ISCHEMIC PRECONDITIONING AND ISCHAEMIA-REPERFUSION: KEY ROLE OF CONNEXIN43 AND MITOCHONDRIAL COMPLEXES

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It has been demonstrated that heart can survive a short period of ischaemia and recover upon reperfusion (IR), but reperfusion can exacerbate the damage that takes place during the ischemic period. There is increasing evidence that mitochondria are implicated in the mechanisms that mediate reperfusion injury, which can be prevented or attenuated by ischemic preconditioning (IP). It has also been described that connexin 43 (Cx43) is located in the cardiomyocyte mitochondria and is important for the cardioprotection mediated by IP. Moreover, IP does not occur in transgenic mice Cx43KI32, which lacks Cx43.

To elucidate the molecular mechanisms implicated in IR, IP, and the role of Cx43 in these processes, we performed a comparative differential expression study of the cardiomyocyte mitochondrial proteome from rats subjected to IP, IR and IP previous to IR. In another set of experiments we analyzed the effect of Cx43 elimination and its implication on IP using a Cx43KI32 transgenic mice model. Rat samples were analyzed by $^{18}O/^{16}O$-labeling and mice samples by iTRAQ. Digestion of proteomes, labeling, peptide separation by IEF and analysis by LIT-MS was performed using a technique previously developed in our laboratory. Quantification and statistical analysis was performed using QuiXoT.

We found that all these processes induced very few significant and low magnitude protein changes, however, the changing proteins were consistently associated with very specific and related functions such as oxidative phosphorylation, fatty acid metabolism and Krebs cycle. A Systems Biology analysis of all quantified proteins with tools developed in our laboratory demonstrated that some complexes from the electron transport chain (ETC) were up-regulated after IP, IR and upon Cx43 elimination, but were unaltered after IP in mice without Cx43. These results show that alterations in ETC play a relevant role in IP and IR through a mechanism that is modulated by Cx43.