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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INITIATION OF DELAYED, ISCHEMIA-INDUCED LIFE THREATENING ARRHYTHMIAS: FOCAL ACTIVITY RELATED TO LEFT VENTRICULAR MECHANICAL FUNCTION

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Summary.

Background. Life threatening ischemia induced arrhythmias occur in two phases. The mechanism of the initiation of the delayed arrhythmias (15-60 minutes following coronary occlusion) is unknown. We hypothesize that the force exerted by the contracting myocardium on the ischemic myocardium induces the initiating premature beat.

Methods and Results. Left ventricular epicardial mapping (11x11 matrix, 5 mm interelectrode distance) of the initiating beats of delayed arrhythmias was performed in 1) open chested pigs (n=7), 2) isolated non working (n=8) and 3) isolated working pig hearts (n=5). Hearts were paced from the left ventricle. No differences in ischemic zone size existed between the groups. Arrhythmias were significantly less frequent and less severe in isolated preparations than in both working preparations. No differences were detected between in situ and isolated working hearts. An epicardial focal origin was detected in 26% of all initiating beats of delayed arrhythmias, six times more often from the border region than from elsewhere. During a pacing protocol with a 700-750 ms pause (isolated hearts with balloon, n=2) more premature beats occurred in the first post pause interval than in any other moment interval.

Conclusions. In isolated hearts delayed arrhythmias were less frequent and less severe than in working preparations. Focal activity was documented in 26% of arrhythmias and emerged from the electrophysiological border. Post pause contractile potentiation was associated with more arrhythmias. We conclude that the initiating beat during the phase of delayed arrhythmias is caused by mechanical interaction between normal and ischemic tissue.
Introduction.

Ventricular arrhythmias following a coronary occlusion constitute a major risk factor for sudden cardiac death. Study of these arrhythmias has focused on those in the first 10 to 15 minutes of regional ischemia. However, a second phase of arrhythmias, 15-60 minutes after occlusion occurs in dogs, but also in other species. The relative contribution of the delayed (IB) type of arrhythmias and the early (1A) arrhythmias to sudden cardiac death in humans is unknown, but animal experiments suggest that mortality in the IB phase is larger than in the 1A phase.

The mechanism of IB arrhythmias has not been studied extensively. For spontaneous arrhythmias the concurrence of a “trigger” (the initiating premature beat) and a “substrate” (pre-existing proarrhythmic conditions) is a prerequisite. Kaplinsky et al. proposed a non-reentrant mechanism for IB arrhythmias on the basis of the absence of local continuous electrical activity (diastolic bridging) preceding a premature ventricular beat. An association between spontaneously occurring ventricular fibrillation (VF) during the IB phase and intercellular electrical uncoupling as documented by a rise in tissue impedance has been described and remains following ischemic preconditioning. Intercellular uncoupling gives rise to conduction slowing and may form the substrate for (micro) reentry.

De Groot et al. have defined the substrate of delayed ventricular arrhythmias by repetitively applying a trigger in the form of a closely coupled premature beat. During the IB phase of arrhythmias a temporary substrate for VF is formed by a period of critical coupling of surviving to severely depressed myocardium. The mechanism of the initiating beat in the IB phase, however, is not known.

Zhou et al. have documented that a sudden increase in left ventricular load is arrhythmogenic during ischemia up to 30 minutes duration. However, the precise mechanism of the effect of ventricular loading was not established. Franz et al. have suggested that myocardium experiencing greater relative stretch may act as “focus” of arrhythmias. Acute volume load in infarcted canine hearts reduced refractory period and increased inducibility of tachyarrhythmias.

We hypothesize that the initiating mechanism of IB arrhythmias is related to the force exerted by the unaffected myocardium on the perimeter of the ischemic tissue. Because compliance of the ischemic tissue decreases at the time of the IB arrhythmias the stretch experienced by the border zone increases. We tested the following implications of this hypothesis: 1) in the IB phase of arrhythmias, more triggers occur in ejecting compared to unloaded ventricles; 2) the initiating beat originates from the interface between viable and ischemic myocardium; 3) the occurrence of premature beats is related to left ventricular contraction. To test this effect we performed left ventricular activation mapping during the first hour following coronary occlusion in isolated perfused ejecting and non-ejecting hearts and in in situ hearts of open chested pigs.

Methods.

Animal preparation

Isolated, Langendorff perfused non working pig hearts (n=8) and in situ, open chested pigs (n=7) were handled as described in chapter 2. In the working isolated preparations (n=5) a compliant plastic bag was introduced into the left ventricle through the left atrium and connected to tubing, filled with prewarmed (37 °C) saline. A clamp allowed control of the ejection resistance.
Figure 7.1. Averaged arrhythmias in five minute bins of observation time following coronary occlusion (at t=0) in the three models used (panels A, B and C). Number of surviving animals indicated by drawn line. During the first 10 minutes of ischemia the hearts were beating spontaneously. VPB, ventricular premature beat (number divided by 10); VT, ventricular tachycardia.
Figure 7.2. Top panel. Hearts grouped according to the most severe arrhythmia in the delayed phase of arrhythmias. No significant difference between both working preparations (in situ and isolated working hearts). Arrhythmia severity in working hearts was significantly larger than in non-working (isolated) hearts. Bottom panel: Number of triggers (first VPBs) corrected for the differences in observation time. No statistical difference occurred between both working preparations. Both were significantly different from the isolated perfused (non working) model.
A pressure transducer was connected to the tube. The saline level and the tube clamp were adjusted to a diastolic pressure of 0-10 mm Hg and a systolic pressure of 30-50 mm Hg.

A short (<30 sec) occlusion of the left anterior descending artery (LAD) below the first diagonal branch identified the ischemic zone. A ligature was left around the LAD. Half of the composite electrode (11x11, 5 mm interelectrode distance) was positioned over the prospective ischemic area. A bipolar stimulating electrode was placed in the non-ischemic left ventricle, close to the multi electrode. Bipolar recording electrodes were placed near the stimulating electrode and on the right ventricular outflow tract.

Protocol

After an equilibrium period of at least 30 minutes following instrumentation the ligature around the LAD was tied. Pacing was discontinued until 10 minutes following LAD-occlusion to reduce susceptibility to early ventricular arrhythmias. At 10 minutes of ischemia ventricular pacing was started. Spontaneous occurrence of ventricular arrhythmias was recorded from 15 until 60 minutes of ischemia (observation time). As many as possible onsets of arrhythmias were captured on disk. After the occurrence of VF the observation period was ended.

The following groups were studied: 1; open chested animals (in situ), 2; isolated perfused hearts (isolated), 3; isolated perfused hearts with intraventricular balloon (isolated working), 4; as group 3 but with a pacing protocol incorporating a pause.

Following each experiment the heart was perfused with a solution containing black ink. The atria, the aorta and the pulmonary vessels were removed and the ventricles were divided in stained and unstained tissue. The ratio of the weight of unstained tissue and total ventricular tissue was calculated.

Analysis of data

Isochronal maps were constructed from all stored activation sequences if the total number of arrhythmias was below 25. When more arrhythmias occurred, and equal fraction of arrhythmias in each 5 minute bin of observation time was selected. Activation times were determined as described in chapter 2.

Hearts were graded according to the following arbitrary arrhythmia severity scale (60 minutes of ischemia). 0; no arrhythmias, 1; <30 ventricular premature beats (VPBs), 2; >30 VPBs, 3; couplets, 4 VT (ventricular tachycardia: 3 or more consecutive VPBs) or VF. A higher grade indicated a more severe arrhythmia. Focal activity was defined as earliest electrical activity within the field of electrodes. Earliest activity at the margin of the electrode was not considered focal. At the site of earliest activation, no R-wave should be present. Reentrant activation was defined according to Pogwizd and Corf. The line separating tissue with ST-elevation from tissue with ST-depression indicated the electrophysiological border.

Statistics

Data are presented as mean±SEM unless stated otherwise. The z-test was used for testing differences between proportions. For nonparametric comparisons of multiple groups Kruskall-Wallis (KW) followed by Dunn's test was used, for parametric comparisons ANOVA and Newman-Keuls test. p<0.05 is assumed a statistical significant difference. NS indicates a non statistically significant difference.
Results.

Incidence of spontaneous arrhythmias

Figure 7.1A shows the incidence of spontaneous arrhythmias during the first hour following occlusion in \textit{in situ} hearts in bins of 5 minutes. Stimulation was started at the end of the 1A phase (10 minutes of ischemia) with a constant cycle length between 350 and 450 ms, depending on the spontaneous sinus rate. During the delayed (1B) phase of arrhythmias VF occurred in 5 of 7 animals (decrease in n). A delayed phase of arrhythmias is obvious. Panel B shows the spontaneous arrhythmias in group 2. Although a 1B phase of arrhythmias is present, the incidence of arrhythmias is much lower. More delayed arrhythmias are present with an intraventricular balloon (figure 7.1C) than without (figure 7.1B).

In figure 7.2 the individual experiments were graded according to an arrhythmia severity scale. The severity of arrhythmias was significantly greater in group 1 and 3 than in group 2 (both p<0.05). No statistical significant differences occurred between groups 1 and 3. Note that the total duration of the grouped 1B phase is 145 (46% of observation time), 335 (93%) and 225 (100%) minutes in group 1 (n=7), 2 (n=8) and 3 (n=5), respectively, as a result of VF. Panel B summarizes the averaged total number of triggers per heart (the first VPBs of all arrhythmias) in the three groups of experiments, corrected for the differences in total duration of observation time. Groups 1 and 3 differ statistically significantly from group 2, but not from each other.

The relative weight of the ischemic myocardium was 30.6±2.0 (mean±SEM, n=7), 31.9±2.7 (n=8) and 29.8±1.5 (n=5) % in group 1, 2 and 3 respectively (p=NS).

Origin of the initiating premature complex

Isochronal mapping was performed of the initiating beats of arrhythmias. The number of isochronal maps made was 269, 117 and 283 for groups 1, 2 and 3 respectively. An isochronal map of the onset of VF (\textit{in situ} heart, 21 minutes following occlusion) is shown in figure 7.3. The electrogram shows the last two stimulated beats and the first spontaneous beats that initiated VF. In panel A the activation wave of the last basic beat traverses the compound electrode from the site of stimulation into the ischemic area. The first premature beat (panel B) has a focal origin at the border between the ischemic and the normal tissue. The second beat of the arrhythmia (panel C) is not apparently focal.

All first premature beats of which maps were made were categorized as either focal or not focal of origin. Seventy-two of 273 (26.4 %) analyzed first premature beats had a focal origin (p=NS between groups). No reentrant origin of the arrhythmias was detected.

The shortest distance of focal origins to the electrophysiological border in \textit{in situ} experiments is shown in 5 mm bins in figure 7.4A. A predominance of focal activity is observed close to the electrophysiological border. Panel B shows that the grouped occurrence of focal activity in groups 1-3 in the border region (extending 1 cm at either side of the electrophysiological border) is significantly larger than of focal activity in tissue outside the border region (z-test). The number of electrode sites inside and outside the border region was 572 and 724 respectively.

The averaged distance to the electrophysiological border was 0.4±1.4, -3.5±0.9 and -4.4±1.1 mm in groups 1, 2 and 3 respectively (mean±SEM, p<0.05 between groups 1 and 2, and groups 1 and 3, NS between 2 and 3).

Figure 7.5 shows an activation map of a premature beat recorded after 26 minutes of ischemia. Two sites underlying the electrode demonstrated early activity. At both sites local electrograms had an initial negative deflection and activation spread in a centrifugal fashion.
Figure 7.3. Top panel. Electrogram of the onset of VF 21 minutes following occlusion. The shaded area (diagram) designates ischemic tissue, the pulse the site of stimulation. Dots in the enlarged multi electrode represent electrode positions, the circle the site of focal origin. The dotted line indicates the electrophysiological border. Panels A, B and C are activation maps of the corresponding beats (top panel). Lines indicate 5 ms isochrones, arrows gross activation sequence. Numbers indicate activation time, the asterisk the site of origin of the premature beat. Note the absence of R-wave in local electrogram recorded from the focus (top panel).
Figure 7.4. Panel A. Number of foci (in situ experiments) in 5 mm bins distance from the electrophysiological border (0 mm). Negative values indicate the ischemic side of the border. The border region is between the dotted lines. Panel B. Number of foci (all experiments) in locations inside and outside the border region. More foci occur within the border region.
Both early foci of activation were positioned close to the electrophysiological border. Of 72 focal activation patterns, 21 demonstrated a secondary origin.

When more than a single premature beat was recorded (all 3 experimental groups) focal activity was recorded in the second beat in 18 of 86 cases, in the third in 9 of 37. Sequential focal activity (focal activity in beat 1 and 2 or in beat 2 and 3) was recorded in 11 cases. Figure 7.6 shows an example (group 3) in which focal activity was documented in two consecutive beats after 34 minutes of ischemia. The isochronal maps demonstrate that the foci were disparate but were located near the electrophysiological border in both cases. Deeply negative T-waves were not observed during the observation period.

**Ectopic activity and contraction**

In group 4 (two hearts) we tested whether more spontaneous premature ventricular beats occurred following the first contraction after a pause (of 700 and 750 ms). Of the 101 triggers during the 1B-phase 39 occurred in the first interval following the stimulation pause (p<0.05 vs the expected 11%). On the other hand, 4 triggers occurred in the second post-pause interval (p<0.05 vs the first post-pause interval). Figure 7.7 is an online recording of 3 premature beats in subsequent stimulation cycles and of pressure in the intraventricular balloon.

Figure 7.8 shows the spontaneous occurrence of ventricular fibrillation. Note the potentiated pressure in the normally paced beat just preceding the arrhythmia.

**Discussion.**

This study demonstrates that the initiating mechanism of the delayed ischemia-induced arrhythmias is related to left ventricular mechanical function. In non-working preparations the incidence of delayed arrhythmias is low. De Groot et al. have demonstrated in the same model that an appropriately timed premature beat could induce VF more easily during than before or after the 1B phase. The substrate for delayed ischemia-induced arrhythmias is formed by the critical coupling between a surviving subendocardial and subepicardial layer and the severely depressed intramural tissue. Thus, the paucity of triggers rather than the absence of a substrate causes the relative arrhythmogenic quiescence during the 1B phase in non-working hearts.

In the in situ experiments the autonomic nervous system may have influenced arrhythmogenesis. The incidence of triggers is similar in isolated perfused working hearts and in in situ, whereas VF occurred more often in the in situ model. This suggests that the autonomic nervous system, although not relevant for the triggering mechanism, acts on the substrate of the arrhythmia possibly by creating heterogeneities by focal release of catecholamines. The topic of the present study relates to the origin of the initiating beats of an arrhythmia only.

A focal origin could be demonstrated in 26% of the first premature beats, other earliest activity being detected at the margin of the electrode. The position of the multi electrode (halfway over the left ventricular ischemic border) precluded detection of foci elsewhere. A reentrant origin of the arrhythmia was never detected in the tissue under the electrode. De Groot et al. have demonstrated that intramural electrical activity is absent in this phase of arrhythmias and that epicardial conduction velocity is such that reentry, if present, could have been detected with the electrode used.

Whenever a focal origin was detected, it was primarily located at the border of ischemic and non-ischemic tissue. Although small differences in the distance of the focal origins and the electrophysiological border occur between various models, a small zone of preferential focal activity
Figure 7.5. Multiple foci. Top panel: electrograms 26 minutes after occlusion. Activation map from the VPB shows two foci (asterisks). Details as in figure 7.3. Electrograms were recorded from correspondingly marked sites.
Figure 7.6. Sequential foci. Top panel. Electrograms of a short run of VT, 26 minutes following occlusion. The panel shows activation maps of the subsequent beats. Details as in figure 7.3. The first and second beats have focal origins marked A and B (circles in middle left panels) close to the border. No R-waves occur at the sites of origin (top panel).
Figure 7.7. Pause protocol. Pressure recording from the intraventricular balloon (19 minutes following occlusion) Left and right ventricular (LV and RV) bipolar electrograms show VPBs in the first post pause interval.
was present in each. In association with the increased number of arrhythmias in working versus non-working models an effect of mechanical interaction between the normal and the ischemic zone is probable. Notably, the fraction of focal origins was not different between the various models suggesting that a similar mechanism caused arrhythmias.

Although the mechanical interaction across the ischemic border continues, delayed arrhythmias cease. Other factors, therefore, facilitate this process. The diastolic current of injury tends to depolarise tissue at the normal side of the ischemic border, but is not strong enough to bring myocardium to excitation threshold. When intercellular uncoupling is complete (end of the 1B phase), the electrotonic influence ceases. Signs of the endsystolic injury current (deeply negative T-waves), which is responsible for the initiation of early ischemia-induced arrhythmias have not been detected during the delayed phase. Alternatively, release of endogenous catecholamines may cause abnormal automaticity. However, release of catecholamines occurs in the severely depressed tissue. Because sufficient excitabile myocardium is present in the ischemic tissue (in the subendocardial and subepicardial regions) focal origin of the arrhythmia should have been detected from within the ischemic tissue, not preferably from the border tissue. Also, the occurrence of repetitive focal activity was rare and was documented maximally up to two beats. Nevertheless, interplay between the release of catecholamines from the ischemic nerve endings and stretch activated channels may play a role in the temporal abundance of triggers. The small differences in the site of focal activity between the various models relative to the ischemic border could relate to subtle differences in the transmural development of ischemia depending on left ventricular loading. Indeed, the distance of focal activity to the electrophysiological border did not differ.

**Clinical significance**

This study provides evidence for the arrhythmogenic effect of mecanoelectrical feedback for the initiation of arrhythmias during the delayed phase of acute ischemia. In the presence of a suitable substrate this mechanism may lead to VF. The arrhythmogenic effect of mechanical interaction between the ischemic and normal myocardium during ischemic has been anticipated but not directly demonstrated. These results have implications for clinical practice. Not only does this paper confirm the important contribution of the delayed (1B) phase of arrhythmias to early mortality following coronary occlusion, it implies that increasing myocardial contractility or increased afterload may be arrhythmogenic. Also, it can be inferred that regional ischemia in hypertrophied hearts (following previous myocardial infarction) is more arrhythmogenic than in normal myocardium. In addition, hypertrophied myocytes are more susceptible to stretch than normal myocardium.

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**References.**

intraventricular pressure (mm Hg)

LV electrogram

RV electrogram

stimulus protocol

1 sec.

Figure 7.8. Pause protocol; details as in figure 7.7. Induction of ventricular fibrillation following a potentiated beat.


13. Carmeliet E: Cardiac ionic currents and acute ischemia: from channels to arrhythmias. *Physiological Reviews* 1999;79:917-1017


25. Lab MJ: Transient depolarization and action potential alterations following mechanical changes in isolated myocardium. *Cardiovascular Research* 1980;14:624-637
