Ischaemia-induced cellular electrical uncoupling and ventricular fibrillation

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Sudden death resulting from ventricular fibrillation (VF) during acute myocardial ischaemia forms an important contribution to mortality associated with infarction. Its temporal distribution is not known, but 30% of mortality occurs within the first 60 minutes. Two distinct phases of arrhythmias have been demonstrated in laboratory animals subjected to coronary occlusion. The mechanism of the second, 1B phase (which is associated with more lethal events than the first, 1A phase) is largely unknown but appears to be related to cellular uncoupling, i.e. the closure of gap junctions.

Gap junctions are intercellular communication channels that are permeable for ions and metabolites and are necessary for normal propagation of electrical activation. It has been suggested that closure of gap junctions results in a largely inhomogeneous substrate in which microreentry forms the electrophysiological mechanism for VF. However, there is growing support for the hypothesis that arrhythmias relate to the persistence of residual coupling rather than to the occurrence of uncoupling. With this, the ischaemic midmyocardium can depress the intrinsically viable tissue of the ischaemic subepicardium and subendocardium and cause conduction slowing and block leading to arrhythmias. Progression of uncoupling terminates this interaction and allows the subepicardium and subendocardium to recover. Indeed, electrophysiological properties recover subepicardially whereas the midmyocardial tissue becomes inexcitable. In addition, activation patterns during VF become restricted to the two-dimensional plane of the subepicardium. These observations support the hypothesis of residual coupling as an arrhythmogenic mechanism during the delayed phase of acute ischaemia. Whether this mechanism is equally important in patients with remodelled and failing hearts can at this time only be speculated upon. However, modifying intercellular coupling might turn out a new antiarrhythmic therapy. (Neth Heart J 2002;10:360-5.)

Key words: ischaemia-induced, ventricular fibrillation

Sudden cardiac death during acute myocardial ischaemia still forms a major contribution to total mortality, thereby presenting a challenging health care problem. Sudden death, defined as 'dead outside the hospital', 'dead on arrival' or 'dead in the ER' occurred in almost half a million cases in the United States in 1998.[1] The majority of cases are caused by circulatory collapse resulting from a life-threatening arrhythmia, mostly ventricular tachycardia or ventricular fibrillation (VF).[2] Indeed, VF was often the first rhythm observed by paramedics at out-of-hospital resuscitation settings.[3] Further insight in the mechanisms of VF in these settings might lead to therapies that can prevent its occurrence or delay its onset until professional assistance is available.

Many studies have been published in the last decades describing the mechanism of early arrhythmias during ischaemia and infarction (for review see[4]). The vast majority of these deal with the arrhythmias that occur within the first ten minutes of coronary occlusion or with those that occur after several days.[4] Apart from the observation that 30% of mortality associated with acute myocardial infarction occurs within the first hour after onset of complaints,[5] not much is known about the distribution of lethal arrhythmias in patients during the acute phase of myocardial ischaemia. From experiments in animals subjected to coronary ligation it is known that arrhythmias occur in two distinct phases during the course of acute myocardial ischaemia, subsequently termed early and delayed (1a and 1b), that are separated by a relatively arrhythmia-free interval.[6-8] Particularly the second phase, between 15 and 45

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minutes after coronary occlusion, might be an effective target for treatment and prevention of mortality. Delaying the onset of this phase of VF might create a time window large enough to reach and treat the patient. Currently, there are no therapeutic options available to prevent or delay the occurrence of these phase 1B arrhythmias. In addition, their mechanism is largely unknown.

A relation between the occurrence of these arrhythmias and the onset of electrical cellular uncoupling (brought about by closure of gap junctions) has been described.[9-11] Evidently, these processes cannot be studied experimentally in patients and the only information available has been obtained from animal models.

Cellular electrical coupling

Rapid electrical activation of the myocardium is necessary for a synchronous contraction of the heart. Discontinuous electrical activation of the heart is not only undesirable for the haemodynamics but is also arrhythmogenic: local inhomogeneity in excitability and refractoriness can create the conditions for reentry. Cardiac myocytes are connected or coupled with gap junctions that form low resistance communication channels between myocytes and allow the passage of ions and small molecules. Thus, gap junctions can be regarded as resistors that electrically connect individual myocytes, thereby allowing propagation of activation. Closure of gap junctions, also referred to as cellular uncoupling, results in increase of intercellular resistivity and therefore in conduction slowing and block,[12,13] which can be a substrate for arrhythmias.[4]

Gap junctions are built up from two hexamers (connexons) that consist of six proteins (connexins) forming a pore. Several connexins have been described in cardiac tissue and they are classified by their molecular weight. In working myocardium, connexin43 (Cx43) is the most abundant connexin, but Cx45 is present as well. Gap junctional conductance is influenced by several substances and intracellular messengers. IP_3,[14] cAMP,[15] ATP,[16] and $Ca^{2+}{}_i$[17]change gap junctional conductance and thereby influence the propagation of electrical activation between myocytes. Under physiological circumstances, however, intact cellular coupling allows synchronisation of action potential duration and equilibration of the intracellular milieu between adjacent cells.

Metabolic and electrophysiological factors are, however, not completely equilibrated over the many cells and cell-cell boundaries from which the heart is composed. There are electrophysiological gradients: the action potential is longer in midmyocardium than in epicardium and endocardium, and longer in endocardium than in epicardium.[18] In addition, in the normal ECG the duration of the T wave demonstrates a repolarisation gradient over the ventricular wall and illustrates that normal cellular coupling does not synchronise myocardial repolarisation completely.

Despite the presence of these physiological gradients, under normal conditions there is an enormous safety in the number of gap junctions present and the number needed for normal propagation.[19] That is, the number of open gap junctions exceeds the number that would be minimally required for conduction. It has recently be calculated that a more than 95% reduction of available gap junctions would be required to achieve a mere 50% reduction in conduction velocity.[19] Other simulation studies predict that conduction velocity can decrease to only a few percent of its control value when gap junctional resistance is increased enough, underscoring the large safety for conduction.[12] Transgenically engineered mice, especially those in which the gene for Cx43 is knocked out, potentially supply an experimental model for studying the role of specific gap junctional proteins in electrical activation of the heart. Evidence reported so far indicates, however, that this approach is not as straight forward as theoretically expected. Homozygous Cx43 knock-out mice (-/-) die shortly after birth from pulmonary atresia.[20] Heterozygous mice do develop normally, but have decreased ventricular conduction velocity and suffer from arrhythmias.[20] When subjected to regional ischaemia, they develop ventricular fibrillation more often than wild type normal mice.[20] A conditional Cx43 knock-out mouse, in which the gene is specifically blocked within the heart, demonstrated conduction slowing and sudden cardiac death as well.[21] Although these data conflict with the predicted effect of gap junction reduction[12,19] and the mechanism of these changes is not known, the data support the role of gap junctions in electrical conduction.

The picture is still far from complete, but we can assume that normal gap junctional coupling is related to normal propagation of activation within the heart, whereas severely reduced coupling can cause conduction slowing and block. The latter factors are of interest for arrhythmogenesis, because they form the necessary ingredients for the occurrence of reentrant activation: the electrophysiological mechanism of VF.

Decreased cellular coupling and arrhythmias

Acute ischaemia is associated with depolarisation of the cell membrane and with subsequent decrease of excitability. When ischaemia proceeds, the myocardium becomes irreversibly damaged. At this point myocytes are electrically inexcitable, uncoupled, and undergo rigor as a consequence of ATP depletion. Recent evidence suggests that cellular uncoupling[22] results from dephosphorylation and intracellular redistribution of gap junction proteins and provides a direct relation between ATP depletion and the occurrence of cellular uncoupling. Both decreased excitability and cellular uncoupling can cause conduction slowing and block and must therefore be regarded as arrhythmogenic factors.

It has been suggested that localised cellular uncoupling causes a grossly heterogeneous substrate

where small groups of cells are electrically insulated from other groups, and where microreentrant activation can take place around one or a few myocytes.[10] Indeed, microreentrant activation was demonstrated in cultured neonatal rat myocytes subjected to the cellular uncoupler palmitoleic acid.[13] However, evidence that microreentry occurs in the intact, regionally ischaemic heart is lacking. The question whether microreentry constitutes the mechanism for VF is not exclusively academic: the therapeutic approach would be completely different when directed at a substrate with generalised depressed excitability (as is the case during the 1A phase) compared with a substrate that consists of many microscopic sites of localised conduction slowing and block.

There are data that demonstrate the association between the proceeding of cellular uncoupling during ischaemia and the occurrence of ventricular arrhythmias. Smith et al. showed in an open chest pig model that spontaneous occurrence of delayed ventricular arrhythmias, which occurred between 19 and 30 minutes of ischaemia in that study, was associated with the onset of a rise of tissue impedance, an indirect measure for coupling.[9] This relation remained intact after a period of ischaemic preconditioning, an intervention that postpones irreversible ischaemia-induced changes:[11,23] both the occurrence of arrhythmias and the onset of cellular uncoupling were postponed by approximately 30 minutes. Interestingly, in this open chest pig model, the delayed phase of arrhythmias was associated with more VF than the early phase. Although the contribution of 1B arrhythmias to mortality in humans is not known, the arrhythmogenic changes during the early, 1A phase are present for a much shorter time in humans than in experimental animals,[24] consequently resulting in less chance to develop VF during this period.

Further evidence for the relation between decreased cellular coupling and ventricular arrhythmias comes from the observation that the duration of cellular uncoupling is increased in hearts from rabbits with heart failure and arrhythmias,[25] whereas also a reduction in the number of gap junctions has been reported in human hearts with heart failure.[26]

Moreover, Peters et al. showed that regions with gap junctional disarray, that is, were distribution of gap junctions as a result of remodelling is not normally aligned, form the lines of conduction block around which reentry occurs during chronic myocardial ischaemia.[27]

For any arrhythmia to occur, the arrhythmogenic trigger (the event that sets off the arrhythmia, often a well-timed premature beat) and the arrhythmogenic substrate (the preexisting tissue conditions that allow a trigger to induce and arrhythmia) need to be present at the same time.[28] We recently explored the arrhythmogenic substrate[29] in the pig heart during the 1B phase of ischaemia by applying an aggressive programmed stimulation protocol.[30] In that study, we found that VF was inducible between 14 and 53 minutes of ischaemia, but after that time the same aggressive pacing protocol no longer induced VF. Meanwhile, tissue impedance, a measure for cellular uncoupling, continued to rise.[30] Thus, despite the presence of the arrhythmogenic trigger, in this case the prematurely paced beats, progression of cellular uncoupling terminated the arrhythmogenic substrate during acute myocardial ischaemia. The observation that tissue impedance continued to rise after termination of arrhythmia inducibility argues against microreentry as underlying mechanism.

Reduced coupling, conduction slowing and arrhythmogenesis: a novel hypothesis

As outlined above, progression of ischaemia is associated with deterioration of cellular functions and ultimately results in cell death. However, not all cells die within the ischaemic zone. Intramural strands of myocardium survive ischaemia, and ultimately have normal electrophysiological properties.[31] In addition, a thin subepicardial and subendocardial layer survive the ischaemic burden.[32] The notion of this surviving layer and the relation between arrhythmias and cellular uncoupling allows formulation of the following hypothesis: The initially viable subepicardium and subendocardium are temporarily depressed by persistence of intercellular communication with the severely ischaemic midmyocardium. The partially open gap junctions allow the midmyocardium, which is larger in mass than the thin outer layers of the ischaemic zone, to electrically depress the subepicardium and subendocardium. In fact, the membrane potential of the subepicardium and subendocardium is reduced through an extracellular current from the depolarised midmyocardium. Complete closure of gap junctions terminates the electrical interaction between subepicardium and midmyocardium thereby preventing this 'drain' of membrane potential. Subsequently, the subepicardium can restore its normal electrophysiology, and the arrhythmogenic substrate vanishes. Figure 1 displays a graphic representation of this hypothesis. Before ischaemia (panel A) normal conduction can take place in normally coupled myocardium. Panel B shows that when ischaemia is instituted, partial uncoupling takes place. The midmyocardium depresses the intrinsically viable subepicardium, in which conduction is now hampered. After complete closure of the gap junctions (panel C), the subepicardium can recover and restore its normal electrophysiological properties, whereas the midmyocardium dies. This recovery hallmarks the end of the arrhythmogenic period. From experimental studies on isolated myocytes, coupled to a computer model cell via an adjustable gap junctional resistance, it is known that indeed such electrotonic depression can take place, provided the mass of the depressor is large enough.[33]

Several implications of this hypothesis in the setting of acute ischaemia have now been tested. The ischaemic

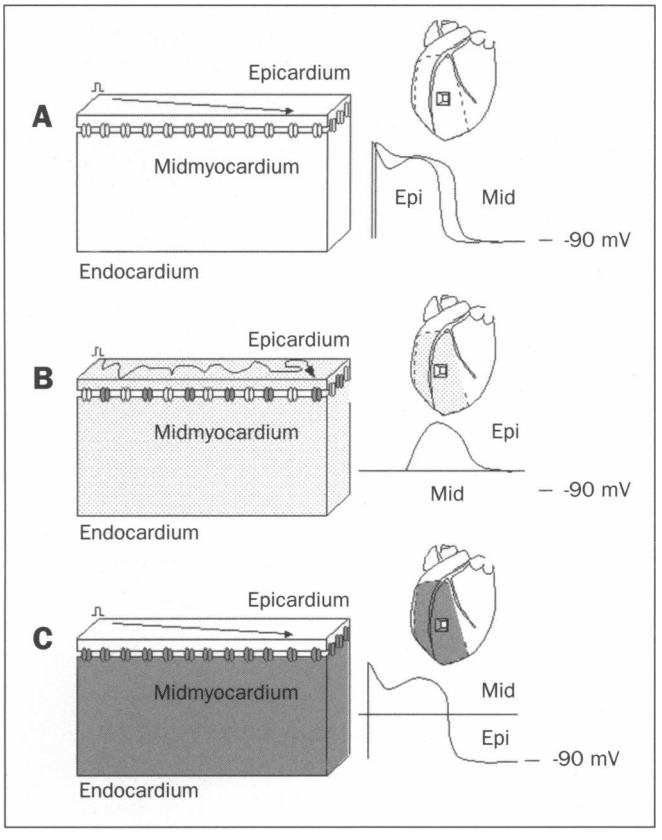

Figure 1. Schematic representation of the hypothesis, adapted from reference 39. A. Before coronary occlusion, cellular coupling is intact (open gap junctions (white)) and both subepicardium and midmyocardium are viable and produce normal action potentials (Epi and Mid respectively). Conduction velocity is fast and undisturbed (arrow). B. During ischaemia, the midmyocardium is depressed, cellular uncoupling advances (closed gap junctions, grey), but is still partially intact (open gap junctions, white) and causes depression of the subepicardium. Conduction velocity is hampered (curled arrow). C. After complete uncoupling, subepicardium and midmyocardium are dissociated. Hence, midmyocardium remains severely depressed, but in subepicardium normal electrophysiological properties can restore (normal action potential, normal conduction velocity).

subepicardium indeed proved partially viable. We demonstrated that after 60 minutes of ischaemia, 69% of the subepicardium contained glycogen, whereas the midmyocardium was devoid of glycogen.[30] Although these values were measured in selected microsections, and thus represent a semi-quantitative measure, they show that large areas in the subepicardium are metabolically viable. This conclusion is supported by our observation that the maximal negative deflection rate of the extracellular electrogram, which is a measure for local excitability, recovered in 91% of the sites that were still excited after 60 minutes of ischaemia.[30] Unlike the subepicardium, no electrical activity and thus no recovery was recorded at intramural sites within the ischaemic zone.[30] Remarkably, there was no heterogeneity in the time course of the tissue impedance rise within the ischaemic tissue, which is consistent with the electrotonic depression hypothesis.

Another implication of the hypothesis is that activation patterns should become restricted to the two-dimensional plane of the viable subepicardium, once the midmyocardium is inexcitable. This was indeed demonstrated in our study of VF.[34] Activation of the region of interest changed from a mixture of planar and breakthrough activation patterns before ischaemia to predominantly quasi-planar activation after ischaemic changes had established.[34] There was a tenfold reduction in the number of breakthrough activation patterns, which arise from the midmyocardium, from approximately six per second before ischaemia to approximately 0.7 per second after 60 minutes of ischaemia, indicating predominantly two-dimensional activation.[34] Further support for two-dimensional activation of the subepicardium during the 1B phase of acute ischaemia was supplied by measuring the lifespan of phase singularity points. High resolution mapping of arrhythmias can reveal their dynamic behaviour. Phase singularity points refer to the phenomena that occur at the break-up of fibrillatory wavelets. At these sites, tissue is not excited but electrotonically affected in such a way that the periodicity of the excitation cycle cannot be determined. In other words, one cannot determine whether such sites are excited, not excited or refractory.[35] Functionally, phase singularity points can anchor wavelets, that is, wavelets can curve around phase singularity points and thereby give rise to reentry. Since phase singularity points arise at wave breaks, their lifespan gives an estimate of the stability of excitatory waves during fibrillation: a short lifespan indicates many rapid wave breaks and a grossly disorganised rhythm, whereas a longer lifespan indicates more stable activation of the tissue.[36] Under normoxic conditions, phase singularity points during VF have a very short lifespan, and only approximately 2% are present throughout a rotation of a wavelet.[37,38] In our VF study during acute regional ischaemia, we demonstrated that the lifespan of phase singularity points increased from approximately 50 ms before ischaemia to approximately 200 ms after 60 minutes of coronary occlusion. This observation demonstrates that the arrhythmia had become more stable and organised,[36] which was interpreted as consistent with two-dimensional activation of the subepicardium.

In summary, there is growing evidence that different regions within the ischaemic zone electrotonically influence each other in a way that intrinsically viable tissue is electrically depressed through persistent coupling with irreversibly damaged tissue. Cellular uncoupling terminates this situation and concludes the period during which arrhythmias are inducible.

It is well possible that this mechanism contributes to arrhythmias in patients with heart failure as well. Apart from the observation that human failing heart

tissue is approximately eight times more sensitive to gap junctional uncouplers, indicating a decreased degree of coupling (J.M.T. de Bakker and R. Derksen, unpublished data), not much information is available on this subject. Also, it is not known whether the mechanism described above is equally important in acute ischaemia in remodelled atherosclerotic hearts, as encountered in patients. One might, however, speculate that during the transition between normal or moderately reduced coupling to total uncoupling, electrotonic depression of viable myocardium takes place and forms an arrhythmogenic substrate.

Conclusion

Arrhythmias during the delayed phase of acute ischaemia form a large source of mortality in laboratory animals.[6,9] Although the distribution of lethal arrhythmias in humans is not known, it is certain that the first hour of ischaemia is associated with 30% of the total ischaemia-induced mortality,[5] and that the ischaemic changes causing arrhythmias during the 1A phase only last for a short period in humans.[24]

The mechanism of the delayed phase of arrhythmias is related to the development of cellular electrical uncoupling, but, as outlined above, the arrhythmogenicity relates to persistence of residual coupling rather than to the onset of uncoupling.[39] There is growing evidence that the arrhythmogenic substrate is formed by electrotonic depression of the outer layers of the ischaemic zone, that is, the subepicardium and subendocardium.[30,34,40] Potentially, intervening in the process of uncoupling between these tissue compartments can modulate the duration of the period in which coupling is critical and arrhythmias occur. The data above are mainly derived from studies in healthy animals and further research is needed to confirm the role of uncoupling induced arrhythmias in patients with acute ischaemia and heart failure.

Nonetheless, modifying electrical coupling might become a novel antiarrhythmic target in a condition that is associated with a high mortality rate. ■

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