

Ischemia-induced arrhythmia: the role of connexins, gap junctions, and attendant changes in impulse propagation[☆]

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Abstract

Sudden cardiac death accounts for more than half of all cardiovascular deaths in the US, and a large proportion of these deaths are attributed to ischemia-induced ventricular fibrillation. As such, the mechanisms underlying the initiation and maintenance of these lethal rhythms are of significant clinical and scientific interest. In large animal hearts, regional ischemia induces two phases of ventricular arrhythmia. The first phase (1A) occurs between 5 and 7 min after arrest of perfusion. This phase is associated with membrane depolarization, a mild intracellular and extracellular acidification and a small membrane depolarization. A second phase (1B) of ventricular arrhythmia occurs between 20 and 30 minutes after arrest of perfusion. This phase occurs at a time when ischemia-induced K^+ and pH changes are relatively stable. The arrhythmia is presumed to relate to the process of cell-to-cell electrical uncoupling because a rapid increase of tissue impedance precedes the onset of the arrhythmia. Of note is that tissue resistance is primarily determined by the conductance properties of the gap junctions accounting for cell-to-cell coupling. Impulse propagation in heart is determined by active and passive membrane properties. An important passive cable property that is modulated by ischemia is intercellular resistance and is determined primarily by gap junctional conductance. As such changes in impulse propagation during myocardial ischemia are determined by contemporaneous changes in active and passive membrane properties. Cellular K loss, intracellular and extracellular acidosis and membrane depolarization are important factors decreasing excitatory currents, while the collapse of the extracellular compartment and cell-to-cell electrical uncoupling increase the resistance to current flow. The time-course of cellular coupling is closely linked to a number of physiological processes including depletion of ATP, and accumulation of intracellular Ca^{2+} . Hence, interventions such as ischemic preconditioning attenuate the effect of subsequent ischemia, delay the onset of cell-to-cell electrical uncoupling and likewise delay the onset of ischemia-induced arrhythmia.

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1. Introduction

Myocardial ischemia occurs when the energy demands of the cardiac tissue are not met by available energy substrate. Most commonly, this occurs with the sudden

occlusion of a coronary artery secondary to a thrombus and all too frequently culminates in life-threatening arrhythmia and sudden death. Consequently, understanding the physiological mechanisms accounting for ischemia-induced electrical instability is an important objective of clinical electrophysiologists and basic scientists alike. Despite several decades of intense study, many questions regarding ischemia-induced ventricular fibrillation (VF) remain unresolved [1], and central to these questions is the role of cell-to-cell electrical coupling for the initiation

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and maintenance of ischemia-induced VF. Yet, in contrast to extensive study of active membrane properties and repolarization, the contribution of passive properties, including cell-to-cell electrical coupling to conduction slowing in ischemia, has received little attention. The purpose of this review is to provide a general overview of the role of cell-to-cell electrical coupling as determined by the biophysical properties of gap junctions and connexins, and the development of arrhythmia in the setting of myocardial ischemia.

2. What are gap junctions and connexins?

Intercellular communication of ions and small signaling molecules occurs through protein conduits, gap junctions, formed by the union of 2 hemichannels (connexons), each composed of 6 protein subunits known as connexins (see review [2]). Connexins form a family of proteins, each with specific biophysical characteristics determining the time- and voltage-dependence of the channel conductance and permeability. In the heart, the most prominent connexins associated with electrically active tissue are connexin 43, connexin 40, and connexin 45. The type of connexin expressed, their rates of expression and degradation [3], distribution, density, and the architecture of the tissue modulate the magnitude of cell-to-cell electrical coupling in heart. These factors are affected by heart disease (see review [4]) and therefore contribute to the conditional susceptibility of diseased myocardium to arrhythmia. Yet, gap junctional conductance is also modulated by metabolites that accumulate or are consumed in ischemic myocardium, for example, H^+ , Ca^{2+} , Mg^{2+} , arachidonic acid, lysophosphoglycerides, long-chain acylcarnitines, and adenosine triphosphate (ATP). Because impulse propagation is determined in part by the magnitude of cell-to-cell electrical coupling, changes in cellular metabolites associated with ischemia are expected to modulate electrical properties and conduction characteristics.

3. How do gap junctions affect electrical properties and conduction?

The transfer of action potentials between contiguous myocytes is determined by the magnitude of inward currents and the degree to which adjacent cells are coupled electrically via gap junctions (see review [5]). In general, a surplus of gap junctions is available to conduct excitatory current, and a mild to moderate degree of cellular uncoupling does not affect the speed of conduction. Paradoxically, further cell-to-cell uncoupling will transiently speed conduction as the downstream electrical load increases, shortening the space constant and abbreviating the time to reach the activation threshold of the excitable membrane. As uncoupling progresses, further impulse propagation slows until failure of conduction ensues. Consequently, cell-to-cell electrical coupling is an important

factor in the conduction in the heart and, in the accompanying paper by Rudy (ISCE symposium 2005), an important factor determining the safety factor for impulse propagation.

During ischemia, conduction slowing relates to changes of cellular excitability, the magnitude of the excitatory inward currents, and the resistive properties of the intracellular and extracellular spaces. During the first 10 minutes of ischemia, only about 30% of the measured decrease of conduction velocity can be attributed to the change of passive membrane properties, that is, the longitudinal extracellular resistance and intracellular resistance (r_i). Therefore, during the early phase of ischemia, the conduction slowing is more likely to be related to changes in active membrane properties secondary to the voltage- and pH-dependence of the sodium channel [6], whereas later in ischemia, cell-to-cell uncoupling plays a more important role [7].

4. How is cell-to-cell electrical coupling measured in whole hearts?

In compact myocardium, the longitudinal r_i is determined by myoplasmic resistance and the gap junctional resistance in series. Because the gap junctional resistance is much greater than the myoplasmic resistance, changes in the bulk r_i reflect primarily changes in cell-to-cell electrical coupling. During ischemia, r_i increases abruptly between 15 to 25 minutes after arrest of perfusion in the ischemic papillary muscle of the rabbit and cellular electrical uncoupling is complete within 10 to 15 minutes [7,8].

The resistivity of myocardium can be determined in situ using the 4-electrode technique (see reviews [9-11]). This method uses a linear array of 4 electrodes in which the outer 2 deliver a constant alternating current or direct current and the inner 2 measure the resulting voltage proportional to the whole tissue impedance or resistance. The method has been used successfully to determine the time course of changes of tissue resistivity, that is, cell-to-cell electrical coupling in ischemic heart [12-16]. Because changes in whole tissue resistance parallel changes in the intracellular longitudinal resistance as determined using linear cable analysis in ischemic papillary muscles [7], the time course of cell-to-cell electrical uncoupling in ischemic whole heart can be inferred from changes in whole tissue resistivity measured in the ischemic zone. Based on such measurements, it is well established that cell-to-cell electrical uncoupling occurs approximately 20 to 30 minutes after the arrest of perfusion in large hearts [13-15].

5. What causes cellular electrical uncoupling in ischemia myocardium?

Some conditions such as increased heart rate hasten the onset of cellular uncoupling, whereas other conditions such as slow heart rate, acidosis, ischemic preconditioning, and

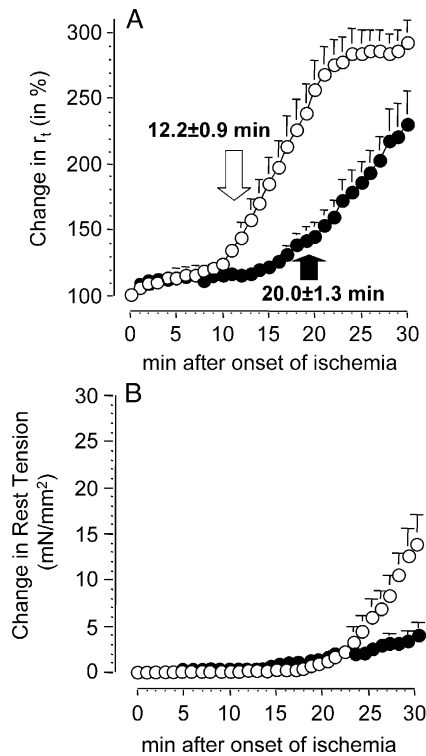


Fig. 1. Inhibition of Na^+/H^+ exchange on ischemia-induced uncoupling. The onset of cell-to-cell electrical uncoupling and contracture was measured in a series of electrically stimulated isolated perfused and ischemic rabbit papillary muscles in the presence of an inhibitor of NHE-1 (dimethyl amiloride, $n = 9$) and its absence (control, $n = 9$). Cell-to-cell electrical uncoupling was inferred from changes in whole tissue resistance, and contracture was measured by a piezoresistive force transducer affixed to the tendon. A, In ischemic muscles, the onset of cell-to-cell electrical uncoupling was 12.2 ± 0.9 minutes in control (open arrow) and 20.0 ± 1.3 minutes in dimethyl amiloride (closed arrow, $P = .0002$). B, Inhibition of NHE-1 delayed the onset of contracture ($P = .0177$) in ischemic muscles. Because the effect of inhibition of NHE-1 on cellular uncoupling during ischemia is observed only in electrically stimulated muscles, the effects of NHE-1 are likely to relate to the delay of the rise in Ca^{2+}_i and/or preservation of the free energy of hydrolysis of ATP. r_t indicates tissue resistance.

calcium and sodium channel blockers delay the onset of cell-to-cell electrical uncoupling. Whether cellular uncoupling during ischemia is a consequence of changes of intracellular ions, accumulation of metabolic by-products, cellular energy depletion, or a combination of these factors is not known with certainty.

Recent studies using Na^+/H^+ exchanger-1 (NHE-1) inhibitors in ischemic porcine hearts [17] and rabbit papillary muscles (Fig. 1A) show that inhibition of the NHE-1 during ischemia delays the onset of ischemia-induced cell-to-cell electrical uncoupling as determined by measures of resistivity. Moreover, inhibition of NHE-1 preserved cellular ATP content, delayed the onset of the ischemia-induced increase in rest tension (Fig. 1B), and maintained conduction until it failed concurrently with the rise in resistance [17]. Based on these studies and others, most evidence suggest an increase of intracellular Ca^{2+} (Ca^{2+}_i) [18]

and/or dephosphorylation of connexin [19] as the likely triggers for cellular uncoupling during ischemia.

6. What is the time course of ischemia-induced arrhythmia?

A bimodal frequency distribution of arrhythmia including VF occurs after the sudden occlusion of a major coronary artery in a large mammalian heart [14,15,20–22]. As shown in Fig. 2A, the first phase of arrhythmia peaks between 5 and 10 minutes after arrest of perfusion and the second phase 10 to 30 minutes later. These 2 distinct phases of arrhythmia are called 1A and 1B, respectively, and the mechanisms accounting for these rhythms are likely to differ. Although 1A and 1B arrhythmias have not been specifically described in man, there is reason to believe that similar mechanisms are operative given that many of the ischemia-related episodes of VF and sudden cardiac death occur shortly after the onset of ischemia [23].

7. What is the mechanism of 1B arrhythmia?

The mechanism explaining the origin of 1B arrhythmias has not been established. Yet, cellular uncoupling is believed to be an important factor because the highest incidence of ventricular arrhythmia occurs during the process of cellular uncoupling, that is, when the tissue resistivity has increased to about 50% of its ultimate change associated with total cell-to-cell uncoupling [14,15,24] as shown in Fig. 3. Such an

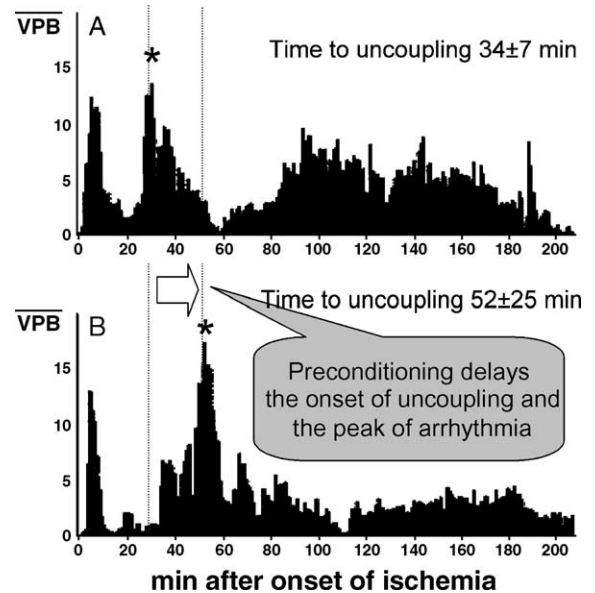


Fig. 2. Time course of ventricular arrhythmia in ischemia. A, Phase 1A and 1B ventricular arrhythmias are shown. The asterisk indicates the onset of cell-to-cell electrical uncoupling as determined by the secondary increase in whole tissue resistivity and phase angle. B, Preconditioning delays the onset of 1B arrhythmias and the onset of cell-to-cell electrical uncoupling. Modified from Cinca et al [14].

observation suggests that electrical cellular uncoupling induces electrophysiologic heterogeneity and is a critical determinant for the formation of the electrical substrate requisite for the initiation and maintenance of VF. Further evidence of an association between cell-to-cell electrical uncoupling and ischemia-induced arrhythmia comes from studies in connexin 43-deficient mice who demonstrate an increase in the frequency and complexity of arrhythmia during ischemia when compared with wild type mice [25].

Electrotonic interaction via cell-to-cell interaction homogenizes the voltage field of juxtaposed cells. The extent to which electrotonic interaction can effectively smooth the spatial nonuniformity created by ionic and voltage gradients depends on the distance over which the gradient extends and the extent of cell-to-cell communication, that is, the length constant. The effects of diffusion at ischemic boundaries are apparent in the subendocardium over a distance much greater than the longitudinal or transverse length constant. Therefore, cellular uncoupling cannot effectively homogenize the electrophysiologic nonuniformity created by diffusion at ischemic boundaries, and because cellular interaction diminishes during ischemia, microscopic heterogeneity in electrical properties increase, particularly transverse to the long axis of the muscle fibers.

As such, cellular uncoupling allows areas of viable myocardium depressed through electrotonic interaction to partially recover, thereby leading to areas of inexcitability, depressed conduction, and minimally affected conduction, all within a well-defined region, that is, the subendocardium and subepicardium, leading to substantial heterogeneity of electrical properties and conduction [24].

Two studies done 2 decades ago provide important insight into the electrotonic interaction within normal and metabolically stressed cardiac tissue. A “preservation phenomenon,” that is, normalization of the action potential duration into a hypoxic zone, was described in cardiac muscle [26]. Atrial premature depolarization was preserved up to 4 mm from the normoxic-hypoxic transition when the hypoxic partition was perpendicular to the fiber axis and a much shorter distance when the partition was parallel to the fiber axis, reflecting the differences in the longitudinal and transverse space constant. Because electrotonic interaction was lost in the hypoxic tissue, action potential duration improved in the normal zone. The concept of electrotonic interaction at ischemic boundaries was advanced further when the length constant in the hypoxic zone was shown to shorten, consistent with cell-to-cell uncoupling [27]. Such effects might explain several observations in ischemic boundaries of regionally ischemic whole heart. For example, extracellular electrograms [28], ST-TQ shifts [29], and conduction velocity [30] tend to normalize at ischemic boundaries after 20 to 30 minutes of ischemia. Because the degree of electrotonic interaction depends on the cell-to-cell communication, factors modulating gap junctional conductance during ischemia are critical to understanding the unique electrophysiologic properties of the border zone.

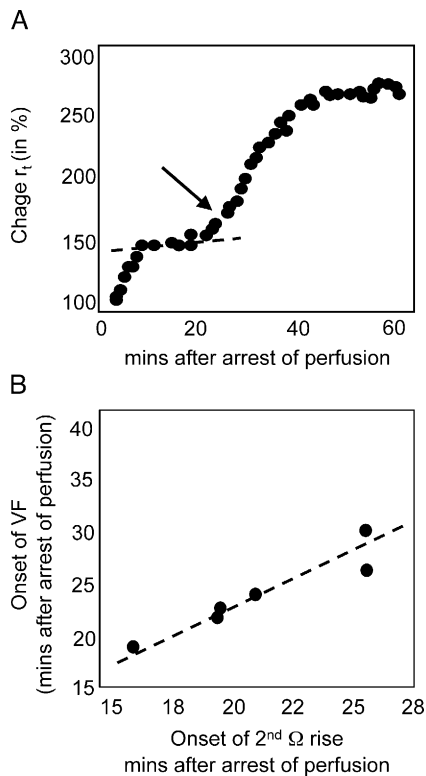


Fig. 3. A, Time-dependence of cell-to-cell electrical uncoupling as determined by the measurement of whole tissue resistance with the 4-electrode method in a regionally ischemic porcine heart. B, The relationship between the onset of the rapid rise in whole tissue resistance and the onset of VF in the ischemic porcine hearts. Modified from Smith et al [15].

8. What physiological and metabolic conditions determine cellular uncoupling in the ischemic border zone?

The ischemic border zone represents a tissue having physiological characteristics primarily determined by biochemical processes linked to the production or use, diffusion, and accumulation or disappearance of gases and metabolites. Over the years, several closely allied laboratories have generated results that provided insight into the mechanisms determining impulse propagation within ischemic boundaries. In brief, these findings are (1) the quantification of marked heterogeneity of extracellular K^+ and pH along ischemic boundaries in the heart [31-34], (2) the effects of carbon dioxide diffusion on intracellular acidosis and cellular K^+ loss [35,36], and (3) the attendant changes on excitability and cell-to-cell electrical uncoupling [37]. Specifically, experimental conditions that either favor or limit the accumulation of myocardial carbon dioxide in ischemic papillary muscle lead to different patterns of conduction slowing and block. These results might be explained by heterogeneity of Ca^{2+}_i and the free energy of hydrolysis of ATP within the ischemic boundary, and in

turn spatial and temporal heterogeneity of the onset of cellular uncoupling. Hence these results are likely to have relevance for the understanding of mechanisms accounting for changes in the velocity of impulse propagation and conduction failure within ischemic boundaries.

In conclusion, cellular uncoupling during ischemia contributes to the onset of serious arrhythmia and might contribute to the normalization of the ST segment in ischemic hearts. Further studies are needed to determine whether conduction ultimately fails because of impedance mismatching (load), wave front curvature, the restitution properties of the ischemic ventricle, regional differences in ion channel type, and distribution or some combination of these mechanisms.

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