Cx43 PHOSPHORYLATION AND CARDIOPROTECTION

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Connexin-43 (Cx43), a transmembrane protein able to oligomerize into hexamers around a central pore, is the main protein forming gap junctional channels in ventricular cardiomyocytes. Altered Cx43 expression or distribution has been shown to impair propagation of electrical impulses and promote arrhythmias during ischemia and reperfusion. However, in the past few years, it has become evident that the role of Cx43 in myocardial ischemia pathophysiology goes beyond its role in arrhythmogenesis. Gap junction uncouplers have been shown to limit infarct size after ischemia in different tissues, including heart and brain, thus supporting a role for gap junctions in intercellular spreading of injury during ischemia-reperfusion. These findings were further strengthened by studies in heterozygous Cx43-deficient mice (Cx43+/−) submitted to chronic ischemia without reperfusion, which showed reduced infarctions in mutant animals. However, the involvement of Cx43 in the pathophysiology of ischemia-reperfusion injury is not restricted to the spreading of injury, and several studies suggest an important role in cardioprotection.

In this context, the study by Srisakuldee et al., published in the current issue of Cardiovascular Research, adds important information on the cardioprotective role of Cx43. The authors demonstrate that both ischemic preconditioning and pretreatment with diazoxide are able to induce, in isolated rat hearts, specific phosphorylation of Cx43 at serines 262 and 368 without modifying total Cx43 expression. Furthermore, hearts treated with another cardioprotective agent, fibroblast growth factor 2 (FGF-2), displayed similar increases in P-S262-Cx43 and P-S368-Cx43 levels when given during normoxia, before ischemia or at the onset of reperfusion, and showed reduced Cx43 lateralization during ischemia. Importantly, a link between these three protective manoeuvres may exist, as they have been proposed to protect hearts by activating...
PKCε, which also phosphorylates Cx43. However, the study by Srisakuldee et al.\textsuperscript{7} goes beyond this initial descriptive data and adds important mechanistic information that can help to understand the cardioprotective role of Cx43. Thus, in elegant experiments carried out in isolated neonatal cardiomyocytes, authors demonstrated that overexpression of wild-type Cx43 was able to reduce injury after simulated ischemia, whereas overexpression of mutated S262A-Cx43, incapable of phosphorylation at that site, increased injury and abolished protection by FGF-2 and by overexpression of PKCε. In view of these results, Srisakuldee et al.\textsuperscript{7} propose that phosphorylation of Cx43 at serine 262 induces an injury-resistant state that can be reached after several protective manoeuvres like ischemic or pharmacological preconditioning, or treatment with FGF-2. Although the exact mechanism by which Cx43 phosphorylation at S262 contributes to cardioprotection is not explained (i.e., whether it affects channel gating, kinetics or Cx43 distribution), this study is the first to address the question of the potential molecular modifications of Cx43 involved in protection.

The study leaves open several important questions. It is not known whether the lack of protection seen in neonatal cardiomyocytes overexpressing mutant S262A-Cx43 can be generalized to the entire heart. Transgenic mice models expressing this defect should be developed to provide a clear answer. In addition, it is not known whether Cx43 mutations other than at S262 would also affect protection by ischemic or pharmacologic preconditioning. Furthermore, the authors suggest a dominant-negative effect of S262A-Cx43 overexpression over endogenous Cx43 in isolated neonatal cardiomyocytes, but no clear proof of the presence of this effect is given. Moreover, it is not known whether all forms of endogenous protection are linked to Cx43 phosphorylation at S262. Although authors conclude that Cx43 phosphorylation at S262 likely mediates PKCε-
A critical question is how S262 phosphorylation can affect cardioprotection; at present, the mechanisms by which Cx43 mediates cardioprotection are not known. Two main groups of possibilities have been proposed. One involves a reduction in chemical coupling induced by treatments such as ischemic preconditioning \(^8\). According to the other proposal, Cx43 may mediate cardioprotection through gap junction-independent mechanisms. This hypothesis is supported by experiments performed in isolated cardiomyocytes obtained from heterozygous, Cx43-deficient mice (Cx43\(^{+/-}\)). As occurred with isolated mice hearts \(^5\), isolated cardiomyocytes obtained from these mutant mice cannot be preconditioned \(^6\). In fact, both hypotheses are not mutually exclusive, and it is possible that both may contribute to some degree to cardioprotection.

Little is known about the gap junction-independent effects of Cx43 on protection \(^9\). Unopposed hemichannels that open under ischemic conditions may mediate the release of several cardioprotective mediators to the extracellular space. Unopposed hemichannels have been considered to be important mediators of protection in other tissues or cells such as astrocytes \(^10\). Deficiency of mitochondrial Cx43 has been shown to abolish protection by diazoxide, a protective drug that supposedly specifically interacts with mitochondrial K\(_{\text{ATP}}\) channels, and to reduce production of reactive oxygen species, known mediators of protection, in response to this drug \(^11\). Importantly, replacement of Cx43 by a different connexin isoform, Cx32, in a knock-in mice model, modifies mitochondrial potassium uptake \(^12\), highlighting the importance of Cx43 in mitochondrial physiology.
At present, it is not known whether the link between Cx43 phosphorylation at S262 and cardioprotection described in the current issue by Srisakuldee et al. 7 affects the complete population of Cx43 or whether it is restricted to Cx43 located at gap junctions, hemichannels or mitochondria. Previous work has shown that most of the Cx43 located both in gap junctions and mitochondria is in the phosphorylated state, resulting in electrophoretic migration at 43-45 kDa, which corresponds with the mobility described for P-S262-Cx43. Thus, both Cx43 pools are candidates for being involved in protective mechanisms.

Regulation of Cx43 goes beyond a single amino acid phosphorylation. More than 20 serine or tyrosine residues are able to be phosphorylated by different protein kinases. Furthermore, it involves regulation by factors like pH, intracellular Ca\(^{2+}\) levels, and post-translational modifications, including S-nitrosylation. The work by Srisakuldee et al. 7 is the first to address this important question and opens an intriguing new field of research.

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