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Review

Role of connexin 43 in cardiovascular diseases

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ABSTRACT

Gap junctions (GJs) channels provide the basis for intercellular communication in the cardiovascular system for maintenance of the normal cardiac rhythm, regulation of vascular tone and endothelial function as well as metabolic interchange between the cells. They allow the transfer of small molecules and may enable slow calcium wave spreading, transfer of "death" or of "survival" signals. In the cardiomyocytes the most abundant isoform is Connexin 43 (Cx43). Alterations in Cx43 expression and distribution were observed in myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia. Recent reports suggest the presence of Cx43 in the mitochondria as well, at least in the inner mitochondrial membrane, where it plays a central role in ischemic preconditioning. In this review, the current knowledge on the relationship between the remodeling of cardiac gap junctions and cardiac diseases are summarized.

Contents

1. Introduction

Gap junctions (GJs) are intracellular structures that provide connections and communication between cells, allowing the passage of ions and small molecules such as ATP, glutathione, cAMP, IP_3 and glucose (Pieperhoff and Franke, 2007). In the heart, GJs mediate electrical coupling between cardiac myocytes, forming the cell-to-cell pathways for orderly spread of the wave of electrical excitation responsible for synchronous contraction (Del Rya et al., 2015). GJ channel is composed of a hemichannel (named connexon) formed of six transmembrane proteins (connexins) embedded in the plasma membrane of one cell joined in mirror symmetry with a connexon hemichannel in the opposing cell membrane (Li et al., 2002). Twenty one genes coding for connexins have been identified, which are classified according to their

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different molecular weights that range between 26 and 60 kDa. Each connexin is constituted by four transmembrane domains, two extracellular loops and one intracellular; amino and carboxy terminal regions are both located in the cytosol (Solan and Lampe, 2005).

In the normal adult heart, there exists three main isoforms: Cx40, Cx43 and Cx45. Cx40 is expressed in the atrial myocytes, in the atrioventricular node, His-bundle and the ventricular conduction system. The expression of Cx45 is mainly localized in the sinoatrial node and the atrioventricular node (Jansen, 2010). Cx43 is the most abundant and is expressed in atrial and ventricular myocytes (Lampe and Martinez, 2004) so this review will focus on its function and role. Cx43 oligomerize in the Golgi/trans-Golgi network and, after assembly, is transported to the non-junctional plasma membrane through the cytoskeleton. Once inserted into the cell membrane, Cx43 spreads in the region where there are GJ plaques by a microtubule/dynein/β-catenin/N-cadherin-dependent pathway (Sáeza et al., 2010). Several protein kinases are implicated in Cx43 phosphorylation, such as mitogen activated protein kinase (MAPk), protein kinase C (PKC), protein kinase A (PKA), casein kinase 1 and Src (Jansen, 2010). Phosphorylation on serine 368 in the COOH-terminal regions of Cx43 provide the regulation of the turnover of GJs communication, such as trafficking, assembly/disassembly, degradation and gating of GJ channels (Popolo et al., 2013). GJ channels preferentially localize to the intercalated disks, discrete regions of cardiomyocyte-cardiomyocyte coupling in the heart, where they interact intimately with adherens junctions (Nambara et al., 2007). This system allows for intercellular communication in cardiovascular tissue and ensures the right maintenance of the cardiac rhythm, regulation of vascular tone and endothelial function, as well metabolic interchange between adjacent cells (Iwasaki et al., 2011). Abnormalities of the normal cardiac rhythm are a common, serious and often fatal complication of many forms of heart disease (Severs et al., 2008). Interest in the role of GJs in heart disease was piqued when images of diseased hearts stained for Cx43 showed that cardiac pathologies were associated with a change in the normal pattern of Cx43 in the ventricular myocardium (Popolo et al., 2014). Rather than being localized at the intercalated disk, Cx43 in human hearts after a myocardial infarction was found on the sides of the myocytes, known as the lateral membranes. Being as intercalated disk localization of Cx43 was considered important for anisotropic conduction in normal heart, this “lateralization” of Cx43 was suspected to be involved in alterations of conduction in the injured heart (Duffy, 2012). In fact, alterations in Cx43 expression and distribution were observed in myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia (Allen, 1992).

Cx43 expression and localization are also altered in aged mice and rat hearts and this increased heterogeneity correlates with age-associated alterations in the heart rhythm and increased atrial fibrillation in patients (Schultz, 2015a). Cx43 expression is higher in female hearts suggesting that Cx43 can be involved in sex-related differences in incidence of life-threatening arrhythmias (Knezl et al., 2008).

Even if most of the functions ascribed to Cx43 in cardiac pathophysiology are related to its function in a GJ, recent literature reports roles and functions for Cx43 outside of intercellular communication (Sakurai et al., 2013; Kalvelyte et al., 2003). The presence of Cx43 in mitochondria is well established and several studies report that mitochondrial content of Cx43 is enhanced by ischemia-reperfusion (Rodriguez-Sinovas et al., 2006) and regulates apoptosis (Goubaeva et al., 2007).

In the last decade, a new localization on Cx43 in cardiac tissue has been described. A recent report suggested that Cx43 is present in the mitochondria from mouse, rat, pig and human left ventricular myocardium, and may play a role in mediating the cardioprotective effect of ischemic preconditioning. Co-immunoprecipitation studies have shown an interaction of Cx43 with translocase of the outer membrane 20 (TOM 20), which is, with Tom5, 6, 7, 22, 40 and 70, part of the translocase of the outer membrane (TOM) protein complex and thereby of the general mitochondrial import machinery (Boengler et al., 2006). TOM 20 is the only known protein complex involved in the entering of nuclear-encoded proteins into mitochondria. The proteins bind TOM through TOM 20 and, after the recognition step, reach the internal membrane or the matrix through the TIM (Translocase of the Inner Membrane) complexes (Rodriguez-Sinovas et al., 2006). It has been demonstrated that ischemic preconditioning induces Cx43 translocation from cytosol to mitochondria with a mechanism that involves heat shock protein 90 (Hsp90) and TOM 20. ATP-dependent Hsp90 in the cytosol is implicated in the import process but mitochondrial receptors for these factors have not been established. Cytosolic Hsp90 is generally involved in the folding of newly synthesized proteins and its role in mitochondrial import may be an extension of this activity. Mitochondrial import machinery involves binding of the target protein to a chaperone (Hsp90/Hsp70), presentation to specific parts of TOM complex, and release into the inner mitochondrial membrane (Ruiz-Meana et al., 2008) (Fig. 1).

Mitochondrial Cx43 modulates the matrix potassium levels and participates in energy metabolism in the heart (Schultz et al., 2015a, 2015b). Yue et al. (2002) have demonstrated that K^+ influx, through mitochondrial ATP-dependent K^+ channels (mitoKATP), causes mitochondrial depolarization in preconditioned cardiomyocytes, an effect associated with reduced mitochondrial reactive oxygen species production and infarct size reduction. These effects are most important for the cardioprotection. Furthermore S-nitrosation of mitochondrial Cx43 increases mitochondrial permeability, especially for potassium, and leads to increased reactive oxygen species formation. The increased amount of S-nitrosation mitochondrial Cx43 by ischemic preconditioning may link nitric oxide and Cx43 in the signal transduction cascade of cardioprotective interventions (Soetkamp et al., 2014). In addition mitochondrial Cx43 play an important role in matrix calcium homeostasis; in fact, calcium overload in subsarcolemmal mitochondria was reduced by blocking Cx43-formed channels with Gap27. (Srisakuldee et al., 2014).

Furthermore, recently other studies indicate that mitochondrial Cx43 translocation is implicated in cardioprotection against doxorubicin-induced cardiotoxicity (Pecoraro et al., 2014).

2. Role of Cx43 in ischemia-induced arrhythmias

Electrical coupling is essential for normal impulse propagation through the heart, together with proper excitability of the cardiomyocytes and normal tissue architecture. Reduced electrical coupling can increase the propensity for arrhythmias rendering the ventricle more susceptible to re-entry. This condition seems to be due to dysfunction and disorganization of Cx43. Indeed, reduction of about 90% in Cx43 expression results in about 50% decrease in the conduction velocity (Van Rijen et al., 2004). While 50% reduction in Cx43 may lead to some conduction slowing, high levels of electrical uncoupling are needed to increase arrhythmogeneity. Arrhythmias are a common complication of myocardial ischemia and infarction in humans. These pathologies are associated with progressive remodeling, loss of sarcomeres and perinuclear accumulation of glycogen. Furthermore, it was verified that the organization of GJs was markedly disordered. They are not aggregated into intercalated disks but are distributed over myocyte surfaces (Severs et al., 2004). Abnormal tissue architecture, e.g. due to increase of fibrosis, may have synergistic