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Mitochondrial Cx43, an important component of cardiac preconditioning☆

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ABSTRACT

Connexin 43 (Cx43) forms gap junction channels that are essential for the propagation of electrical depolarization in cardiomyocytes, but also with important roles in the pathophysiology of reperfusion injury. However, more recent studies have shown that Cx43 has also important functions independent from intercellular communication between adjacent cardiomyocytes. Some of these actions have been related to the presence of Cx43 in the mitochondria of these cells (mitoCx43). The functions of mitoCx43 have not been completely elucidated, but there is strong evidence indicating that mitoCx43 modulates mitochondrial respiration at respiratory complex I, production of radical oxygen species and ATP synthesis. These functions of mitoCx43 modulate mitochondrial and cellular tolerance to reperfusion after prolonged ischemia and are necessary for the cardioprotective effect of ischemic preconditioning. In the present review article we discuss available knowledge on these functions of mitoCx43 in relation to reperfusion injury, the molecular mechanisms involved and explore the possibility that mitoCx43 may constitute a new pharmacological target in patients with ST-segment elevation myocardial infarction (STEMI). This article is part of a Special Issue entitled: Gap Junction Proteins edited by Jean Claude Herve.

Contents

1. Cx43 and ischemia-reperfusion injury

Connexin 43 (Cx43) was the first connexin to be discovered as the monomer responsible for the formation of hexameric hemichannels and gap junction (GJ) channels in the heart [1]. Since then, it has been recognized as a key element in the propagation of electrical impulse in the heart, an essential aspect of cardiac function. However, Cx43 is also present in many cell types in which propagation of electrical impulses does not occur physiologically, and it was soon proposed that Cx43 could be involved in physiological functions other than electrical impulse propagation [2–6]. These include the exchange of molecules between adjacent cells, or between cells and the extracellular space [7–9]. Both of these exchanges have been described to have a role in ischemia-reperfusion (IR) injury.

Early observations demonstrated altered GJ-mediated impulse propagation during ischemia, although there is evidence of residual GJ permeability in cardiomyocytes during and even after development of

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rigor contracture [10]. The first evidence of the involvement of Cx43 in reperfusion injury in cardiomyocytes was the observation that reperfusion-induced hypercontracture can propagate to adjacent cardiomyocytes during reperfusion in a gap-junction dependent manner [11]. The mechanism proposed was the passage of Na^+ ions to the adjacent cell, with subsequent exchange with Ca^{2+} when the ability of the adjacent to extrude Na^+ was impaired by damage of the Na^+ pump [12]. Many studies have confirmed since then Cx43 GJ-mediated propagation of cell death in cardiomyocytes and in other cells during ischemia-reperfusion and other related conditions [13–19]. However, this phenomenon is far from being completely understood, and gap-junction mediated communication has been also proposed to favor salvage of cardiomyocytes under certain conditions, likely by dilution of the cytosolic alterations [20]. In addition, altered Cx43 GJ communication during reperfusion may lead to reperfusion arrhythmias, which complicates translation of transient, selective Cx43 GJ blockade during initial reperfusion to clinical practice [21–23]. Not only Cx43 GJ have been involved in IR injury, but there are also many studies supporting a role of sarcolemmal Cx43 hemichannels in anoxic or ischemic injury in cardiomyocytes (reviewed elsewhere, as in [4,6,24]).

Since Cx43 participates in the pathophysiology of IR injury it was hypothesized that it could also be involved in the mechanism of cardioprotective interventions, in particular ischemic preconditioning (IPC). IPC (cycles of brief ischemia-reperfusion applied before sustained coronary occlusion) is consistently protective against reperfusion-induced cell death among animal species and IR conditions, but its mechanism has remained elusive for three decades. Initially, it was suspected that delayed restoration of full GJ permeability during reperfusion could contribute to IPC protection, but different experiments, in particular those including monitoring of changes in myocardial impedance during IR, failed to provide any evidence supporting this hypothesis [25]. However, subsequent experiments with genetically modified animals clearly indicated that Cx43 was necessary for IPC exerting its cardioprotective effect [26–29], and this appeared to be related to attenuated reactive oxygen species (ROS)-signaling during IPC in animals deficient in Cx43 [30]. Altogether, these series of studies seemed to indicate that functions of Cx43 other than GJ-mediated intercellular communication (GJMICC) were important for IPC-induced cardioprotective signaling. More recent studies showed that these GJ independent effects of Cx43 could depend on its unexpected localization at the inner mitochondrial membrane [31,32].

Different groups, using different techniques, have provided evidence of the mitochondrial location of Cx43 (mitoCx43) [31–34]. We know, thanks to studies using immuno-electron microscopy, either in tissue [31] or in isolated mitochondria [35], showing its presence within the organelle crests, and to subfractionation and biochemical analysis of digitonin- and proteinase K-treated mitochondria [32,36], that Cx43 is located at the inner mitochondrial membrane [32] of subsarcolemmal mitochondria [36] where it forms hemichannels, and that translocates to that position using the TOM pathway [32]. Evidence suggests that it is oriented with the C-terminus at the intermembrane space [37]. The physiological role of mitoCx43 has not been elucidated, but available data show that it modulates K^+ influx to the mitochondrial matrix and mitochondrial respiration [37,38]. Available data indicate that the mitoCx43 increases respiration, ATP levels, and ROS generation, and can contribute to reperfusion injury. In this review article we will discuss evidence showing that Cx43, and in particular its mitochondrial localization is a key element of cardioprotective signaling induced by IPC.

2. Cx43 is necessary for preconditioning protection

Cx43 and GJ are known to be regulated by phosphorylation by different kinases, including PKC and p38-MAPK [39]. Since these kinases are also involved in the intracellular signaling pathways activated by IPC, it was early hypothesized that GJ were, indeed, taking part in its protective effect [40]. As an example, PKC directly phosphorylates Cx43 on Ser368 [41], and this phosphorylation event also occurs during IPC [42]. However, it was soon realized that gap junctional channels were not a requisite for IPC protection, as isolated cardiomyocytes submitted to simulated ischemia-reperfusion, which do not show functional GJ, still could be preconditioned [43]. Nevertheless, this does not mean that GJ are not involved in protection, as IPC is generally more potent in whole hearts than in isolated cardiomyocytes [44]. Even more, Cx43 has been demonstrated to be essential for IPC protection.

Several lines of evidence support a role for GJ in the trigger phase of IPC. Infusion of heptanol, a GJ uncoupler, given for 5 min before the preconditioning cycle in isolated perfused mice hearts, abolished the protective effect of IPC [45]. Similarly, the protective effect of a δ-opioid receptor agonist, (D-Ala^2, D-Leu^5)-enkephaline acetate (DADLE), against infarction, was abolished by pretreatment with heptanol [44]. Moreover, the antiarrhythmic effect of IPC observed in dogs submitted to transient coronary occlusion was also attenuated by pretreatment with intracoronary carbenoxolone, another GJ uncoupler [46]. These data are suggestive that gap junctional channels need to be open during the trigger phase of IPC for effective transport of signaling molecules within the area at risk [44]. However, care should be taken when considering the effects of GJ uncouplers under these conditions, especially in the case of heptanol. This alcohol has been demonstrated to possess clear anti-ischemic actions, attenuating the increase in cytosolic Ca^{2+} induced by simulated ischemia in rat cardiomyocytes, and delaying rigor onset and cell-to-cell electrical uncoupling in isolated rat hearts submitted to global ischemia [14]. Such anti-ischemic actions would render preconditioning cycles ineffective.

Contribution of unapposed Cx43 hemichannels to the trigger phase of IPC should be also considered. Indeed, both heptanol and carbenoxolone also block Cx43 hemichannels, rendering interpretation of results obtained with these drugs complex. Non-junctional Cx43 hemichannels open under a variety of stimulus, including ischemia or metabolic inhibition [4,5,47], and release a number of intracellular components, including ATP [44,48], that may act on neighboring cells in a paracrine/autocrine manner [4,5]. Furthermore, it has been shown that preconditioning protection in glial C6 cells was linked to a marked increase in the amount of Cx43 hemichannels [49]. However, it has been suggested that the contribution of Cx43 hemichannels in the myocardium should be negligible [44], as more than 80 min of ischemia are needed to induce a peak of ATP release, inhibitable by Cx43 hemichannel inhibitors, in rat neonatal cardiac myocytes submitted to ischemic stress [50], while only 50% rod-shaped ventricular rabbit myocytes showed propidium iodide accumulation after 90 min treatment with metabolic inhibitors [51].

Thus, the role of Cx43 in the trigger phase of IPC can be considered rather controversial. In contrast, and although our knowledge about the end-effectors of IPC protection is very limited, it has been postulated that Cx43 may be one of them [52]. The first works suggesting that Cx43 was necessary for IPC protection were done by Schwanke and coworkers [26,27]. In these studies, Cx43 deficiency in heterozygous $Cx43^{+/-}$ mice abolished preconditioning protection [26,27]. However, the authors did not explore the mechanisms of this effect. It was initially argued that the role of Cx43 was dependent on GJMICC. In this sense, ischemic or pharmacological preconditioning have been linked with higher myocardial Cx43 levels [53] and increased extent of Cx43 phosphorylation during the sustained ischemic event [42,44,54–56], effects that were associated with a reduction in GJ permeability [55,56] and redistribution of Cx43 from intercalated disks to the lateral free plasma membrane [57]. Furthermore, PKC-mediated phosphorylation of Cx43 on Ser368 has been suggested to result in a change in single channel behavior that contributes to a decrease in intercellular communication [41]. All these findings would be indicative of a reduction in GJ permeability that would prevent diffusion of death factors between adjacent cells during ischemia-reperfusion, thus increasing cell survival.