ELSEVIER

Cardiovascular Research 50 (2001) 362–372

Cardiovascular Research

www.elsevier.com/locate/cardiores
www.elsevier.nl/locate/cardiores

Late ventricular arrhythmias during acute regional ischemia in the isolated blood perfused pig heart
Role of electrical cellular coupling

Joris R. de Groot[a,*], Francien J.G. Wilms-Schopman[b], Tobias Opthof[c], Carol A. Remme[a], Ruben Coronel[a]

[a]*Experimental and Molecular Cardiology Group, Academic Medical Center, Amsterdam, The Netherlands*
[b]*The Interuniversity Cardiology Institute, The Netherlands*
[c]*Department of Medical Physiology, University Medical Center Utrecht, The Netherlands*

Received 16 October 2000; accepted 19 January 2001

Abstract

Objective: Acute ischemia comes with two phases of life-threatening arrhythmias, early (within 10 minutes, 1A) and late (after about 15 minutes, 1B). The mechanism of the latter is unknown and in this paper, we test the hypothesis that a phase of intermediate coupling between surviving epicardium and inexcitable midmyocardium underlies 1B arrhythmias. **Methods:** Pig hearts ($n=26$) were retrogradely perfused with a blood Tyrode's mixture. The left anterior descending artery was occluded. We investigated (1) inducibility of ventricular fibrillation (VF) with programmed stimulation, (2) tissue impedance (Rt) heterogeneity within the ischemic zone, (3) multiple subepicardial and midmyocardial electrograms, (4) subepicardial lactate dehydrogenase (LDH) and glycogen content. **Results:** In nine of ten hearts, one–three premature stimuli caused VF between 14 and 53 min of ischemia. This typically happened when the Rt of the ischemic zone had increased up to 40% of its final value. More uncoupling terminated the period of VF inducibility. The excitability of the surviving subepicardial layer was depressed during the same period with partial uncoupling, but recovered when the uncoupling from the midmyocardium had progressed further. **Conclusions:** We show that 1B-VF can be induced within a distinct time window and coincides with a distinct range of Rt rise. Subepicardium is electrically depressed, presumably through coupling with midmyocardium, complete uncoupling causes subepicardial recovery and terminates the substrate for 1B-VF. Hence, we suggest that the substrate for 1B-VF consists of intermediate coupling of subepicardium and midmyocardium. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ischemia; Arrhythmia (mechanisms); Cell communication; Ventricular arrhythmias; Sudden death

1. Introduction

Sudden cardiac death is associated with ischemia-induced ventricular fibrillation (VF) in the majority of cases [1]. During acute myocardial ischemia, ventricular arrhythmias occur in two distinct phases separated by a period without arrhythmias [2]. The absence of subepicardial continuous diastolic activity ('diastolic bridging') preceding a ventricular premature beat in the second of these episodes (the 1B phase, lasting from 15 to 45 min of ischemia) was interpreted as an indication that a mechanism other than reentry underlies arrhythmias in this phase [2]. In contrast, Kaplinsky et al. [2] did record diastolic bridging, a clear indication of a reentrant mechanism, in the earliest of the two phases [3]. Notably, Kaplinsky [2] did not make a distinction between the arrhythmogenic mechanism of the 'trigger' (the first premature beat) and the mechanism of maintaining the arrhythmia [4].

*Corresponding author. Tel.: +31-20-566-3266; fax: +31-20-697-5458.
E-mail address: j.r.degroot@amc.uva.nl (J.R. de Groot).

Time for primary review 43 days. For this article Prof. Juan Cinca acted as Guest Editor.

0008-6363/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0008-6363(01)00222-X

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

Smith et al. [5] have documented an association between intercellular electrical coupling (measured as a rise in tissue impedance) and the 1B phase of arrhythmias. The association remained following a period of ischemic preconditioning [6]. Although this association does not establish a causal relationship between the phenomena, it is suggestive that the intercellular uncoupling leads to conduction slowing and that (micro-) reentry underlies 1B arrhythmias. Indeed, closure of the gap junctions leads to slowed intercellular activation [7–10]. A (micro-) reentrant mechanism requires a large inhomogeneity of cellular uncoupling [11].

Following coronary occlusion, thin subepicardial and subendocardial layers functionally survive longer than the midmural portion of the left ventricular wall [12–15]. We hypothesize that the disparate rate of loss of function of the subepicardium relative to the midmural tissue provides the basis of the arrhythmogenic substrate in the 1B phase of arrhythmias. During the period of rise in tissue impedance, a critical degree of coupling between the severely depressed intramural layer and the moderately depressed subepicardial layer may lead to a temporary decrease of excitability and conduction velocity in the subepicardial layer [16,17]. After termination of the process of uncoupling, subepicardial conduction recovers. In this study, we specifically studied the following implications of the hypothesis: (1) subepicardial tissue survives longer than midmural tissue; (2) ischemia-induced cellular uncoupling is heterogeneous within the ischemic zone; (3) local subepicardial electrophysiological characteristics improve with the progression of cellular uncoupling following a temporary deterioration; (4) during critical electrical coupling, local subepicardial electrophysiological characteristics are temporarily depressed. In relation to a critical degree of coupling between subepicardium and midmyocardium, programmed electrical stimulation leads to a higher inducibility of VF.

The background of focussing on the substrate for these arrhythmias in this study lies in the notion that a plethora of triggering events will not induce an arrhythmia in the absence of a suitable electrophysiological substrate. However, in the presence of an electrophysiological substrate, a single premature beat may be enough to initiate VF.

Therefore, we studied the inducibility of VF rather than the spontaneous occurrence and mapped transmural activation characteristics, intramural tissue impedance and subepicardial activation in isolated regionally ischemic pig hearts. We conclude that: (1) during the process of uncoupling, electrophysiological properties recover in regions of surviving subepicardium, and that (2) the inducibility of 1B ventricular arrhythmias coincides with partially risen tissue impedance and ceased intramural activity. These findings support our hypothesis that residual, intermediate coupling of the surviving tissue to the irreversibly damaged myocardium underlies the substrate for 1B ventricular arrhythmias.

2. Methods

2.1. Experimental animals and surgical procedures

Pigs of either sex ($n=26$) were handled in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1996). Sedation with azaperone (200 mg i.m.) and metomidate (100 mg i.m.) preceded intravenous anesthesia with pentobarbital (20 mg/kg). Hearts were extirpated and perfused with a blood Tyrode's mixture, according to Langendorff, as described previously [18]. A ligature was placed distal to the first diagonal branch of the left anterior descending artery (LAD). An occlusion of 30 s localized the cyanotic border and the size of the ischemic zone. A bipolar hook electrode was inserted in the non-ischemic myocardium, 5 mm from the cyanotic border. The ventricle was stimulated at a basic cycle length of 450 ms. Myocardial temperature was maintained at 37°C. Cellular uncoupling was measured through change in tissue impedance (Rt) measured with the four-electrode technique [19–21].

2.2. Experimental groups

Three groups were investigated, because the size of the ischemic zone and the invasive interventions did not allow for the performance of all measurements in the same heart.

2.2.1. Group 1 (six pigs)

Heterogeneity in Rt rise was measured. Ten electrode arrays with four equidistant platinum pins (diameter 0.7 mm, length 5 mm, interelectrode distance 2 mm, proximal ends insulated) were inserted perpendicular to the epicardial surface, within the ischemic zone [central ischemic zone (CZ, $n=25$) and border zone (BZ, $n=21$)] and non-ischemic zone (NZ) ($n=10$), after which, the heart was allowed to recover for 30 min. Electrodes with drifting values ($n=4$) were excluded from analysis. Alternating current (30 μA, 1 kHz) was applied on the outer two electrodes and the voltage difference was measured between the inner two. Changes in Rt result from changed extracellular impedance or from changed intercellular impedance. It has been demonstrated previously that, during ischemia, an initial modest increase in Rt results from increased extracellular impedance, whereas the second Rt rise (after approximately 15 min of ischemia) corresponds to cellular electrical uncoupling [7]. In the configuration used, electrodes can be regarded as point sources for current delivery [22,23] and polarization effects on their surfaces are minimized.

Every 30 s, a recording consisting of the average of five periods of the derived voltage sine was taken from all electrode arrays and data were stored on the hard disk of a personal computer for offline analysis. The system was calibrated in saline with known resistivity (65 Ω.cm). Recording started 15 min prior to coronary occlusion.

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

Ischemia-induced Rt changes depend on location within the ischemic zone, thus, the relative change in tissue impedance (ΔRt) is calculated by: $\Delta Rt=(Rt_m-Rt_{ctrl})/(Rt_{end}-Rt_{ctrl})$, where Rt_m is the measured value, Rt_{ctrl} is the control value and Rt_{end} is the final value after 90 min of ischemia. Onset and second rise of Rt were calculated from the derivative of the sequential measurements. Heterogeneity was defined as the difference in the time course of the second rise and the maximal slope of the rise of Rt at different sites within the ischemic zone.

2.2.2. Group 2 (ten pigs)

Subepicardial and midmyocardial electrograms were studied. Nine multielectrode needles were introduced in CZ perpendicular to the epicardial surface. After introduction of needles (needles contained ten stainless-steel electrodes each, with an interelectrode distance of 1 mm), the heart was allowed a 30-min recovery period. A 105 multielectrode array (9×12 matrix, stainless steel, interelectrode distance 1 mm) was sutured to the epicardium of CZ to record epicardial activation. Control measurements were taken before LAD occlusion. After occlusion, recordings were made every 10 min up to 60 min ischemia. In seven experiments, recordings were made once every 2 min, starting 30 min after coronary occlusion. A 'virtual ground' connected to the pulmonary artery served as a reference. Data (1.8 s duration, sample frequency 1 or 2 kHz) were AC amplified with a custom-made acquisition system and were stored on the hard disk of a personal computer for offline analysis with a custom-made analysis program. Local activation times, defined as time between the stimulus artifact and the steepest negative deflection, were automatically detected in the electrogram and activation maps were generated.

Based on electrogram morphology, we considered a dV/dt_{min} less steep than -2.5 V/s as a sign of remote activity and local inexcitability. We used absolute values of dV/dt_{min} as an estimate of local action potential upstroke velocity. It should be noted that these measures are scaled by the amplitude of the extracellular electrogram, i.e., when electrogram amplitude decreases during ischemia, so does dV/dt_{min}. However, local recovery was determined from the increase in dV/dt_{min}, thus, a decrease of electrogram amplitude could not be held responsible for such changes. Bipolar electrograms were calculated by subtracting an electrogram from its neighbor.

2.2.3. Group 3 (ten pigs)

Programmed stimulation was performed from the bipolar hook electrode to determine the minimum number of ventricular premature beats that produced VF during 90 min of ischemia. Stimulation was started 10 min after coronary occlusion to avoid 1A arrhythmias. After ten cycles, up to three premature stimuli (PS) with the shortest possible coupling interval that produced capture (1 ms accuracy) were applied. After induction of VF, the heart was DC defibrillated, and was allowed 3 min recovery before resumption of programmed stimulation. When three PS failed to induce VF, another attempt was undertaken within 2 min.

Multiple unipolar electrograms were simultaneously recorded with a multielectrode array (121 stainless-steel electrodes, 11×11 matrix, interelectrode distance 2 mm) overlying the electrophysiological border zone (BZ, the area 1 cm from the cyanotic border within the ischemic region [24]) and the central ischemic zone (CZ, the ischemic region surrounded by BZ). Rt was continuously recorded from CZ (see experimental group 2). Electrograms were recorded and activation maps made, as in group 1.

We performed histochemical analysis on 11 hearts (seven from group 2 animals after 60 min of ischemia, four from group 3 animals after 90 min of ischemia). Hearts were cut into 1-cm-thick slices parallel to the base. Slices were submerged for 8 min in phosphate buffer with 2,3,5-triphenyltetrazolium chloride (composition in mmol/l: $NaH_2PO_4 \cdot 2\ H_2O$, 67.1; $Na_2HPO_4 \cdot 2\ H_2O$, 142; 2,3,5-triphenyltetrazolium chloride, 6.3) for the presence of lactate dehydrogenase (LDH) and were subsequently photographed. Rinsing the slices in cold saline terminated the reaction. A transmural biopsy of approximately 1 cm^3 was taken from the CZ of two slices in each heart, cut in 7-μm-thick microsections, and stained with periodic acid Schiff (PAS) for glycogen. In all sections, epicardium and endocardium were clearly discernable. In each experiment, four sections were microscopically studied (100× magnification). Total subepicardial surface and subepicardium with glycogen were measured with a vernier in all slices. The amount of glycogen containing subepicardium was divided by total subepicardial surface, which was expressed as the percentage of total epicardial surface within the section. Hence, a semi-quantitative estimate of the amount of subepicardial glycogen is derived.

2.3. Statistics

Unless stated otherwise, data are presented as the mean±S.E.M. To determine differences between groups, an unpaired *t*-test was used. When data were not normally distributed, a Mann–Whitney test was used. For multiple comparisons, ANOVA for repeated measures was used. $P<0.05$ indicated statistical significance.

3. Results

3.1. Viable subepicardial layer

The presence of lactate dehydrogenase (LDH) was investigated in 11 sliced hearts (see Methods). Fig. 1A

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

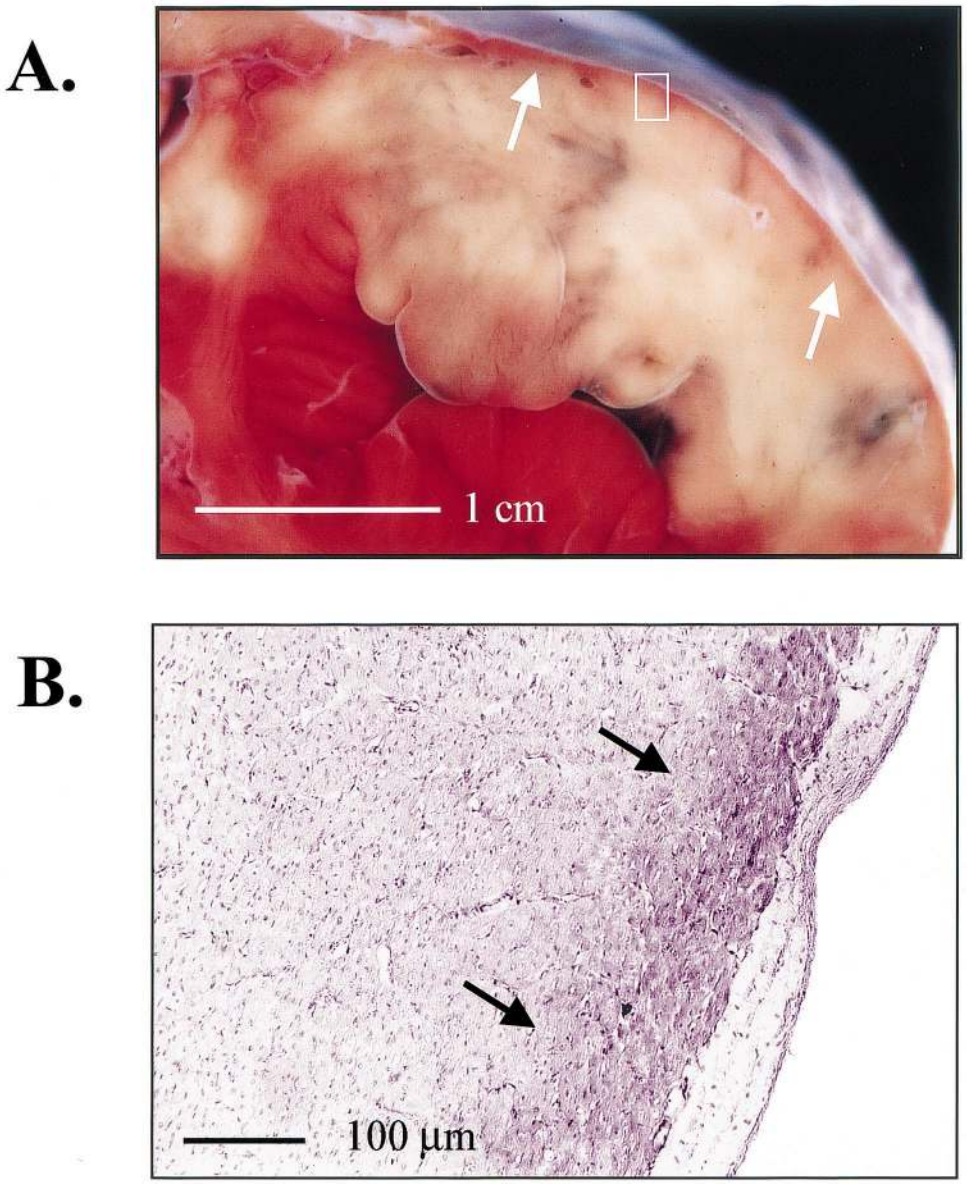

Fig. 1. (A) Slice of heart stained with 2,3,5-triphenyltetrazolium chloride after 60 min of ischemia. LDH-containing tissue is stained red. Note the thin red subepicardium (white arrows). (B) Periodic acid Schiff staining of a microsection from the same heart, indicated by the white square in panel A. Note that glycogen is present in the six–eight cell layers subepicardially only.

shows the presence of subepicardial LDH overlying the ischemic zone. Subepicardial LDH was observed in 10 of 11 hearts, although not in every slice.

Fig. 1B shows a section (PAS staining) from the same heart as in panel A. The darker coloration of the subepicardium (arrows) shows glycogen in up to eight subepicardial cell layers, whereas midmyocardium lacks glycogen. Subepicardial glycogen was present in 69±19% (mean±S.D.) of subepicardium in the slices studied after 60 min and in 48±27% after 90 min of ischemia ($P<0.01$). Glycogen and LDH were absent in the midmyocardium after 60 and 90 min of ischemia.

3.2. Homogeneity in cellular uncoupling

Rt changes in NZ, BZ and CZ were studied in group 1 over 90 min. An immediate increase in Rt of more than 10%, attributed to changes in extracellular impedance [7], occurred after coronary occlusion in 28 out of 46 sites in CZ and BZ, but was absent in NZ. Time to second Rt rise, which is associated with onset of cellular electrical uncoupling [7], did not differ between BZ and CZ in any experiment (on average, 13.1±0.7 and 13.2±0.7 min, respectively, $P=$NS), nor did time to maximal slope (36.9±1.4 and 37.5±2.3 min, $P=$NS). Rt reached a

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

plateau after approximately 75 min, in BZ and CZ. However, despite the similar time courses and the absence of differences before ischemia (370±25 Ω.cm and 332±13 Ω.cm in CZ and BZ, respectively), a large difference in the amount of Rt rise was observed, with higher values in CZ than in BZ. For example, at the start of the second Rt rise, Rt had increased by 27.1±14.6% to 478±40 Ω.cm in CZ and by 9.9±7.0% to 365±16 Ω.cm in BZ ($P<0.001$), whereas at the steepest Rt rise, Rt in CZ had increased by 75.5±22.5% to 652±45 Ω.cm and in BZ by 44.0±27.9% to 483±32 Ω.cm ($P<0.001$).

With the four-electrode configuration used, Rt is recorded in a large tissue volume. The border zone contains normal and ischemic myocardium [24], which is the probable cause of intermediate Rt values. The identical time course of Rt rise in CZ and BZ suggests that uncoupling occurs homogeneously within the ischemic zone.

3.3. Subepicardial electrograms

We hypothesized that coupling of subepicardium to midmyocardium underlies the substrate for 1B arrhythmias. Fig. 2A shows selected bipolar electrograms of ventricular paced beats in a heart from group 2. After 60 min of ischemia, the subepicardial site was still excited. The change in polarity of the electrogram during ischemia indicates a change in activation sequence. The intramural electrogram increased in duration after 20 min; a very broad complex after 30 min preceded inexcitability after 40 min.

Panel B shows a substantial decrease in excitable midmyocardial sites. After 1 h, virtually no excitability remains in the midmyocardium. At the subepicardium, excitability is maintained at more than 80% of the sites for as long as 30 min. During the whole period, subepicardial excitability is maintained over a significantly larger number of sites, although there is a decrease after 30 min. After 60 min, 26% of subepicardial sites were still excitable. In 91% of these 266 sites, the slope of the electrogram was minimal before 60 min of ischemia and subsequently recovered.

Panel C shows the change in dV/dt_{min} of the epicardial sites that were excitable after 60 min of ischemia. Overall, dV/dt_{min} decreased from 16±0.5 V/s before ischemia to a minimum of 3.5±0.2 V/s after 45±0.7 min of ischemia. Thereafter, it increased to 5.5±0.2 V/s ($P<0.001$ vs. 45 min). Note that this recovery follows the time of maximal Rt rise (after approximately 37 min) and precedes the termination of the 1B period (see below). These data

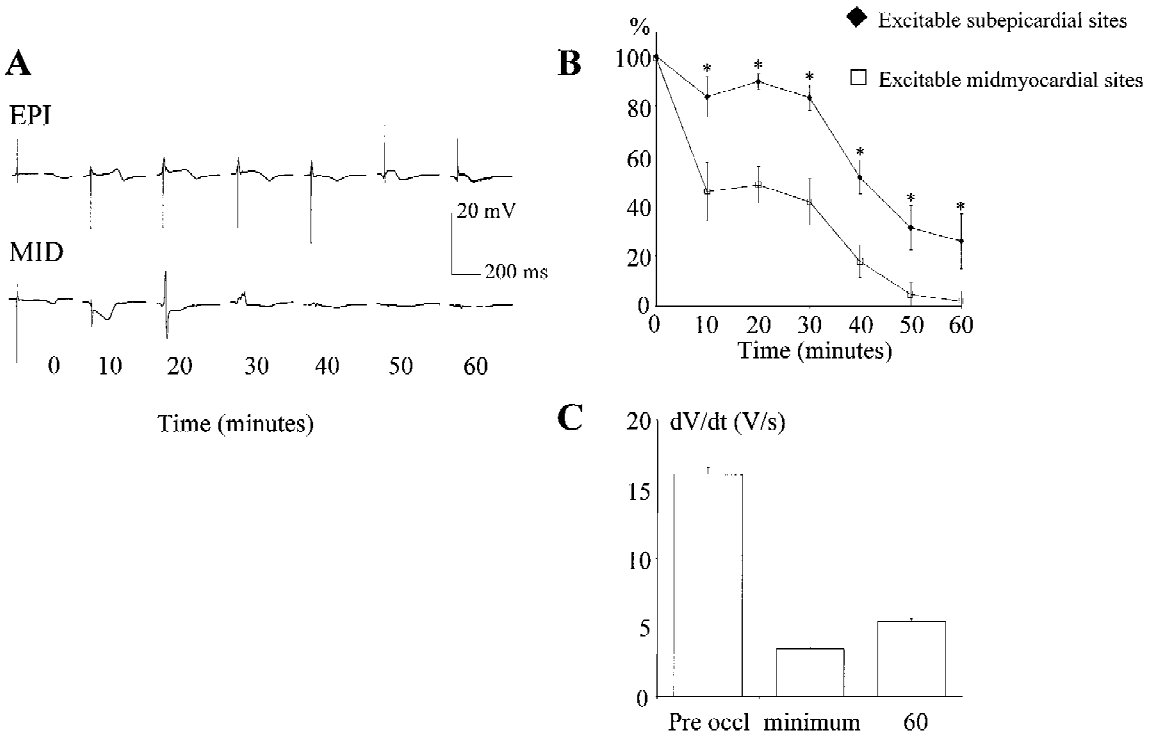

Fig. 2. (A) Bipolar electrograms from subepicardium (EPI) and midmyocardium (MID) during 60 min of ischemia [numbers indicate time (min)]. Note that, after 60 min, the subepicardial electrogram remains essentially unchanged whereas midmyocardial excitability ceased after 30 min. (B) Percentage of excitable subepicardial and midmyocardial sites during 60 min of ischemia (mean±S.E.M.). Asterisks indicate $P<0.01$, subepicardium versus midmyocardium. (C) dV/dt_{min} in the surviving subepicardial sites before ischemia, at minimum (45±0.7 min) and after 60 min of ischemia. Note that ischemia causes a decrease in dV/dt_{min} before 45 min, but that recovery occurs with progression of ischemia ($P<0.001$ vs. 45 min).

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

suggest that subepicardial recovery is caused by uncoupling of the subepicardium from the irreversibly damaged midmyocardium.

3.4. Conduction delay during the 1B phase

Fig. 3A shows activation maps of ventricular paced epicardial electrograms after 30, 40 and 50 min of ischemia. After 30 min, conduction slows (crowding of isochrones) in the lower right quadrant of the map. After 40 min, slowing of local conduction is even more pronounced. After 50 min, conduction in the area with delay after 40 min has recovered. Hence, after 50 min of ischemia, local conduction in the subepicardium overlying the centrally ischemic zone improved, although conduction slowed in the upper left quadrant and in the right half of the map.

Panel B shows selected unipolar electrograms (sites circled in panel A). Recovery of local activation (40–50 min) is evident from the increase in dV/dt_{min} concomitant with an increase in local conduction velocity. Panel C shows the change of conduction time (ΔCT) for isochronal maps in panel A. ΔCT was calculated as averaged absolute differences in activation time of a site and its immediate neighbors. Local ΔCT is increased after 40 min of ischemia (grey shaded area), whereas it normalizes after 50 min in that area, despite subsequent conduction slowing in other areas in the activation map.

3.5. Ventricular fibrillation during the 1B phase

With programmed electrical stimulation, we tested the hypothesis that the above-demonstrated changes provide the arrhythmogenic substrate for VF. Fig. 4A shows sequential unipolar electrograms, recorded in a typical experiment from group 3. Before ischemia, VF was not inducible with three premature stimuli (PS). However, the number of PS inducing VF decreased from three to one between 17 and 32 min of ischemia, after which it increased again. In this experiment, the lowest number of PS to induce VF was 1 (after 32 min), and VF was inducible up to 90 min of ischemia. Panel B shows the time course of relative Rt rise (ΔRt) in the same experiment. VF was induced with the lowest number of PS at low ΔRt; at ΔRt above 0.30, more PS were needed to induce VF. Thus, in this experiment, the 'substrate' for 1B arrhythmias was optimal at relatively low ΔRt.

Fig. 5A summarizes VF induction in ten experiments.

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

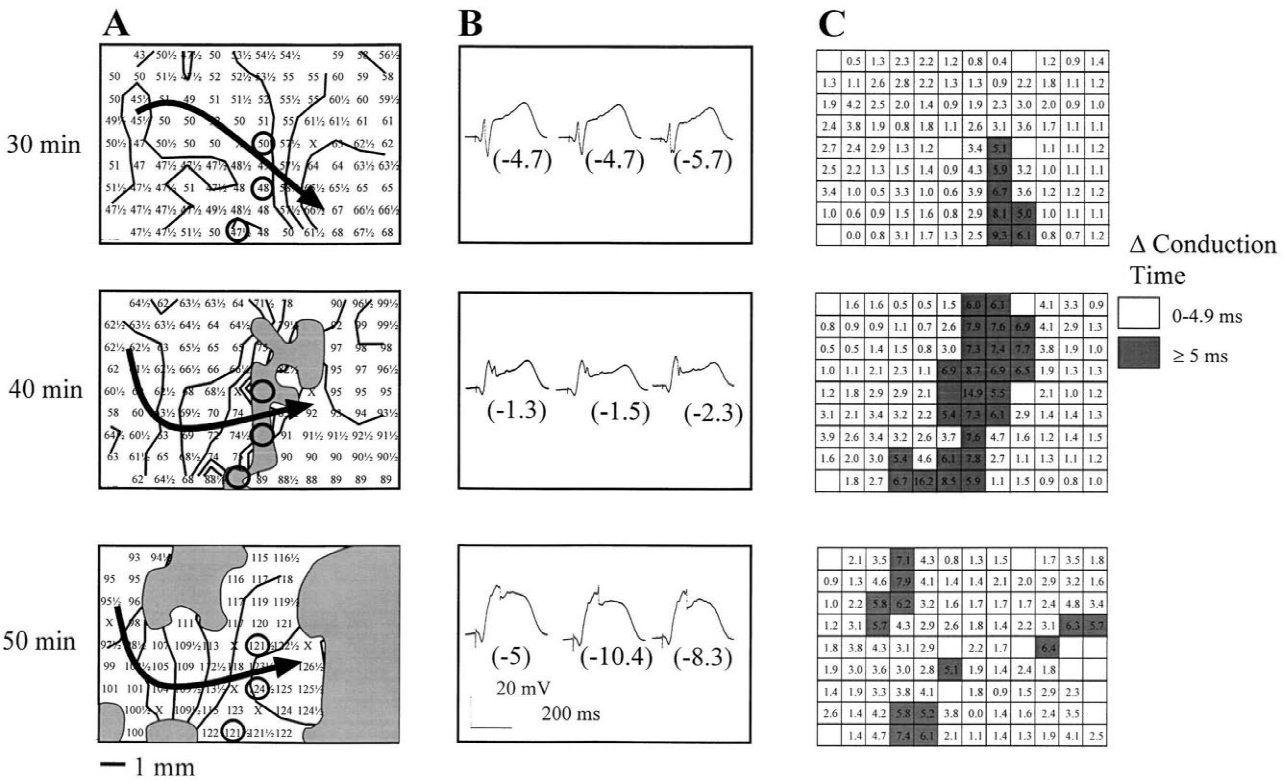

Fig. 3. (A) Activation maps of ventricular paced beat after 30, 40 and 50 min of ischemia. Numbers indicate activation times, and sites with dV/dt_{min} less negative than −2.5 V/s are shaded grey. Lines indicate 5 ms isochrones. Arrows denote gross activation sequence. (B) Selected electrograms from activation maps in panel A (circles). Numbers indicate dV/dt_{min} (V/s). (C) Change in conduction time (ΔCT, see text) from activation maps in panel A. Increase in ΔCT corresponds to a decrease in conduction velocity in the corresponding activation map. Note that dV/dt_{min} of local electrograms, local conduction velocity and ΔCT worsen after 40 min but recover after 50 min of ischemia.

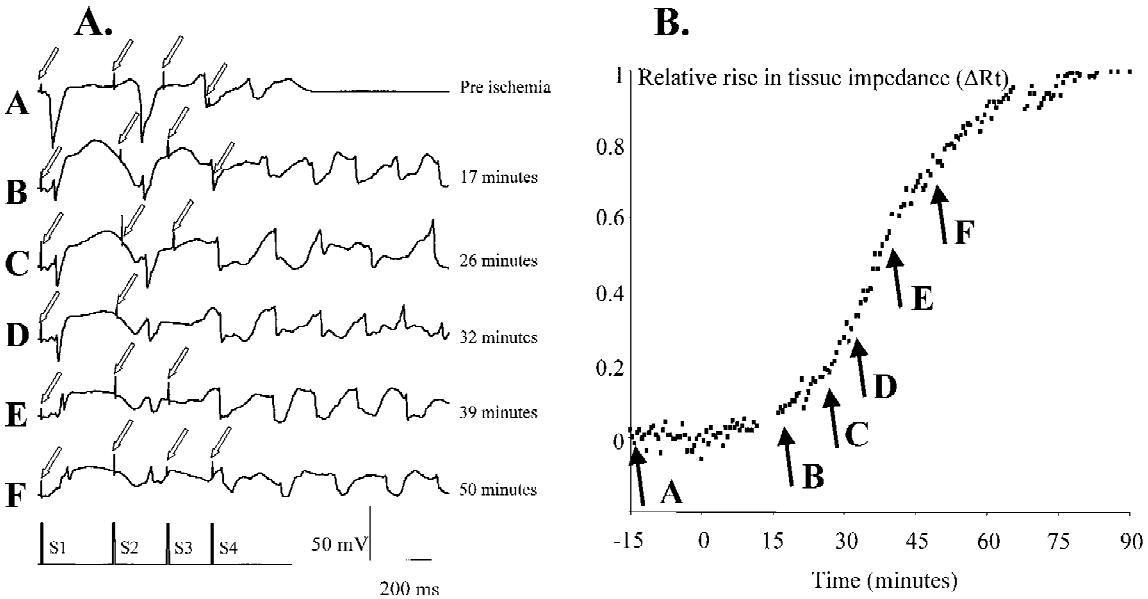

Fig. 4. (A) Unipolar electrograms recorded from the same electrode within the central zone during attempts to induce VF. VF is not induced with three short coupled, premature stimuli before occlusion (A). At 17, 26 and 32 min of ischemia, VF was provoked with a decreasing number of premature stimuli. Thereafter, the number of premature stimuli to induce VF increased again at 39 and 50 min of ischemia. Arrows denote stimulus artifact. Inset: graphic representation of stimulus protocol (S1, S2, S3, S4). (B) Corresponding ΔRt time course. Arrows indicate the moments that VF was induced, letters refer to the tracings in panel A.

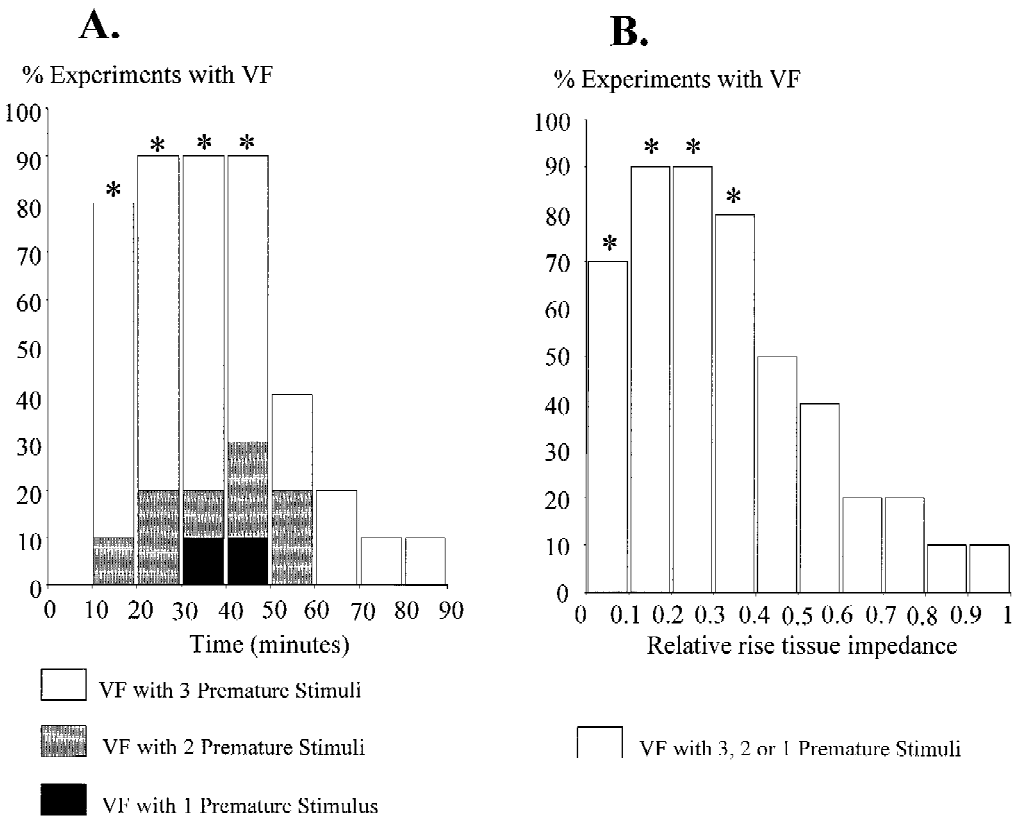

Fig. 5. (A) Percentage VF inducibility (premature stimulus from non-ischemic zone; first attempts after 10 min of ischemia) during 90 min of ischemia ($n=10$). Between 10 and 50 min of ischemia, VF was inducible in nine of ten experiments. (B) VF inducibility related to a relative rise in tissue impedance (ΔRt, see Methods section for calculation). Bars indicate VF with up to three premature stimuli. VF was inducible in nine of ten experiments at ΔRt up to 0.4. VF induced did not differ from zero when ΔRt exceeded 0.4. Asterisks indicate $P<0.001$ vs. preischemia.

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

Three PS always failed to induce VF before coronary occlusion. Between 10 and 50 min of ischemia (attempts started 10 min after occlusion), VF was induced in nine of ten hearts ($P<0.001$ vs. preocclusion). On average, VF was inducible between 13.7±1.6 and 52.8±4.9 min of ischemia, which therefore marks the time window during which the substrate for 1B-VF was present. Interestingly, local electrical recovery precedes the end of the period during which VF could be induced and inexcitability of the midmyocardium does not preclude VF induction (compare Figs. 2 and 3).

Panel B summarizes VF induction versus ΔRt. At ΔRt up to 0.4, VF was inducible in nine of ten hearts ($P<0.001$ vs. preocclusion). At higher values, inducibility of VF did not differ from that before coronary occlusion. Obviously, the substrate for 1B arrhythmias is present only at a moderate state of uncoupling.

In five pigs from group 3, activation maps of 56 VF episodes of 1B-VF were investigated. Fig. 6 shows an activation map of VF, 40 min after coronary occlusion. In four consecutive activations, reentry occurred around slim lines of functional conduction block. There are fixed lines of block (observed in 79% of VF episodes) in consecutive beats. Complete macroreentrant circuits around areas or lines of block were observed three times (5%). The length of these circuits was 33±9.3 mm and calculated conduction velocity was 27±6.8 cm/s. Complex activation (more than three activation wavefronts in an activation map) was present in 45% of the episodes studied and was associated in all but one case with slim lines of functional conduction block. In 79% of episodes, broad activation fronts were observed in at least one of the first six to ten beats of VF. No evidence for a microreentrant mechanism was found.

4. Discussion

We have found that (1) a surviving layer is present at the end of the time of 1B arrhythmias. (2) 1B-VF is based on reentry around slim lines of functional block, that (3) 1B-VF can be provoked between 14 and 53 min of regional ischemia when (4) the relative rise in tissue impedance is increased by not more than 40%. Typically, (5) the surviving subepicardial myocytes are transiently depressed during this vulnerable period. (6) Their electrical recovery heralds the end of the temporal window of induction of 1B arrhythmias.

4.1. Sudden cardiac death

Little conclusive evidence is available about the exact timing of sudden death relative to the onset of myocardial ischemia in human subjects. Often, patients suffer from chest discomfort before dying, and approximately 25% of patients die before reaching the hospital [25]. During acute ischemia, lethal ventricular arrhythmias occur in two distinct phases [2]. The early or 1A phase has been studied extensively (for review, see [26]), its mechanism being

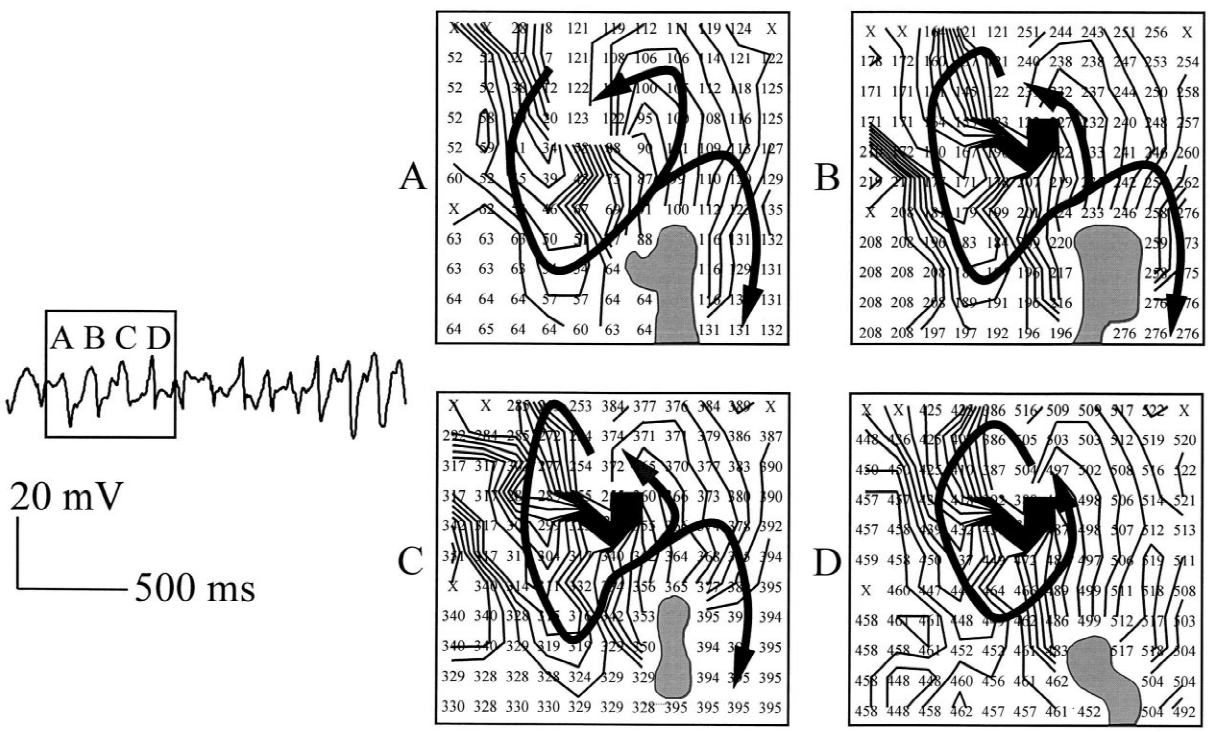

Fig. 6. Activation maps during 1B-VF. Four subsequent beats in panels A to D, see left electrogram. Areas with dV/dt_{min} less negative than −2.5 V/s are shaded grey. The arrows indicate activation sequence. Numbers and lines are as in Fig. 3A. Note that lines of functional block anchor reentrant activation.

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

depression of excitability primarily caused by increased extracellular potassium [27,18] facilitating reentry around a zone of conduction block [28]. A different, thus far unknown, mechanism is probably operative during the delayed or 1B phase [2,29]. The spontaneous occurrence of 1B arrhythmias has been reported to last from 12 to 30 min of ischemia [2,5,29–31]. There is evidence that the time course of ischemia-induced electrophysiological changes is advanced in diseased human hearts compared to those of healthy laboratory animals [32]. Most likely, 1B arrhythmias are not only the principle cause of death in animals during ischemia [2,5], but also play an important role in sudden cardiac death in humans.

4.2. Mechanism of 1B arrhythmias

For spontaneous occurrence of VF, both the arrhythmogenic 'trigger' (i.e., the premature beat that induces the arrhythmia) and 'substrate' (i.e., preexisting electrophysiological circumstances that allow the arrhythmia to maintain) should be present [4]. An abundance of triggers will not induce an arrhythmia in the absence of an electrophysiological substrate and, conversely, in the presence of a suitable substrate, one single trigger can be enough to provoke ventricular fibrillation. Therefore, we applied the 'trigger' through programmed stimulation to exclusively investigate the 'substrate', defined as inducibility of VF, as described previously [18]. Increasing the size of the ischemic zone or changing the pacing mode may increase the occurrence of spontaneous VF, but does not overcome the problem of separating the trigger from the substrate. Because invasive interventions within the ischemic zone, such as introducing multielectrode needles, and many impedance electrode arrays in group 1 and 2 may affect the electrophysiological substrate for arrhythmias, we only tested VF inducibility in group 3, in which we restricted the invasive interventions to a minimum. Ideally, all experimental procedures should be carried out in the same heart, however, given the limitations mentioned above, we chose to conduct these investigations in different experimental groups.

Our study shows a temporal relation between the inducibility of 1B-VF and intermediate electrical coupling. The substrate of 1B-VF is present following the onset till about 40% of total uncoupling. In spontaneously occurring 1B-VF, a relation with the onset of cellular uncoupling was previously suggested [5]. These authors also described that VF only occurred when Rt had increased modestly [5].

An increase in gap-junctional resistance may create conduction slowing and -block, and can be arrhythmogenic if changes occur heterogeneously [11,18]. We have shown that temporal heterogeneities in Rt rise are absent. Rt changes reflect changes in either inter- or extracellular impedance. It has been demonstrated that the onset of cellular uncoupling correlates with the second rise in Rt [7]. Structural properties of the myocardium do not permit the measurement of Rt with a spatial resolution of less than 2 mm [22]. Hence, microscopic heterogeneities cannot be excluded. However, microreentry was never observed in activation maps of 1B-VF. In addition, we did demonstrate macroreentry around slim lines of functional block and, in the majority of VF episodes, the impulse was propagated as a large activation front, which makes a microreentrant mechanism improbable.

We demonstrated that intramural tissue is inexcitable when the 'substrate' for 1B-VF is still present (Figs. 2 and 5). Thus, intramural ischemic myocardium is not a prerequisite for 1B arrhythmias, although the excitable ischemic myocardium will of course be involved in the reentrant circuits, as is the non-ischemic myocardium.

4.3. Epicardial border zone

This study demonstrated that, after 60 min of ischemia, the subepicardium overlying the ischemic zone, in contrast to midmyocardium that becomes inexcitable, remains in part viable. Despite the decreased number of excitable subepicardial sites, the 26% of sites that were still electrically excitable demonstrated an increase in dV/dt_{min} between 45 and 60 min (Fig. 2). Subepicardium proved viable (presence of LDH) and contained glycogen 69% of subepicardium contained glycogen and LDH (Fig. 1, panel B), in line with previous findings [12,13,33,34]. The subepicardial layer that survived ischemia, also termed the epicardial border zone, was observed for the first time by Harris [35] and has been demonstrated in the healing infarction where it exhibits uniform anisotropy [15] and can serve as a 'substrate' for monomorphic ventricular tachycardias in the subacute phase of ischemia [36]. During monomorphic ventricular tachycardia (VT), functional lines of activation block, similar to those observed in this study during VF (Fig. 6), are caused by redistribution of gap junctions and gap-junctional disarray in the five-day-old infarction model [9]. Although these data are obtained in a different model during a different phase of ischemia, it follows that enhanced transverse, as well as diminished longitudinal, coupling of subepicardium favors reentry. Apart from remodeling of gap junctions and subsequent anisotropy, conduction can also be hampered by intercellular communication. Indeed, during intact coupling, depolarized midmyocardial cells exert an electrotonic load on the subepicardium [16,17] and may depress excitability. The fixed localization of the observed lines of block (see Fig. 6) and their disappearance with altered activation sequence suggests that altered gap-junctional resistance and changed anisotropy are the underlying cause. The observation that VF seems to be locally anchored around these lines of block underlines the notion that structural rather than functional changes underlie substrate for 1B-VF. With progression of ischemia-induced uncoupling, we showed that not only the local electrogram improves, but also that local conduction velocity increases

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

(Fig. 3). It should be realized that local dV/dt_{min} depends on the amplitude of the extracellular electrogram. However, during progression of ischemia and subsequent decrease of electrogram amplitude, this would decrease dV/dt_{min}, whereas we report increased values. Moreover, after subepicardial recovery, 1B-VF inducibility ceases, thus, the 'substrate' for 1B arrhythmias disappears. It is highly unlikely that such recovery is caused by ischemic changes other than cellular uncoupling, because potassium and lipid metabolites continue to rise [37–39], pH does not normalize [40] and the time course of subepicardial recovery is beyond that of catecholamine release [41,42]. The small increase in collateral flow during this phase of ischemia is confined to BZ and is not found in CZ [29].

We therefore suggest that depression of excitability of the subepicardium through residual coupling to the depolarized midmyocardium forms the substrate for 1B ventricular arrhythmias. A comparable mechanism is most likely operative at the subendocardium, but our mapping was restricted to the subepicardium. It is unlikely that, at the lateral border, excitability is also depressed with the same magnitude through this mechanism: the load from the normal zone will probably counteract the load from the ischemic zone. Thus, 1A and 1B arrhythmia share a common mechanism: depressed excitability [26]. However, it is caused by increased potassium, hypoxia and acidification in the former [26], and we suggest that it is caused by coupling to an inexcitable depolarizing 'load' in the latter. We have demonstrated that temporary electrophysiological deterioration of the subepicardium overlying the ischemic zone favors reentrant ventricular arrhythmias. The disappearance of the 'substrate' for 1B arrhythmias coincides with the recovery of the subepicardium. The proposed mechanism of subepicardial recovery through uncoupling from the midmyocardium would explain the transient nature of these arrhythmias.

Acknowledgements

The authors gratefully acknowledge Charly Belterman, Wim ter Smitte and Carel Kools for expert technical assistance, Sara Tasseron for the preparation of microsections, Drs André Linnenbank and Mark Potse for development of the analysis program, and Professor Michiel J. Janse for valuable discussion.

References

[1] Zipes DP, Wellens HJJ. Sudden cardiac death. Circulation 1998;98:2334–2351.
[2] Kaplinsky E, Ogawa S, Balke W, Dreifus LS. Two periods of early ventricular arrhythmia in the canine acute myocardial infarction model. Circulation 1979;60:397–403.
[3] Josephson ME, Horowitz LN, Farshidi A. Continuous local electrical activity. A mechanism of recurrent ventricular tachycardia. Circulation 1978;57:659–665.
[4] Coumel P. The management of clinical arrhythmias. An overview on invasive versus non-invasive electrophysiology. Eur Heart J 1987;8:92–99.
[5] Smith WT, Fleet WF, Johnson TA, Engle CL, Cascio WE. The 1b phase of ventricular arrhythmias in ischemic in situ porcine heart is related to changes in cell-to-cell electrical coupling. Circulation 1995;92:3051–3060.
[6] Cinca J, Warren M, Carreño A et al. Changes in myocardial electrical impedance induced by coronary artery occlusion in pigs with and without preconditioning. Correlation with local ST segment potential and ventricular arrhythmias. Circulation 1997;96:3079–3086.
[7] Kléber AG, Riegger CB, Janse MJ. Electrical uncoupling and increase of extracellular resistance after induction of ischemia in isolated, arterially perfused rabbit papillary muscle. Circ Res 1987;61:271–279.
[8] Saffitz JE, Corr PB, Sobel BE. Arrhythmogenesis and ventricular dysfunction after myocardial infarction. Is anomalous cellular coupling the elusive link? Circulation 1993;87:1742–1745.
[9] Peters NS, Coromilas J, Severs NJ, Wit AL. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. Circulation 1997;95:988–996.
[10] Lerner DL, Yamada KA, Schuessler RB, Saffitz JE. Accelerated onset and increased incidence of ventricular arrhythmias induced by ischemia in Cx43-deficient mice. Circulation 2000;101:547–552.
[11] Kuo C-S, Munakata K, Reddy P, Surawicz B. Characteristics and possible mechanism of ventricular arrhythmia dependent on the dispersion of action potential duration. Circulation 1983;67:1356–1367.
[12] Fujiwara H, Ashraf M, Sato S, Millard RW. Transmural cellular damage and blood flow distribution in early ischemia in pig hearts. Circ Res 1982;51:683–693.
[13] Schaapherder AFM, Schumacher CA, Coronel R, Fiolet JWT. Transmural inhomogeneity of extracellular $[K^+]$ and pH and myocardial energy metabolism in the isolated rat heart during acute global ischemia; dependence on gaseous environment. Basic Res Cardiol 1990;85:33–44.
[14] Fiolet JWT, Baartscheer A, Schumacher CA, Ter Welle HF, Krieger WJG. Transmural inhomogeneity of energy metabolism during acute global ischemia in the isolated rat heart: dependence on environmental conditions. J Mol Cell Cardiol 1985;17:87–92.
[15] Ursell PC, Gardner PI, Albala A, Fenoglio JJ, Wit AL. Structural and electrophysiological changes in the epicardial border zone of canine myocardial infarcts during infarct healing. Circ Res 1985;56:436–451.
[16] Tan RC, Osaka T, Joyner RW. Experimental model of effects on normal tissue of injury current from ischemic region. Circ Res 1991;69:965–974.
[17] Tan RC, Joyner RW. Electrotonic influences on action potentials from isolated ventricular cells. Circ Res 1990;67:1071–1081.
[18] Coronel R, Wilms-Schopman FJG, Dekker LRC, Janse MJ. Heterogeneities in $[K^+]_o$ and TQ potential and the inducibility of ventricular fibrillation during acute regional ischemia in the isolated perfused porcine heart. Circulation 1995;92:120–129.
[19] Rush S, Abildskov JA, McFee R. Resistivity of body tissues at low frequencies. Circ Res 1963;12:40–50.
[20] Plonsey R, Barr R. The four-electrode resistivity technique as applied to cardiac muscle. IEEE Trans Biomed Eng 1982;29:541–546.
[21] Ackmann JJ. Complex bioelectric impedance measurement system for the frequency range from 5 Hz to 1 MHz. Ann Biomed Eng 1993;21:135–146.
[22] Steendijk P, Mur G, Velde ETvd, Baan J. The four-electrode resistivity technique in anisotropic media: theoretical analysis and

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022

application on myocardial tissue in vivo. IEEE Trans Biomed Eng 1993;40:1138–1148.
[23] Fallert MA, Mirotznik MS, Downing SW, Savage EB, Foster KR, Josephson ME, Bogen DK. Myocardial electrical impedance mapping of ischemic sheep hearts and healing aneurysms. Circulation 1993;87:199–207.
[24] Janse MJ, Cinca J, Morena H et al. The 'border zone' in myocardial ischemia. An electrophysiological, metabolic and histochemical correlation in the pig heart. Circ Res 1979;44:576–588.
[25] Löwel H, Lewis M, Hörmann A. Prognostische bedeutung der Prähospitalphase beim akuten myokardinfarkt. Ergebnisse des Augsburger Herzinfarktregisters 1985–1988. Deut Med Wocheschr 1991;116:729–733.
[26] Janse MJ, Wit AL. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. Physiol Rev 1989;69:1049–1169.
[27] Coronel R, Fiolet JWT, Wilms-Schopman FJG et al. Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in the isolated perfused porcine heart. Circulation 1988;77:1125–1138.
[28] Janse MJ, Van Capelle FJL, Morsink H et al. Flow of 'injury' current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischemia in isolated porcine and canine hearts. Evidence for two different arrhythmogenic mechanisms. Circ Res 1980;47:151–165.
[29] Russell DC, Lawrie JS, Riemersma RA, Oliver MF. Mechanisms of phase 1a and 1b early ventricular arrhythmias during acute myocardial ischemia in the dog. Am J Cardiol 1984;53:307–312.
[30] Menken U, Wiegand V, Bucher P, Meesman W. Prophylaxis of ventricular fibrillation after acute experimental coronary occlusion by chronic beta-adrenoceptor blockade with atenolol. Cardiovasc Res 1979;13:588–594.
[31] Patterson E, Kalcich M, Scherlag BJ. Phase 1B ventricular arrhythmias in the dog: localized reentry within the mid-myocardium. J Intervent Cardiac Electrophysiol 1998;2:145–161.
[32] Sutton PMI, Taggart P, Opthof T et al. Repolarisation and refractoriness during early ischaemia in humans. Heart 2000;84:365–369.
[33] Wilensky RL, Tranum-Jensen J, Coronel R, Wilde AAM, Fiolet JWT, Janse MJ. The subendocardial border zone during acute ischemia of the rabbit heart: an electrophysiologic, metabolic, and morphologic correlative study. Circulation 1986;74:1137–1146.
[34] Lowe JE, Cummings RB, Adams DH, Hull-Ryde EA. Evidence that ischemic cell death begins in the subendocardium independent of variations in collateral flow or wall tension. Circulation 1983;68:190–202.
[35] Harris AS. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. Circulation 1950;1:1318–1328.
[36] Fenoglio JJ, Pham TD, Harken AH, Horowitz LN, Josephson ME, Wit AL. Recurrent sustained ventricular tachycardia: structure and ultrastructure of subendocardial regions in which tachycardia originates. Circulation 1983;68:518–533.
[37] Hill JL, Gettes LS. Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. Circulation 1980;61:768–778.
[38] Yamada KA, McHowat J, Yan G-X et al. Cellular uncoupling induced by accumulation of long-chain acylcarnitine during ischemia. Circ Res 1994;74:83–95.
[39] Corr PB, Yamada KA, Creer MH, Sharma AD, Sobel BE. Lysophosphoglycerides and ventricular fibrillation early after onset of ischemia. J Mol Cell Cardiol 1987;19(Sup V):45–53.
[40] Watanabe I, Johnson TA, Buchanan J, Engle CL, Gettes LS. Effect of graded flow reduction on ionic, electrical, and mechanical indexes of ischemia in the pig. Circulation 1987;76:1127–1134.
[41] Schömig A, Dart AM, Dietz R, Mayer E, Kübler W. Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A: Locally mediated release. Circ Res 1979;55:689–701.
[42] Wilde AAM, Peters RJG, Janse MJ. Catecholamine release and potassium accumulation in the isolated globally ischemic rabbit heart. J Mol Cell Cardiol 1988;20:887–896.

Downloaded from https://academic.oup.com/cardiovascres/article/50/2/362/274427 by guest on 16 August 2022