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Review

Acute ischemia-induced gap junctional uncoupling and arrhythmogenesis

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Abstract

Sudden cardiac death forms a major cause of mortality. Myocardial ischemia-induced ventricular fibrillation (VF) is frequently the underlying mechanism. Ventricular arrhythmias arise in two distinct phases during the first hour of ischemia. The first, the 1A phase, has been extensively studied, and few studies relate to the 1B phase. The latter is associated with intercellular electrical uncoupling, mediated by decreased conductance of gap junction channels.

Although the relation between gap junctional uncoupling and decreased conduction velocity appears clear under normoxic conditions, additional factors contribute to conduction slowing during ischemia, and VF occurs preferentially at moderate levels of uncoupling. A potential mechanism of arrhythmias depends on temporary electrotonic depression of intrinsically viable tissue by the large bulk of the ischemic zone. This causes conduction slowing and conduction block in the surviving layers, leading to arrhythmias. These arrhythmias then resolve with progression of uncoupling. It is unknown whether either accelerated uncoupling or maintenance of gap junctional communication is antiarrhythmic. Ischemic preconditioning postpones both gap junctional uncoupling and occurrence of VF. Given the burden of sudden death and the large number of casualties in the low-risk population, there is, even in the era of implantable cardiac defibrillators, need for further understanding the mechanism of ischemia-induced VF.

Keywords: Ischemia; Gap junctions; Ventricular arrhythmia; Cellular uncoupling; Connexin; Arrhythmia mechanism

1. Introduction

Cardiovascular mortality remains the leading cause of death in the industrialized world [1,2]. Sudden death, defined as 'death outside the hospital,' 'dead on arrival,' or 'dead in the emergency department,' was responsible for almost 300,000 casualties in the United States alone in 1998 [3]. Ventricular arrhythmias, ventricular fibrillation (VF) in particular, are the cause of cardiac arrest in the majority of cases [1]. Acute myocardial ischemia is the major cause of sudden cardiac death [1,4]. Large-scale clinical trials with antiarrhythmic drugs have failed to reduce the incidence of sudden death and even caused an increased mortality in the treated groups [5–7]. Implantable defibrillators are efficient against ventricular arrhythmias [8,9], but risk stratification of sudden death is only possible in the high-risk population, whereas most deaths occur in the far larger low-risk population [10]. Therefore, our understanding of the underlying mechanism of ischemia-induced ventricular arrhythmias appears to be incomplete. In this review, we will first discuss the mechanism of ventricular arrhythmias and gap junctional closure during acute myocardial ischemia, then look at the direct influence of gap junctional uncoupling on conduction velocity and the creation of heterogeneities. After that, we will review the data supporting the role of gap junctions in the genesis of ischemia-induced arrhythmias.

1.1. Electrophysiologic mechanism of ventricular fibrillation

Reentry is the underlying electrophysiological mechanism of ventricular fibrillation [4]. Reentry was first

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defined by Mines [11] in 1914 as a persisting electrical impulse that reactivates an area of previously activated myocardial tissue that is no longer refractory, resulting in a circus movement of activation. The length of such circle depends on its wavelength, defined by the mathematical product of refractory period and conduction velocity (plus an excitable gap when present) [12]. The requirements for reentrant activation in the intact heart are a region of unidirectional block and (regionally) slow-enough conduction velocity to allow the activation impulse to travel around the zone of block. It is facilitated by a short refractory period. The ultimate proof of reentry is its termination by interruption of the circle [11]. Our understanding of reentry has been extended by the introduction of different concepts of its mechanism such as single rotor reentry [13] and fibrillatory conduction [14,15] and the leading circle concept [16], but Mines' principles apply in all these forms of ventricular fibrillation.

For an arrhythmia to occur, both a suitable substrate (the preexisting circumstances that allow perpetuation of the arrhythmia) and a trigger (the event that sets off the arrhythmia within the substrate) need to be present [17]. Factors such as catecholamines, $[K^+]_o$, pH, and drugs might modulate both trigger and substrate. During acute myocardial ischemia, the conditions necessary for the initiation of reentry, both trigger and substrate, occur in concert and, indeed, ventricular fibrillation is often encountered [4].

In the course of ischemia, gap junctions close. This may result in electrophysiological effects. Its first results were noted as early as 1875 and 1879 by Engelmann [18], who stated that the cells live together but die singly, and by Burden-Sanderson and Page [19] and De Mello et al. [20], respectively, who described the phenomenon of 'healing over'—the reversal of (electrical) signs of myocardial injury following a stab wound of the heart Gap junctional uncoupling may contribute to attaining conditions favorable for the initiation of reentrant activation and ventricular fibrillation by slowing of conduction (both directly and indirectly) and the creation of heterogeneities.

1.2. Closure of gap junctions in ischemia

Tissue impedance, the composite measure of resistance and reactance and an indirect measure for intercellular coupling, increases in a biphasic manner following coronary occlusion [21]. Immediately after interruption of coronary flow, a first rise of approximately 10–25% occurs, attributed to collapse of the vasculature [21]. Fleischhauer et al. [22] showed that the impedance of the perfusion fluids contributes little to total tissue impedance. However, collapse of the vasculature affects the relation between the intracellular and extracellular volume and might therefore affect whole tissue impedance. The second rise in impedance, after approximately 15 min, was attributed to closure of the gap junctions, and thus to decrease of intercellular conductance [21].

1.2.1. Ischemia-related factors responsible for closure of gap junctions during ischemia

Although many factors change simultaneously during early ischemia, several individual factors that uncouple gap junctions have been identified. Increase of diastolic cytoplasmatic $[Ca^{2+}]$ is associated with gap junctional uncoupling [23,24]. Diastolic $[Ca^{2+}]$ increases between 15 and 25 min of ischemia in rabbits [24] and closely precedes gap junctional uncoupling, which lasts between 10 min (rabbit) and 40 min (pig) [25–27] and results in conduction slowing and conduction block [24].

Ischemia-induced intracellular acidification also decreases gap junctional conductance [28]. Decreased intracellular pH also renders gap junctions more sensitive to increased $[Ca^{2+}]_i$ [29].

Lysophosphoglycerides and arachidonic acid metabolites accumulate in the intercalated disks of ischemic cells after a few minutes of ischemia and decrease gap junctional conductance [30]. Catecholamines increase cAMP and $[Ca^{2+}]_i$, which in turn decrease gap junctional conductance [31].

Cx43 proteins dephosphorylate during ischemia [32,33] and transfer from the intercalated disks to intercellular pools [33]. The latter reports show a direct link between energy deprivation as the common pathway leading to membrane depolarization, Ca^{2+} overload, anaerobic glycolysis leading to decreased pH_i, and gap junctional uncoupling.

1.3. Gap junctions and conduction velocity

Conduction velocity partially depends on intercellular conductance, the combined resistance of the cytoplasm, and gap junctions.

The number of available gap junctions is much larger than needed for propagation of the action potential under normoxic conditions. Weingart and Maurer [34] showed action potential propagation within a pair of coupled cells at gap junctional conductance of >1.3 nS, albeit with a considerable delay. Rudy and Quan [35] showed in a computer simulation that conduction velocity decreases discontinuously at high gap junctional resistance. Indeed, Jongsma and Wilders [36] confirmed that, under non-ischemic conditions, approximately 90% decrease of gap junctions is required to decrease conduction velocity with 50%. In another study, it was demonstrated that the safety for conduction is much higher when gap junctional coupling is reduced than when I_{Na} is decreased [37]. Thus, slow conduction without conduction block remains possible even at very low gap junctional conductance. These simulation studies corroborate studies by Gutstein et al. [38] in genetically engineered conditional Cx43 −/− mice where a 90% reduction of Cx43 was associated with a decrease of approximately 50% in transversal and longitudinal conduction velocity. This is consistent with the studies of Morley et al. [39] and Thomas et al. [40] who showed little effect of heterozygous Cx43 reduction on

conduction velocity. However, others found that in Cx43 +/− mice, a 50% reduction of Cx43 protein corresponded to a decrease of conduction velocity of approximately 25% [41,42].

The former observations are further supported by studies demonstrating conduction slowing (more prominent in transverse than in longitudinal conduction) upon administration of heptanol or palmitoleic acid [43–46]. Increase of gap junctional resistance with palmitoleic acid decreased conduction velocity to such an extent in cultured neonatal cells that activation fronts could propagate around the perimeter of a single cell [47]. Gap junctional conductance depends in a dynamic manner on transjunctional voltage [48,49] and effect that is larger when there are less gap junctions [49]. Most data on conduction slowing through gap junctional uncoupling relate to normoxic conditions. In the acutely ischemic intact heart, very slow conduction has not been demonstrated. Whether this is because gap junctional coupling remains sufficient for propagation and other factors are responsible for conduction slowing, or whether conduction block occurs before gap junctions uncouple is not exactly known.

1.4. Unmasking of heterogeneities

Gap junctions allow intercellular exchange of ions and small molecules (with a molecular weight of up to 1000 Da [50]) and current, and cause equilibration of ionic concentrations [51,52] and energy-rich phosphates and propagation of the action potential. Their closure, by either pharmacological interventions or by pathological circumstances such as acute ischemia, might create an intercellular gradient in both nutrients and metabolites as in ions. Han and Moe became aware of this equilibrating effect. It was hypothesized that when two adjacent cells have a very different duration of the action potential, the current flowing from the cell with the longer action potential towards the cell with the shorter action potential during the plateau phase would allow the latter cell to depolarize and to generate a premature beat. Mendez et al. argued that this could not be the case between well-coupled cells because the current flowing from the cell with the longer action potential would prolong the short one and would itself shorten the longer action potential [53]. This would lead to an equilibration of action potential durations between adjacent cells. Thus, for a voltage gradient between cells to occur, partial gap junctional uncoupling is required. Indeed, closure of gap junctions causes gradients that are large enough to produce arrhythmias [51].

However, it appears that gap junctional conductance does not decrease to such an extent during ischemia, at least not while cells are excitable. In coupled cell pairs subjected to simulated ischemia, gap junctional coupling remained large enough to equilibrate action potential duration between the paired cells up to the moment of inexcitability [54]. Moreover, the moment of 'ischemia'-induced rigor was exactly the same in two paired cells, whereas there was a large variation in a group of single myocytes [54]. Thus, gap junctional coupling remains sufficient to equilibrate action potential duration and moment of ischemia-induced rigor in cell pairs, suggesting that intercellular communication remains intact and prevents intercellular gradients on a cellular level. In support of the above, gap junctions were still permeable for sodium ions, on one hand, causing calcium overload via the Na^+/Ca^{2+} exchanger [52], and for luciferine yellow, on the other hand [55], at the moment rigor occurred. It remains to be determined what degree of uncoupling is required to cause physiologically significant heterogeneities in intact hearts.

In addition to the potential generalized effect of uncoupling on the unmasking of heterogeneities, gap junctional uncoupling is not equally distributed within the ischemic tissue. The increase in tissue impedance was significantly smaller in the ischemic border zone than in the central zone, although the time course of rise was identical [27]. Hence, ischemic and nonischemic myocardium interdigitate at the ischemic border [56] and ischemic tissue impedance increases, whereas non-ischemic tissue impedance remains normal. Also, subepicardium and subendocardium are relatively unaffected by the ischemic burden through diffusion of oxygen and nutrients from surrounding tissues [57,58]. Irreversibly damaged cells will eventually die and reversibly challenged myocytes will dissociate from irreversibly damaged cells and survive ischemia (healing over) [19]. The ischemic myocardium that uncouples from the rest of the heart can therefore functionally no longer contribute to arrhythmogenesis.

2. Arrhythmogenesis during myocardial ischemia

2.1. Two phases of arrhythmias

Arrhythmias occur in two distinct phases during the first hour of coronary occlusion [4,59,60] The first phase, called immediate ventricular arrhythmias by Kaplinsky et al. [59], later referred to as 1A [60] arrhythmias, lasts from 2 to 8 min of ischemia in the dog. After a relative arrhythmia-free interval, a second, delayed phase, now referred to as 1B [60], occurs from 15 to 45 min of coronary occlusion in dogs and pigs [25–27,59]. This phase has remained relatively poorly studied but appears to be more arrhythmogenic than the 1A phase [25,59]. The 1B phase of arrhythmias coincides with the increase of tissue impedance and therefore was thought to be causally related with gap junctional uncoupling [21]. Fig. 1, derived from the work of Smith et al. [25], shows the distribution of arrhythmic events in nine open-chested pigs. Arrhythmias occurred at the start of the rise of the tissue impedance. However, others have shown

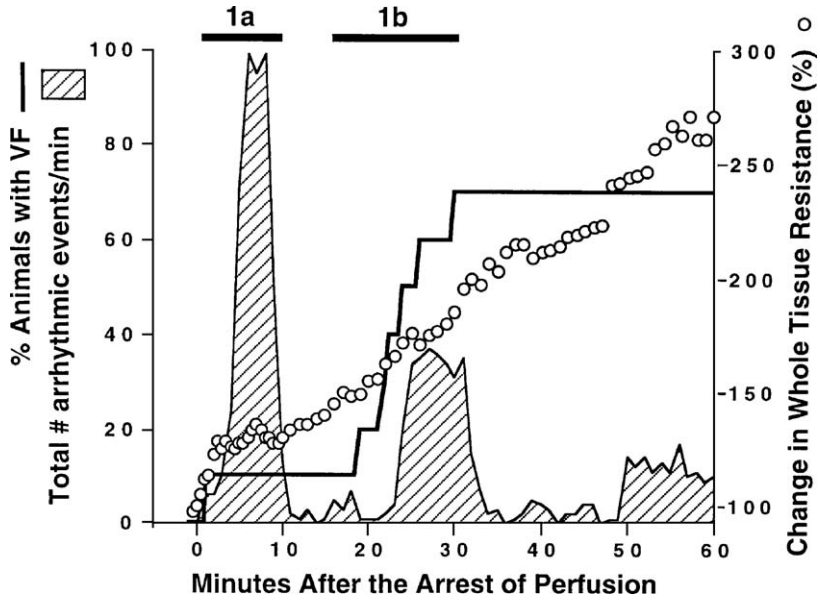


Fig. 1. Incidence of ventricular arrhythmias in regionally ischemic open-chested pig hearts (dashed area). Black line denotes percentage of animals with VF. The open circles indicate average rise in tissue impedance in all animals. The black line indicates the time course and number of animals with VF (reproduced with permission from Ref. [25]).

that the peak of 1B arrhythmias is of shorter duration than the duration of tissue impedance rise [26,59].

Both the ischemia-induced rise in tissue impedance and phase 1B ventricular arrhythmias can be successfully postponed after ischemic preconditioning [26]. Fig. 2 shows the distribution of PVCs during 4 h of regional ischemia in pigs without (upper panel) and with ischemic preconditioning (lower panel).

The two phases of arrhythmias have been described in dogs [59,61], pigs [25,26], sheep [62], and rats [63]. The bimodal distribution is less clear in cats [64] and rabbits [65], and may differ in individual animals [66]. It is unclear whether a similar distribution of ischemia-induced arrhythmias exists in man, as this is obviously extremely difficult to study.

2.2. Arrhythmogenic triggers

In case of ischemia-induced VF (both 1A and 1B), the trigger exists most often from a timely administrated or spontaneous premature ventricular complexes (PVCs) [4] that can be reentrant or nonreentrant in origin [67–69]. The mechanical stretch exerted by the viable myocardium on the rigid ischemic zone may result in PVCs arising preferentially from the ischemic border during the 1B phase [70]. Indeed, the number of PVCs was larger in working hearts than in isolated nonworking hearts, the triggers are initiated at the interface between the ischemic and the viable tissue, and premature beats occur preferentially following potentiated contractions in the viable myocardium [70]. However, a recent study has shown that gadolinium, a blocker of stretch-sensitive channels, did not abate the 1B phase of arrhythmias [71].

2.3. Arrhythmogenic substrates

2.3.1. Role of functional changes in gap junctional coupling

The mechanism by which gap junctional uncoupling causes conduction slowing and arrhythmias in the regionally ischemic heart has not completely been elucidated. Recently, a novel hypothesis was put forward involving the heterogeneity of the myocardium and the evolution of a surviving subepicardial and subendocardial layer [27]. Conduction slowing in the surviving tissue is caused by electrotonic interaction between the large mass of depolarized–dying–intramural cells and nonischemic subepicardial and subendocardial cells [72]. It has been established that a rim of subepicardial and subendocardial tissue survives ischemia and infarction [27,57] and that intramural sites become electrically inexcitable during prolonged ischemia, whereas subepicardial cells remain activated [25]. If the viable myocytes are electronically depressed by electrotonic interaction, slow conduction in the intrinsically viable layer would ensue, which would recover with progression of gap junctional uncoupling (decrease of electrotonic interaction), concomitant with the decrease of arrhythmias.

Several observations support this hypothesis: (1) between two coupled cells, electrical depression of one cell can be transmitted via reduced gap junctional coupling to the other, provided that the mass of the depressant is large enough [72]; (2) VF could be induced with programmed stimulation between 14 and 53 min of ischemia [27]. Thereafter, the same induction protocol failed to induce VF [27]. Fig. 3 shows electrograms of VF inducibility in a typical isolated regionally ischemic pig heart. The number of PVCs to induce VF decreases from three during control to one at 32 min of ischemia, after which more PVC are required to

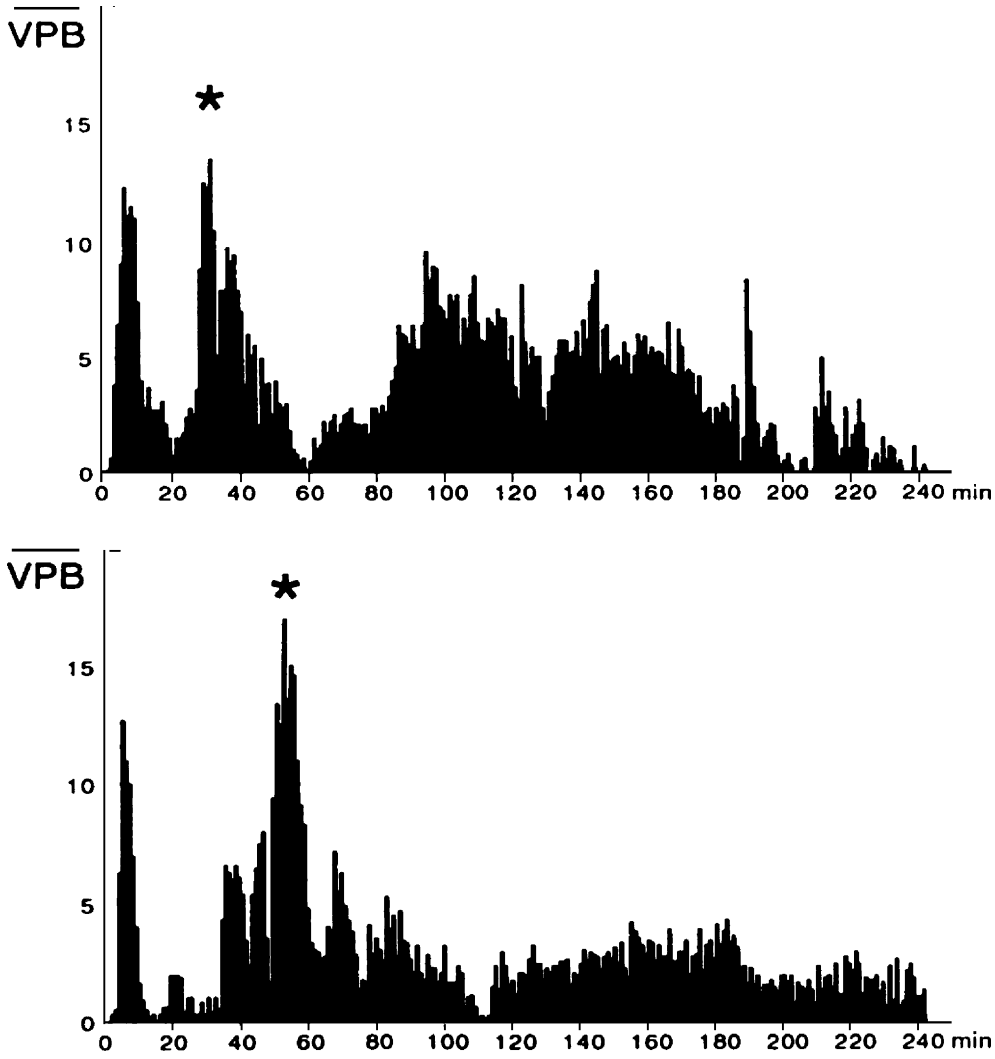


Fig. 2. Incidence of ventricular arrhythmias in regionally ischemic pig hearts without (upper panel) and with ischemic preconditioning (lower panel). The peak of arrhythmogenesis associated with the 1B phase of ischemia is postponed in the preconditioned group (reproduced with permission from Ref. [26]).

induce VF. The right panel shows the rise in tissue impedance in the same experiment. Fig. 4 shows overall data. Thus, by providing the necessary triggers for VF, these experiments indicate that the substrate for VF is evolving during the 1B phase, and that the optimum substrate is present when uncoupling is moderate; (3) spontaneous VF occurred at increase of tissue impedance of <50% in a study by Smith et al. [25]; (4) a decrease followed by an increase in dV/dt of the subepicardial unipolar extracellular intrinsic deflection coincided with a relative rise in tissue impedance of more than 40% of its final value and with inability to induce VF with programmed stimulation in regionally ischemic pig hearts [27]; and (5) in a computer simulation of a multicellular fiber with 1B-like properties, premature beats induced sustained arrhythmias only within a limited window of moderately decreased gap junctional conductance [73]. Strong gap junctional coupling suppressed formation of delayed after depolarizations through hyperpolarization and increased coupling charge, but low gap junctional conductance prevented conduction of ectopic activity to the nonischemic tissue [73].

An alternative hypothesis includes the occurrence of microreentry due to very slow conduction induced by gap junctional uncoupling [21,47]. Although in cultured myocytes exposed to palmitoleic acid very slow conduction was observed [47], microreentry is unlikely to be the underlying mechanism of 1B VF for the following reasons: (1) the reduction of I_{Na} through prolonged recovery of inactivation rather than gap junctional uncoupling might play an important role in conduction slowing because tissue impedance has only increased moderately during the 1B phase; (2) very slow conduction has been shown in vitro [34,46,47] and in silico [36,37], but not in regionally ischemic intact hearts; (3) tissue anisotropy prevents the occurrence of conduction block at critical gap junctional coupling [74]. Gap junctional uncoupling increases anisotropy by decreasing transversal rather than longitudinal conduction velocity [38,43,44]; (4) paradoxical restoration of conduction was observed with

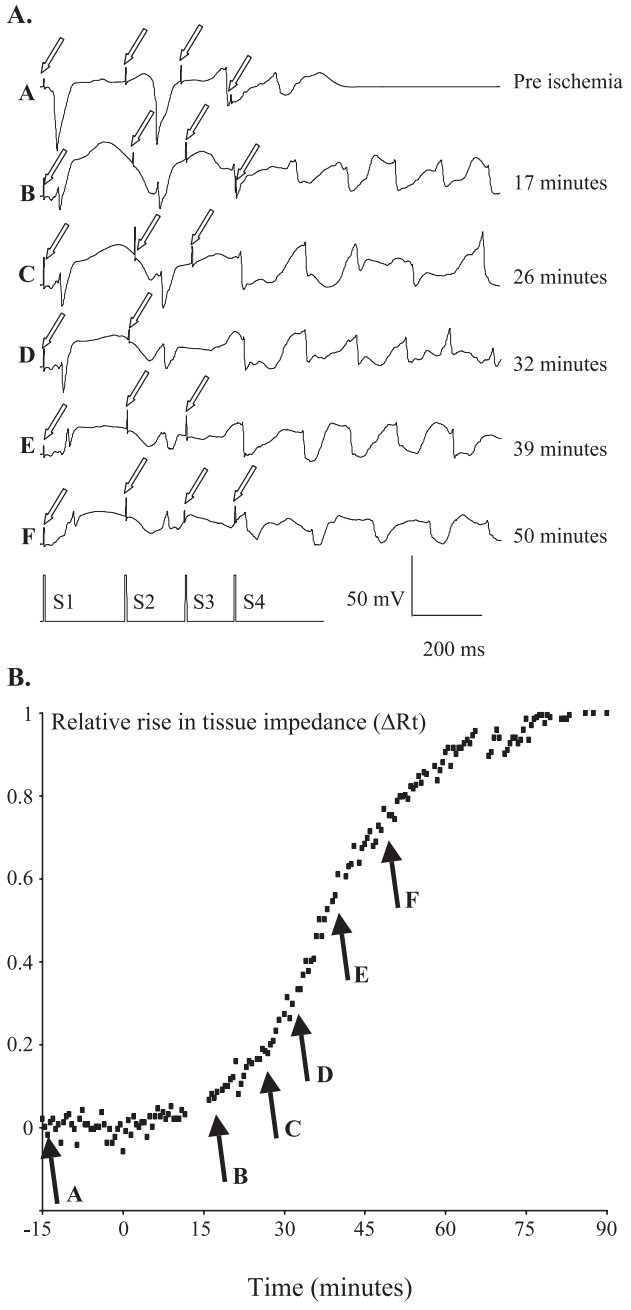


Fig. 3. Panel A: Unipolar electrograms of premature stimulation in a regionally ischemic pig heart. Arrows denote stimulation artifacts. Three PVC did not induce VF before ischemia. The number of PVC decreased from three to one at 32 min of ischemia, after which more stimuli are needed to induce VF. Panel B: Corresponding rise in tissue impedance in the same experiment as in panel A. Letters indicate when electrograms in panel A were recorded (reproduced with permission from Ref. [27]).

progressing gap junctional uncoupling [46]; (5) VF occurred or could be induced at a window of moderate uncoupling only [25,27,73]; (6) epicardial mapping of 1B ventricular arrhythmias showed reentrant circuits with wavelengths in the order of magnitude of centimeters [27] and VF could not be induced in isolated left ventricular preparations up to 9 g and macroreentry around slim lines of activation block was observed [75]; and (7) Ruiz-Meana et al. [52] showed persistent dye coupling after ischemia-induced rigor, demonstrating open gap junctions.

2.3.2. Role of structural changes in gap junctional coupling

As a consequence of tissue anisotropy (more gap junctions along the fiber than perpendicular to fiber direction), conduction velocity and safety for conduction differ in longitudinal and transversal directions, and blockade of conduction in either transversal or longitudinal direction might have differential effects on arrhythmogenesis [46,76,77].

In Cx43 +/− mice, significantly more spontaneous and induced arrhythmias were observed during 1 h of regional ischemia [42]. Fig. 5 shows the incidence of arrhythmias in regionally ischemic wild-type versus Cx43 +/− mice. Both spontaneous and induced VT are more frequent in the Cx43 +/− animals. The hearts of these animals were morphologically normal and conduction velocity under normoxic circumstances was only marginally reduced; therefore, the increased arrhythmogenicity resulted from the interplay between acute ischemia and the genetic background of reduced gap junctional coupling [42]. Thomas et al. [40] showed in cultured Cx43 +/− cells that despite a 43% reduction in expressed level of Cx43 protein, no reduction of conduction velocity was observed.

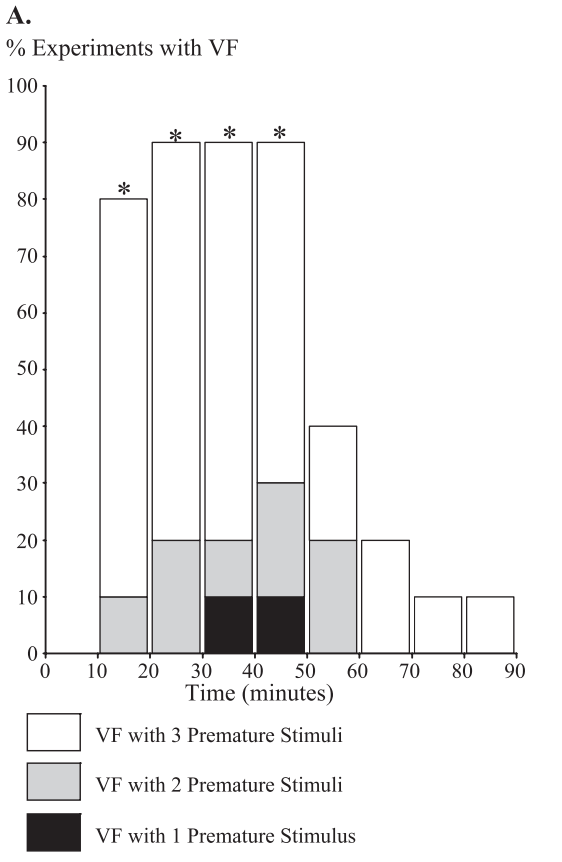
The number of gap junctions decreases and lateralization occurs in failing hearts [78]. Therefore, load mismatches that cause either slow conduction, conduction block, or changed restitution of conduction velocity might arise. Derksen et al. [80] demonstrated that pathologic conduction curves, associated with high vulnerability of VF [79], occur in hearts with interstitial fibrosis and result from load mismatch. Interstitial fibrosis causes cellular uncoupling through insulation of myocardial fibers, and is associated with increased activation delay dependent on the type and amount [81]. Structural remodeling of gap junctions does not occur during the immediate phase of acute ischemia. It is conceivable, however, that arrhythmogenesis in the 1B phase of acute ischemia in hearts of patients with heart failure is more severe because of the preexisting morphological changes. Indeed, the progression of ischemia-induced changes is different in these hearts compared to normal hearts [82]. It has, moreover, been shown that gap junctional uncoupling decreases defibrillation success—a finding of particular importance for the growing population of patients with internal defibrillator [83].

2.4. Modulation of ischemia-induced arrhythmias

2.4.1. Preconditioning

Ischemic preconditioning delayed both gap junctional uncoupling and the occurrence of 1B VF in pigs [26] and was associated with decreased infarct size. Preconditioning may act through various pathways: (1) postponing the increase in $[Ca^{2+}]_i$ and the closely associated rise in tissue

impedance [24]; (2) delayed gap junctional uncoupling in preconditioned hearts is likely related to diminished dephosphorylation and intracellular redistribution of Cx43 during prolonged ischemia [33,84]; (3) preconditioning is abolished with glibenclamide, a blocker of the K_{ATP} channel [85]; (4) opening of the K_{ATP} channel postponed the second rise in $[K^+]_o$ and the decreased catecholamine release from ischemic nerve endings in the isolated rabbit heart, but the decrease in ventricular arrhythmias did not reach statistical significance [86]. The role of closure of gap junctions as a preconditioning factor is unclear. Preconditioning preserved phosphorylation of Cx43, suggesting that the gap junctions remain opened [84,87]. Closure of gap junctions with heptanol in concentrations of 0.5 mM abolished the infarct size reduction by ischemic preconditioning [88], but at a concentration of 1 mM, heptanol was cardioprotective [89].



2.4.2. Reduction of wall stress

Arrhythmogenic triggers during the 1B phase are expected to decrease in occurrence when the wall tension on the ischemic border is lowered [70]. Indeed, unloading the heart with nitroprusside was effective in reducing sudden cardiac death [90].

2.4.3. Autonomic nervous system

Catecholamines, noradrenaline in particular, are released from the ischemic nerve endings with a time course similar to the onset of gap junctional uncoupling [86,91–93] and increase $[Ca^{2+}]_i$ via a G-protein-dependent pathway. Gap junctional conductance increases upon catecholamine-induced increase in intracellular cAMP concentration [31,94]. Consequently, conduction velocity increased and dV/dt max did not change [94]. Parasympathetic stimulation has the opposite effect and decreases gap junctional coupling via a cGMP-dependent pathway [31]. Adrenergic blockade decreases ventricular arrhythmias, but in many studies, heart rate and blood pressure are altered as well [95,96] and the beneficiary effect appeared more prominent on phase-1A than on phase-1B arrhythmias [60,97]. Beta blockade during acute ischemia reduces mortality, partly because of reduction of ventricular rupture.

2.4.4. Other factors

Fatty acid metabolites accumulate in the proximity of intercalated disks and decrease gap junctional conductance, and may modulate arrhythmogenesis [30,98,99]. Preliminary studies with carbenoxolone, an ancient antiulcer drug that can be used as a specific blocker of gap junctions in cardiac muscles [100], showed that infusion in the ischemic zone just prior to coronary occlusion resulted in decreased transversal, but not longitudinal, conduction velocity com-

Fig. 4. Panel A: Percentage of VF inducibility in isolated pig hearts during 90 min of regional ischemia. VF is inducible with one (white bars), two (grey bars), or three premature stimuli (black bars) between 10 and 50 min of ischemia, after which the number of animals in which VF could be induced declined. Panel B: VF inducibility related to relative rise in tissue impedance. VF could be induced up to 40% of relative rise in tissue impedance; at higher degrees of uncoupling, inducibility declined (reproduced with permission from Ref. [27]).

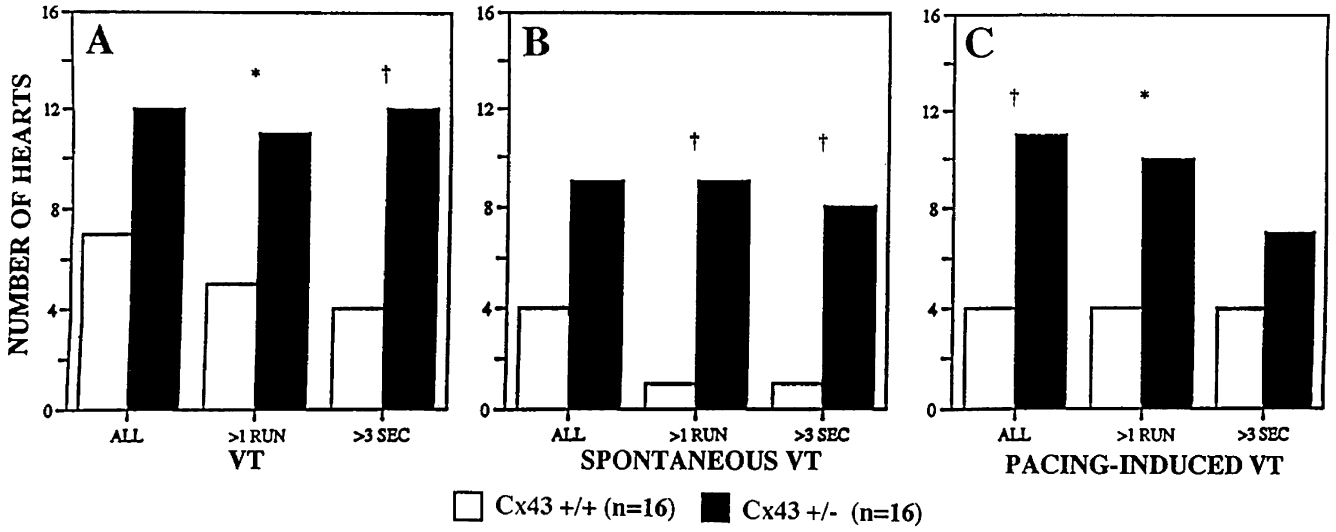


Fig. 5. Incidence of spontaneous and induced VT in wild-type (open bars) and Cx43 +/− mice (closed bars) subjected to regional ischemia. Panel A shows all occasions of VT, panel B and C show spontaneous and pacing induced VT respectively. More arrhythmias were observed in the Cx43 +/− group (reproduced with permission from Ref. [42]).

pared to untreated ischemic hearts, but no reduction in VF (J.R. de Groot, unpublished observation). An increased anisotropic ratio after partial gap junctional uncoupling has been reported previously [41,43,44].

It appears that commonly used antiarrhythmic drugs have little or no effect on gap junctional conductance [101].

3. Clinical relevance

3.1. Lack of studies in humans

The natural time course of ischemia-induced arrhythmias as well as the presence of a 1B phase of arrhythmias and its potential contribution to mortality are unknown in man. It appeared that 30% of sudden cardiac death during the first 24 h of infarction occurred within the first 60 min [102]. This large percentage suggests that at least some of the victims succumb during the 1B phase of arrhythmias. Electrophysiological changes associated with the 1A phase in animals occur much more rapidly in patients undergoing thoracotomy subjected to regional ischemia of short duration [103]. It can be speculated therefore that because 1A changes occur more rapidly, the time window during which 1A arrhythmias can occur is restricted, and 1A arrhythmias occur less frequently in man than in the known animal models. This speculation might contrast with data of out-of-hospital cardiac resuscitation where sudden collapse without prior symptoms is frequently encountered [104]. The number of 1B events in the study by Waalewijn et al. might be underestimated by the patients who where able to seek help and experienced their cardiac arrest in the hospital. However, given the paucity of human data, these scenarios remain highly speculative.

3.2. Can we affect the course of ischemia-induced arrhythmogenesis?

A moderate degree of gap junctional uncoupling is associated with ventricular arrhythmias [27], whereas more advanced uncoupling is antiarrhythmic. Pharmacologic uncoupling therefore may present as a novel target in antiarrhythmic therapy. Pharmacological uncoupling might be selective to the ischemic tissue by the preferential effect of uncoupling on already poorly coupled tissue: the same degree of uncoupling leads to a more pronounced decrease of conduction velocity in poorly coupled than in normally coupled tissue [36]. No clinical trials have, to our knowledge, tested the antiarrhythmic effect of uncoupling agents. Carbenoxolone, a saponin derivative, has been shown to selectively block gap junctions in cardiac tissue with no effect on the major transmembrane currents [100]. Its administration to isolated perfused healthy rabbit hearts did cause a small decrease in conduction velocity. In ischemic tissue, it does not decrease VF inducibility (J.R. de Groot, unpublished observation).

On the other hand, increased gap junctional coupling might be antiarrhythmic by maintaining conduction velocity during acute ischemia for a longer time. However, a large increase in gap junctional conductance could paradoxically cause conduction block through decreased safety for conduction because the current generated by a cell is insufficient to activate the many cells it is coupled to (current sink is too large). There are several agents that increase gap junctional coupling, including agents that increase intracellular cAMP [31,94]. Certain endogenous and synthetic peptides have been reported to decrease dispersion in refractoriness. Indeed, Dhein et al. [105] showed that AAP10 reduced dispersion of activation recovery intervals in a dose-dependent manner up to 10 nmol/l in regionally

ischemic rabbit hearts. It was shown that AAP10 increases gap junctional conductance [106] via a PKC-dependent mechanism [107] and prevents conduction slowing in hypoxic papillary muscles [108]. However, there was no significant reduction in VT or VF by AAP10 [105] nor by HP-5 in regionally ischemic rabbit hearts [109]. A recent study showed that another peptide, ZP123, an AAP analog with a longer plasma half-life compared to AAP10, increased gap junctional conductance with 69% [110]. ZP123 reversed conduction block and decreased the inducibility of reentrant monomorphic VT 1–4 h after coronary occlusion in open chest dogs [110]. The effects of ZP123 were restricted to ischemia, with no change in conduction velocity before ischemia. In summary, what these peptides have in common is that they reduce dispersion of action potential duration and maintain conduction velocity, but their use against acute ischemia-induced VF has not yet been shown convincingly.

3.3. Need of understanding VF in the internal cardiac defibrillator era

The absolute number of sudden deaths in the low-risk population is much larger than that in the high-risk population because the low-risk population is so much larger [10]. With this in mind, there is certainly a need for better understanding the mechanism of VF in normal hearts. The observation that ischemic and pharmacological preconditioning postpones both gap junctional uncoupling and the 1B VF is promising [26]. First, it creates a larger window during which the reversibly damaged myocardium can be salvaged. Second, there is more time to find medical assistance and to reach a hospital, ambulance, or defibrillator. However, the sequence of ischemia and reperfusion appears pivotal for ischemic preconditioning and, to the best of our knowledge, there are no drugs that precondition the heart once ischemia has already started.

From the hypothesis that arrhythmias are caused by a temporal electrotonic effect, mediated via residual gap junctional coupling, it follows that rapid uncoupling of the ischemic tissue would prove antiarrhythmic. This would narrow the arrhythmogenic time window. At this point, however, gap junctional uncoupling therapy is a pure theoretical speculation because there are no drugs that act preferentially within the ischemic zone without affecting the rest of the heart and the rest of the body. Moreover, experimental evidence that increased gap junctional uncoupling is antiarrhythmic is lacking. Once the unanswered questions about this issue are addressed, novel targets in medical therapy may contribute substantially to a decrease of sudden cardiac death in the population at large.

4. Conclusions

Gap junctions are essential for normal propagation of the activation impulse in the heart, and disruption of gap junctional coupling results in discontinuous conduction and arrhythmias. Several factors that modulate gap junctional resistance and affect conduction velocity in vivo and in vitro have been identified.

Myocardial ischemia causes depletion of high-energy phosphates, decreased pH_i, and rise of $[Ca^{2+}]_i$. These factors are associated with dephosphorylation of gap junction proteins and the rise of tissue impedance, a hallmark of intercellular uncoupling. Gap junctional uncoupling causes conduction slowing and therefore provides circumstances leading to reentry, but its exact mechanisms during ischemia are unknown. VF occurs at the start of rise of tissue impedance [25].

Very slow conduction caused by gap junctional uncoupling, as has been demonstrated in vitro [34,46,47] and in silico [36,37], has never been shown in intact hearts, making microreentry an unlikely mechanism. Cx43 +/− mice have slower conduction velocities under normal circumstances and increased arrhythmogenesis during ischemia [42].

The concept of residual coupling between ischemic and nonischemic tissue, whereby the ischemic inexcitable cells electrotonically depress the intrinsically viable tissue, may form an arrhythmogenic mechanism for gap junction uncoupling related to arrhythmias [72]. Indeed, VF inducibility was restricted to the first 40% of tissue impedance rise [27] and gap junctional coupling remains intact longer than excitability [54]. These experimental findings are supported by simulations that provide a mechanism explaining why arrhythmias only occur at modest but not complete uncoupling [73]. However, the evidence for temporary electrotonic interaction between viable and irreversibly damaged myocardium remains circumstantial, and the change in gap junctional conductance between the ischemic subepicardium and the midmyocardium is unknown.

Thus, although many studies have improved our understanding of the role of gap junctional uncoupling in the occurrence of arrhythmias in the regionally ischemic heart, the picture is yet far from complete. The main question that needs to be answered is: Can we locally and specifically modulate gap junctional coupling, either by reducing or by increasing it, and thereby suppress or even prevent lethal arrhythmias during the 1B phase of myocardial ischemia?

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