EDITORIAL COMMENTARY

Connexin, connection, conductance: Towards understanding induction of arrhythmias?

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Myocardial ischemia can result in three different forms of injury to the heart: arrhythmia, contractile dysfunction, and myocardial infarction. Arrhythmia and contractile dysfunction are reversible conditions that appear early (within a few minutes) when ischemia occurs in a beating heart.

Ventricular tachyarrhythmias are common and potentially life-threatening complications of myocardial ischemia and infarction. Although ventricular tachycardia at times can start by a nonreentrant mechanism (e.g., from delayed or early afterdepolarization), most runs of ventricular fibrillation during early myocardial ischemia are initiated by intramural reentry due to slow conduction block within the ischemic zone.¹ At the cellular level, the proposed events responsible for ischemic arrhythmias are calcium overload, partial depolarization of the cell membrane, and cellular uncoupling. Although advances in antiarrhythmic agents and implantation of direct-current defibrillators have resulted in improved prevention of death due to arrhythmia in myocardial ischemia, morbidity and mortality due to arrhythmias are still high.

In the heart, an electrical impulse is propagated in the longitudinal direction of the myocytes, leading to a synchronous and efficient contraction. A considerable part of the electrical impulse is conducted through gap junction channels. Gap junctions are tightly packed clusters of intercellular channels that directly connect the cytoplasms of adjacent cells. The channels are constructed of a multigene family of integral membrane proteins, the connexins (Cx), with over 20 isoforms in mammals.² These channels can also be gated in response to various stimuli, including changes in voltage, pH, and Cx phosphorylation.³ As the channels mediate the cell-to-cell propagation of current flow that governs orderly contraction of the healthy heart, considerable attention has been directed to the possible role of these junctions as a contributory factor in the formation of a proarrhythmic substrate. The study by Procida et al⁴ in this issue of †Heart Rhythm provides further support that this could be the case.

Connexin 43 (Cx43), the most common Cx, is expressed in heart and brain as well as in many other tissues, and a number of phosphorylation sites are known.⁵ The phosphorylation status of Cx43 differs between tissues, and the phosphorylation changes with the physiological context of the tissue, including ischemia in the heart and the brain.⁶ The phosphorylation of Cx43 is rapidly altered by manipulation of several signal transduction pathways, often with concomitant effects on the number of open intercellular channels or their pore size.⁶ Furthermore, the formation and degradation of gap junctions is a very dynamic process, with reports of half-lives of less than 2 hours even in the heart.⁷ Therefore, regulation of gap junction turnover is likely to be critical in the control of intercellular communication, but the timing does not correlate with the very early arrhythmias in acute ischemia. In heart and brain, Cx43 is under normoxia present in a heavily phosphorylated form, and within a few minutes of ischemia, the protein is dephosphorylated to a considerable extent.⁸ The present results of Procida et al lend support to the notion that certain phosphorylations of Cx43 may be vital for the proper functioning of Cx43 in the heart, and ischemic dephosphorylation of these positions partly explains the electrical uncoupling seen in myocardial ischemia. Until recently, the phosphorylation sites in Cx43 have mainly been established by indirect methods, like phosphopeptide mapping and site-directed mutagenesis. The first major mass spectrometric study⁹ of rat heart Cx43 indicated three novel phosphorylation sites (S296, S297, S306). Heart ischemia caused loss of phosphorylation at S306 within minutes, later followed by dephosphorylation of S297 and S306. The group has now performed site-directed mutagenesis, changing S297 to alanines, and has done electrophysiology in transfected HeLa cells. While no specific effects could be found by the mutation S297A, S306A showed a reduction of macroscopic conductance by 60%. The mutated Cx43 channels showed decreased single-channel conductance, decreased open-state probability, and altered opening kinetics. A number of other potential explanations (e.g., protein expression, mislocaliza-
tion, etc.) were excluded. This work indicates that S306 is involved in determining channel properties. Certainly, more detailed follow-up studies could potentially link the dephosphorylation of specific residues to the different pathophysiological events (e.g., atrioventricular block, conduction velocity, arrhythmia, asystole), even more so because pharmacological interference by the antiarrhythmic peptide analog rotigaptide prolonged the time until the onset of asystole and prevented the dephosphorylation at several residues but not at S306.

A line of evidence suggests that Cx43 is a part of the protective defense system against ischemic10 and other toxic injuries.11 We cannot as yet link a specific position and its phosphorylation status to the protective effect of Cx43 against the ischemic insult, although the overall evidence suggests that there is a connection. Previous results have indicated that ischemic preconditioning could delay the onset of pathological consequences of ischemia, and this was paralleled by an initial increase in dephosphorylation followed by a partial protection against dephosphorylation of Cx43.12,13 As Cx43 is the target of numerous kinases and phosphatases and interacts with other proteins of various kinds, it is tempting to suggest that Cx43 is a so-called signal transduction hub that receives input from many sources and is able to integrate them into a cellular response (Supplementary Figure 1). If this is true, one could imagine several therapeutic approaches to alleviate the consequences of ischemia. Antiarrhythmic peptide-like compounds (like rotigaptide) probably interact with a receptor that subsequently initiates the intracellular responses that have Cx43 as one of their targets. Cx43 dephosphorylation in response to low-flow ischemia was significantly prevented by rotigaptide.9 During conditions of acute cardiac ischemia, rotigaptide effectively prevented induction of both ventricular and atrial tachyarrhythmia.14 Rapid intervention with antiarrhythmic compounds and drugs that reestablish the normal phosphorylation status of Cx43, and thereby also reestablish its normal functions, could limit ischemic damages. Combined with cell therapy,15 a considerable improvement in the treatment of cardiac (and possibly brain) ischemia could be achieved.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2009.08.021.

References