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Role of heat shock protein 70 in the anti-ischemic effect of the tubulin-binding agent Taxol on cardiomyocytes


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Our previous data suggested that Taxol protects electromechanical and mitochondrial functions of rat cardiomyocytes against ischemia-reperfusion. To date however, the possible involvement of heat shock proteins in this beneficial influence of Taxol has not been investigated. Therefore, we have studied the effect of Taxol on the production of heat shock proteins 70 (HSP70) from newborn rat cardiomyocytes (CM) in an in vitro substrate-free hypoxia-reoxygenation model of simulated ischemia (SI) and reperfusion (SR). In these conditions, we observed an initial rise in HSP70 mRNA expression during SI and a further induction during SR. Exposing CM to Taxol before SI reduced these increases in HSP70 mRNA expression induced by SI and by SR. This reducing effect was additionally enhanced by pretreating CM by the combination of Taxol and cyclosporine A which suggested that a side effect on mitochondria might contribute to the action of Taxol. Finally, Taxol present during SR only was still able to decrease the post-SI rise in HSP70 mRNA expression. These data suggested that the counterpart of reducing ischemia-reperfusion myocardial injury by tubulin-binding agent Taxol may be a decrease in the cellular expression of the stress molecular markers.

Effect of different glucose+fatty acid combinations on the functional and metabolic recovery of postischemic cardiomyocytes

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We have previously observed that post ischemic functional and metabolic recovery of isolated cardiomyocytes (CM) depends on the nature of the energetic substrate supplied at the time of reperfusion. However, these preliminary studies took in consideration only one single source of energy, i.e., either glucose or different individual fatty acids. Therefore, we intended to extend these preliminary results by studying the effects of the combination of these substrates on the recovery of post ischemic CM in an in vitro model of ischemia-reperfusion. CM were exposed to substrate-free hypoxia simulation of ischemia (2.5 h) and then after their functional and metabolic recovery during reoxygenation (R; 2.0 h) was assessed without substrate (CTRL) or in the presence of one of the following substrate mixtures: glucose (GLC) + oleic acid (OLE), GLC + octanoic acid (OCTA) or OLE+OCTA. During R, the resumption of electromechanical activities was hastened and improved by the three combinations of substrates. However, the OCTA+OLE mixture delayed the recovery as compared with the two other combinations. OCTA+GLC provided a complete recovery of action potential (AP) rate. OLE+GLC induced the fastest resumption of AP amplitude and also the best metabolic recovery, as assessed by MTT test. After 2 h of R, OCTA+OLE improved CM viability evaluated by methylene blue staining as compared with the other conditions. Finally, these combinations of 2 substrates, especially OLE+GLC, induced better post ischemic recovery than single substrate or than the absence of substrate, suggesting complex regulation of the different metabolic pathways during reperfusion. Moreover, these data stressed that the apparent potency of each substrate combination for alleviating reperfusion injury depends on the myocardial function under consideration.

H9c2 cardiac myoblasts undergo apoptosis in a model of simulated ischemia: inhibition by phorbol ester PMA


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Cardiac myocytes undergo apoptosis under condition of ischemia. Little is known, however, about the molecular pathways that mediate this response. A common and critical event in the execution phase of apoptosis is the activation of a family of aspartate-specific proteases termed caspases, which participate in a cascade where initiator caspases activate effector caspases and ultimately cleave a set of structural and functional proteins. In the present study we report that H9c2 rat ventricular myoblasts undergo apoptosis in a model of simulated ischemia, consisting of serum withdrawal and hypoxia, and that is prevented by phorbol-12-myristate-13-acetate (PMA). Simulated ischemia was achieved by culturing the cells in serum-deficient DMEM in an anaerobic workstation (BugBox, Jouy, France) saturated with 5% CO2/95% N2 at 37°C for the indicated time periods. Induction of apoptosis was determined by measuring the activity of caspases and by detecting the 3'-OH end of DNA fragments in the apoptotic nuclei by the method of terminal transferase-mediated dUTP nick end labeling (TUNEL). Hypoxia alone did not induce significant apoptosis, but largely increased the proapoptotic action of serum deprivation. H9c2 cells apoptosis is evidenced by an increase in TUNEL-positive nuclei and by activation of caspases 3, 6, 7 and 9, and loss of mitochondrial functions. In this model of simulated ischemia, cardiomyoblasts apoptosis was associated with a p53-independent Bax accumulation and a down-regulation of Bcl-xL, whereas the levels of cIAP-1, cIAP-2 and XIAP proteins did not change. Phorbol-12-myristate-13-acetate (PMA) is recently known to modulate apoptosis. In our experimental model, PMA significantly reduced the induction of apoptosis elicited by ischemia, inhibiting caspase 3 cleavage, Bax accumulation, Bcl-xL down-regulation as well as restoring cell viability. This research was supported by grants from MIUR and Compagnia di San Paolo, Turin.