics of ventricular fibrillation.

Figure 4.4A shows the biventricular electrogram of direct current induced ventricular fibrillation after 30 minutes of ischemia. In panel B four action potentials of four representative sites are displayed (marked a-d in panel D). Panel C shows the Fast Fourier Transform power spectrum of the entire recording (10 s) of this episode of VF in these four pixels. Dominant frequency of direct current induced ventricular fibrillation was 7.7±1.3 Hz before coronary occlusion. The dominant frequency map of this episode of VF is displayed in figure 4.4D. Figure 4.4E shows the course of the number of domains with one distinct dominant frequency. Figure 4.4F shows the course of dominant frequency before and at 15, 30 and 60 minutes of ischemia in 5 experiments in which VF was induced. In 4 out of 5 experiments in which VF was induced, ischemia caused a small but significant decrease in dominant frequency to 6.8±1.4 Hz after 15 minutes (p<0.001). At thirty minutes of coronary occlusion, dominant frequency started to improve again to 7.4±1.5 Hz, and at 60 minutes of ischemia, dominant frequency has restored to preocclusion values, 7.8±1.7 Hz. However, the number of domains with a distinct dominant frequency increased from 7.6±3.6 before occlusion to 10.3±14 after 60 minutes (figure 4.4E, p<0.05), indicating a larger dispersion in dominant frequencies in the subepicardium after 60 minutes of ischemia.

Figure 4.5A shows phase maps of a breakthrough activation (yellow region at the right side of the map) after 30 minutes of ischemia, and of the same breakthrough 10 ms later, when the region activated by this breakthrough activation has rapidly expanded. Panel B displays the number of breakthrough activations during ventricular fibrillation (n=4 hearts). The first 1.25 seconds of a total of 59 episodes of VF were studied, and the number of breakthroughs decreased during the course of ischemia. Before occlusion the frequency of breakthrough activations was 6.2±4.4 Hz, which decreased to 2.7±2.8 after 30 minutes (p<0.05) and further to 0.7±1.2 after 60 minutes of ischemia (p<0.05 vs 30 minutes). Panel C shows that meanwhile the life span of phase singularity points increased from 48±52 ms before ischemia to 99±38 and 193±103 ms after 30 and 60 minutes
respectively (both $p<0.05$ vs pre occlusion). Hence, during the course of the 1B phase conduction during ventricular fibrillation becomes more confined to the two dimensional layer of the subepicardium, and the arrhythmia demonstrates less wave breaks and becomes more organized and stable.

**Histological features of the ischemic subepicardium.**

We histologically examined micro sections of tissue from the ischemic zone. No differences were found between nuclei of myocytes in the midmyocardial tissue and in the subepicardial rim (not shown). However, given the duration of ischemia one does not expect changes in nucleus morphology to have occurred already.

**Discussion.**

In this contribution, we report for the first time an extensive study of thousands of optical action potentials during 60 minutes of regional ischemia in the isolated blood perfused heart. We demonstrate that in the mechanically quiescent heart action potential duration and conduction velocity decrease during the first 60 minutes of ischemia, while the dispersion of action potential duration increases. Ventricular fibrillation becomes confined to the two dimensional layer of the subepicardium. Dominant frequency of ventricular fibrillation initially decreases within the ischemic zone, but recovers fully after 60 minutes of ischemia compared to pre occlusion values. The number of domains with a distinct dominant frequency does however increase, indicating an increased heterogeneity in the ischemic subepicardium. Activation patterns of ventricular fibrillation become confined to the two dimensional subepicardium during the delayed phase of acute ischemia.

**Substrate of delayed ventricular arrhythmias during acute ischemia**

For any arrhythmia to occur, a relevant trigger and an electrophysiological substrate need to be present. It has been suggested that the substrate for delayed ventricular arrhythmias is different from that during the first ten minutes of ischemia. In an earlier study we demonstrated that the subepicardium overlying the ischemic zone recovered, terminating the period during which delayed arrhythmias could be induced. The current study was undertaken to more specifically study the time course of electrophysiological changes in the subepicardium. We found an initial increase in action potential duration, but after 5 minutes of ischemia APD decreased progressively up to approximately 74% of control, compatible with earlier studies. The absence of gross recovery of APD in this preparation could relate to the absence of mechanical activity: diffusion of potassium and protons that are extruded by the ischemic tissue is hampered because of the lack of contraction. However, dispersion of action potential duration increased in the absence of decrease of mean action potential duration during ischemia, indicating that locally recovery in action potential duration took place while at other sites action potential duration decreased even further.

For the wavelength of an arrhythmia, refractory periods rather than action potential duration are the functional measure. Little information is available on the relation between refractory period and action potential duration in the ischemic subepicardium during the delayed phase of ischemia, although the early phase of ischemia has been studied extensively. Decreased excitability may underlie the decrease in dominant frequency of ventricular fibrillation observed. Our observation that mean dominant frequency initially decreases but fully recovers during 60 minutes of ischemia is consistent with recovery from post repolarization refractoriness. Alternatively, a decrease in the
A

$\text{t=0}$

$\text{t=10 ms}$

B

Frequency of breakthrough activation (Hz)

0 15 30 45 60

0 2 4 6 8

C

Life time PS (ms)

0 50 100 150 200 250

0 15 30 45 60

Duration of ischemia (min)
excitable gap of the arrhythmia may be responsible for the increase in ventricular fibrillation frequency. In the latter case this would indicate that the size of anatomic or functional barriers around which the reentrant activation wanders had decreased in circumference. We observed lines of activation block, but no evidence for decrease in their size was found. Also, no increase in conduction velocity was found.

We demonstrated an increased variance in action potential duration during ischemia, indicating increased heterogeneity. Recently it was demonstrated that within a two dimensional model of myocardium, wave breaks can occur when action potential restitution is changed within a largely heterogeneous substrate. Similar changes in the arrhythmogenic substrate may have altered dominant frequency of direct current induced ventricular fibrillation.

Role of DAM in ischemia-induced electrophysiological changes

DAM is an excitation contraction uncoupler with relatively minor effects on transmembrane ionic currents in the concentrations used in this study. The addition of DAM changes the energy expenditure of the heart, which is of particular importance during ischemia. The quiescence of myocytes is per se energy saving, although during ischemia the ischemic zone stops contracting within the first few minutes of coronary occlusion. Thus, the contribution of mechanical activity is limited. In addition, a direct energy saving effect of low concentrations of DAM was proposed by Vanoverschelde: DAM induced blockade of myofibrillar ATPase results in reduced accumulation of $H^+$ and $Ca^{2+}$.

The objective of the current study was to investigate electrophysiological changes during the delayed phase of ischemia. Hence, the change in time course through the use of DAM is of importance. We found that the onset of rise of tissue impedance, which is an indirect measure of cellular uncoupling, is postponed by approximately 25 minutes compared to contracting hearts. Thus, caution should be taken when extrapolating our results to the beating heart.

Two dimensional activation of the subepicardium

We demonstrated that during the delayed phase of acute ischemia the number of breakthrough activations during ventricular fibrillation decreased significantly. This is consistent with a decreasing contribution of the ischemic midmyocardium to subepicardial activation. Intramural reentry has been proposed as mechanism for arrhythmias during the delayed phase of acute ischemia, but we previously demonstrated that midmyocardial electrical activity ceases while parts of the ischemic subepicardium remained excitable.

In addition, the dynamics of ventricular fibrillation changed during this phase of ischemia: the lifetime of phase singularity points increased. Phase singularity points are sites within excitable media with an arbitrary phase, surrounded by excitable elements that have a continuously changing phase. They arise when waves break or curve and can form the anchoring point of figure-of-eight reentry. However, the lifetime of phase singularity points in the normoxic heart is very short, such that in only approximately 2% a complete reentrant revolution takes place. This is caused by continuous breakdown of wavelets. Prolonged lifetime of phase singularities, as we have demonstrated during the course of acute ischemia, indicates fewer waves breaks and a more organized arrhythmia, consistent with two-dimensional activation. In the partially frozen pig heart, it
was indeed shown that wavelets live longer in two than in three dimensional ventricular fibrillation. We studied the behaviour of direct current induced ventricular fibrillation during ischemia, hence, we cannot conclude on altered inducibility of ventricular fibrillation. However, it can be inferred that the arrhythmogenic vulnerability should decrease when activation patterns become two-dimensional, as is the case in the beating regionally ischemic isolated heart.

Delayed ventricular arrhythmias during acute ischemia are inducible only at a relative rise in tissue impedance of less than 40%. Indeed, relative tissue impedance in this study was found to increase by 41% during the 60 minutes of ischemia that we investigated. Hence, we suggest that the transition of three dimensional into two-dimensional conduction as observed in our experiments relates to the diminishing of the substrate for delayed ischemia-induced arrhythmias.

Limitations

We demonstrated that lines of slow conduction and block arise during ventricular basic and premature stimulation, and that during ventricular fibrillation these zones function as barriers that anchor reentry. However, we did not study the induction of ventricular fibrillation, thus the exact role in arrhythmogenesis of these heterogeneities remains to be established. Potentiometric measurements and measurement of tissue impedance were not performed in the same hearts because the impedance electrodes would have obscured the field of recording.

Conclusions

We showed that action potential duration and conduction velocity decrease during 60 minutes of ischemia. However, the dispersion of action potential duration increased between 30 and 60 minutes of ischemia while APD did not further decrease, suggesting both recovery and deterioration locally. Conduction during ventricular fibrillation becomes confined to the two dimensional subepicardial layer when ischemia progresses and the life time of phase singularities significantly increases, consistent with a more organized and stable form of conduction. These data suggest that the transition of three to two dimensional conduction terminates the period during which ischemia induced delayed ventricular arrhythmias occur.

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