Dispersion of repolarization and arrhythmogenesis

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BACKGROUND The relation between induction of arrhythmias and dispersion of repolarization is not completely understood.

OBJECTIVE The purpose of this study was to study the relation between heterogeneity in repolarization and arrhythmogenesis under conditions of selective regional action potential prolongation and shortening.

METHODS Pig hearts were perfused in a Langendorff setup. The left anterior descending artery (LAD) was cannulated and perfused. Sotalol (220 μM) was infused in the aortic cannula, and pinacidil (20 μM) was infused through the LAD, causing a gradient in repolarization time between the two myocardial regions. Premature stimulation was performed from the LAD region.

RESULTS No transmural repolarization gradients developed after infusion of the drugs. High-density epicardial activation/repolarization mapping (176 unipolar electrodes, 2-mm interelectrode spacing) revealed a maximum repolarization gradient of approximately 120 ms over 14 mm. The critical parameter for differentiating between the occurrence of reentry and the mere occurrence of a line of activation block between the two myocardial regions (and no reentry) was not the magnitude of the repolarization gradient but the timing of arrival of the premature activation wave at the distal side of the line of activation block relative to the repolarization time of the premature beat proximal to the line of block. No spontaneous arrhythmias were observed despite the presence of the repolarization gradient.

CONCLUSION It is not the repolarization gradient but the restitution characteristics of the tissue with the shorter action potential, in combination with the time of arrival of the premature wavefront at the distal side of the line of block, that determines the occurrence of reentry.

KEYWORDS Arrhythmia; Electrophysiology; Conduction; Refractoriness; Heterogeneity

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Introduction

Dispersion in repolarization has been associated with life-threatening arrhythmias.[1,2] Two mechanisms explaining arrhythmogenesis in the presence of large regional differences in repolarization times have been proposed. First, a premature beat initiated in the tissue with early repolarization is conducted toward the tissue with late repolarization and fails to propagate into the latter until it has recovered from inexcitability (unidirectional block).[3,4] As a consequence, the impulse travels around the refractory tissue and reenters the tissue where the impulse has originated. Second, the differences in repolarization times generate an electrotonic current that flows intracellularly from the tissue with late, toward the tissue with early repolarization, which depolarizes the latter. If the threshold potential for activation is reached, a spontaneous premature beat is initiated and an arrhythmia is started. This mechanism for focal arrhythmogenesis has been termed *phase 2 reentry*.[5]

Early evidence for the role of repolarization heterogeneity in arrhythmogenesis has been reported by Han and Moe[1] and Kuo et al.[6] The latter authors recorded six monophasic action potentials simultaneously in dog hearts during global hypothermia and regional hyperthermia. In the presence of regional differences of 111 $\pm$ 16 ms, arrhythmias were inducible by an early premature stimulus delivered at the site with the shortest action potential. In a subsequent study, two premature stimuli did not cause sufficient heterogeneity to produce arrhythmias.[7] The discrepancy between these studies is unexplained, but methodologic factors may have played a role because modification of myocardial temperature may also have affected conduction velocities. Therefore, the exact role of repolarization heterogeneity in producing arrhythmias remains unresolved, but the general consensus is that a larger heterogeneity in repolarization is more arrhythmogenic.[8] Clinical correlates of repolarization heterogeneity support this notion.[9–11]

In this study, repolarization heterogeneity was created by selectively perfusing separate vascular beds of the heart to affect repolarization without changing conduction velocity. The results indicate that repolarization heterogeneity leads

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to unidirectional block but not necessarily to reentry. The study demonstrates that it is not the absolute difference in repolarization times but the combination of activation delay and the restitution characteristics of the tissue with the earlier repolarization that determines whether reentry occurs.

Methods

Ethical statement

This study was approved by the local ethical committee on animal experimentation and conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Experimental setup

Pigs (n = 10; weight 37.5 ± 1.8 kg [mean ± SEM]) were premedicated with 350 mg ketamine (Nimatek, Animal Health BV, Bladel, The Netherlands), 80 mg azaperone (Stresnil, Janssen, The Netherlands, Janssen-Cilag, High Wycombe, UK), and 0.5 mg atropine (Centrafarm Services, Etten-Leur, The Netherlands) intramuscularly and anesthetized with 20 mg/kg pentobarbital (Nembutal, CevaSate Animale Naaldwijk, The Netherlands) intravenously. The animals were intubated and ventilated with room air and isoflurane (Forene, Abbott, Hoofddorp, The Netherlands). Expiratory CO_2 was monitored. Heparin 5,000 international units (LeoPharma, Breda, The Netherlands) was injected intravenously. Blood was collected, and the heart was isolated after a midsternal thoracotomy.

The heart was perfused in a Langendorff setup using a mixture of blood and Tyrode's solution (pH 7.35–7.45) and defibrillated. Atrioventricular block was created by crushing the AV nodal area.

The left anterior descending artery (LAD) was freed over a distance of 5 mm, above the first diagonal branch. A ligature was passed underneath the LAD, and a cannula was introduced through a small incision into the LAD. The cannula was fixed by tying the ligature and was connected to the perfusion system via a miniature heat exchanger.

The temperature of both perfusion areas was controlled by separate heat exchangers in each perfusion limb. Infusion pumps were connected to the side branch of the LAD cannula and to the aortic cannula for administration of pinacidil and sotalol, respectively. Pump rates were adjusted to obtain effective concentrations of 220 μM sotalol and 20 μM pinacidil. Total coronary flow was 191 ± 7.8 mL/min (n = 10). The LAD-perfused myocardium was 37.6° ± 0.30°C; the remainder of the heart was 37.4° ± 0.23°C (both n = 10). The absence of ST-T segment changes in the LAD region indicated the absence of regional ischemia.

Because the perfusion system was recirculatory and the blood–Tyrode's mixture was limited to approximately 3,500 mL (range 2,975–3,910 mL), limited time (range 17.5–23 minutes) was available for selective perfusion of the heart without admixture of the compounds.

Electrophysiologic recordings

A rectangular grid of 11 × 16 electrodes (gold, 2-mm interelectrode distance) was sutured over the border between the myocardium perfused by the LAD and the myocardium perfused by the circumflex artery. The border was identified prior to application of the electrode by a 30-second occlusion of the LAD leading to regional cyanosis. Correct positioning of the electrode was verified by creating a 5-minute occlusion of the LAD and analyzing the border between the region with and that without electrophysiologic signs of ischemia.[12]

Unipolar cathodal stimulation was performed through one of the electrodes in the electrode grid overlying the LAD tissue. One to three stimulus positions were tested sequentially. The anode was placed at the aortic root. Premature beats were introduced after each train of eight beats with coupling intervals ranging from the basic cycle length (600 ms) down to the refractory period. Control recordings were made of a basic beat and a premature beat (at the refractory period) prior to the intervention. After repolarization heterogeneity was created by starting selective perfusion with the drugs, as many recordings as possible were made of the premature beats and of any arrhythmias in order to relate repolarization heterogeneity to arrhythmogenesis. Local unipolar electrograms were recorded against a reference electrode at the aortic root using a data acquisition system (Biosemi, Amsterdam, The Netherlands, sampling rate 2,000 Hz, filtering DC 1 kHz [3dB]). When ventricular fibrillation (VF) occurred, the heart was immediately defibrillated by a DC shock.

Analysis of the electrograms was performed offline using a custom-made analysis program.[13] Local activation times (ATs) were measured at the moment of the minimum dV/dt of the initial deflection and local repolarization times (RTs) at the moment of the maximum dV/dt of the T wave.[14] When determination of activation time was difficult because of fractionation of the signals, Laplacian electrograms were constructed to aid in the detection of local activation.[15]

In six experiments, needles were inserted through the left ventricular free wall. Each needle contained four silver electrodes (4-mm interelectrode distance). Needles were inserted along lines parallel to the base of the heart at three levels: apical, basal, and intermediate.

Drugs

Sotalol (Sotacor vials 10 mg/mL) was obtained from Bristol-Myers Squibb (Woerden, The Netherlands). Pinacidil (pinacidil monohydrate P154) was obtained from Sigma Aldrich (Zwijndrecht, The Netherlands).

Data analysis

Data are presented as mean ± SEM. Statistical significance was tested with the t-test. $P < .05$ was considered significant.