APOPTOSIS INDUCED BY HYPEROSMOTIC STRESS

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We have previously shown that hyperosmotic stress by sorbitol triggers cardiomyocyte apoptosis. However, in this cell death process, deleterious and protective signaling pathways activated by hyperosmotic stress remain unexplored. Our results showed that free radical production, evaluated by DCF fluorescence, was stimulated in cultured neonatal rat cardiomyocytes exposed to sorbitol (Sor, 600 mOsm), effect that was prevented by N-acetylcysteine (NAC, 5 mM). The •OH radical generation was demonstrated by Electron Spin Resonance (ESR) using DMPO as spin trap. NAC, SN50 (a specific NFκB blocker) and AdIκBα (adenovirus that overexpress an IκBα mutated form) inhibited both the expression of a NFκB-LUX reporter gene and the nuclear accumulation of p65-NFκB isoform induced by hyperosmotic stress. Sor (600 mOsm) also increased DNA fragmentation (assessed by agarose gel electrophoresis) and the number of anexin V positive cardiomyocytes (evaluated by flow cytometry); these effects were attenuated by NAC and increased by SN5O and AdIκB. Lastly, cell viability was decreased by Sor and potentiated by NFκB inhibitors. We conclude that hyperosmotic stress stimulates free radical generation in cultured cardiomyocytes and also activates the protective NFκB pathway to attenuate apoptosis. FONDECYT 1010246, FONDAP 15010006, Chilean Society for Cardiology Grant, PG106 UCH. VE, AC and CQ are CONICYT fellows.

when XO activity reached a pick. Using Allopurinol, specific inhibitor of XO activity, we obtained a reduction of the apoptotic process, evidenced by TUNEL and DNA fragmentation. JNK and p38, stress activated protein kinases involved in apoptosis, were phosphorylated during weaning. The expression of p53, a proapoptotic gene, was induced too. In conclusion, high XO activity may be one of the triggers in the apoptosis process by increasing ROS production and controlling the p53 expression. XO maintains apoptosis by inducing RNS production and controlling p38 phosphorylation.