Role of mitochondrial Connexin 43 in an *in vitro* model of hypoxia

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Gap junctions (GJs) are intracellular structures that provide communication between cells, allowing the passage of ions and small molecules. In the heart, GJs are concentrated at intercalated discs, discrete regions of cardiomyocytes-cardiomyocytes coupling, where they interact intimately with adherents junctions (Nambara et al., 2007). Cardiac function is guaranteed by this important intercellular junctions system which provide the right maintenance of the cardiac rhythm and regulation of vascular tone (Iwasaki et al., 2011). GJ channel is consisted of a hemichannel composed of six transmembrane phosphoproteins (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). In the cardiac ventricle the most abundant isoform is Connexin 43 (Cx43) (Severs et al., 2008). Alterations in Cx43 expression and distribution were observed in myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia (Kostin et al., 2003). A recent report suggest that Cx43 is present in the mitochondria and is important in the cardioprotection mechanism providing cytoprotection and preventing the increase of reactive species oxygen (ROS). Cx43 translocates from cytosol to mitochondria with a mechanism that involves Hsp90/TOM20 machinery system (Pecoraro et al., 2015).
The aim of this study was to evaluate the involvement of mitochondrial Cx43 (mCx43) in a cellular model of hypoxia. Chemical hypoxia was induced by cobaltum chloride ($CoCl_2$) added to incubation medium of rat cardiomyoblast cell line (H9c2) in a dose- and time-manner (50-100-150μM; 3-6-24 h). In order to evaluate the involvement of mCx43 experiments were performed both in absence and in presence of radicicol [5μM], an Hsp90 inhibitor.
MTT assay showed that cellular vitality in our experimental condition was higher than 70% and Western blot analysis showed that $CoCl_2$ induced HIF-1α expression to confirm a condition of hypoxia.
Immunofluorescence analysis indicates that $CoCl_2$ induced an increase of mitochondrial localization of Cx43 which was inhibited by radicicol, indicating a translocation of Cx43 to mitochondria membrane. Mitochondria plays a pivotal role in the ROS production in the heart, so oxidative stress is a component of several forms of cardiac disease (Sovari et al., 2011). Facs analysis showed that $CoCl_2$ rose mitochondrial ROS production in a dose- and time-dependent manner and the presence of radicicol significantly ($P<0.05$) increases mitochondrial ROS production. Oxidative stress also modifies the intracellular regulation of Ca^{2+} ($[Ca^{2+}]i$) homeostasis and induces mitochondrial calcium overload (Thornton et.,2014). In our experimental conditions $[Ca^{2+}]i$ was evaluated by means of FURA-2AM. $CoCl_2$-treatment significantly ($P<0.001$) affects mitochondrial $[Ca^{2+}]i$ levels in a dose- and time- dependent manner. In fact, delta increase in $[Ca^{2+}]i$ induced by carbonylcyanide p-trifluoromethoxy-pyhenylhydrazone (FCCP; 0.5 μmol/L), a mitochondrial calcium depletory, was significantly ($P<0.001$) higher than control cells indicating an higher mitochondrial calcium storage. The inhibition of Cx43 translocation to mitochondria, induced by radicicol, produces a further calcium overload in mitochondria.
In conclusion, our data suggest that mCx43 plays a pivotal role in cytoprotection by regulating mitochondrial ROS accumulation and calcium overload, both implicated in mitochondrial degeneration with a subsequent cellular damage.

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