Transient exposure to extracellular hydrogen peroxide is associated with a persistent increase in intracellular calcium and superoxide from the mitochondria in ventricular myocytes without apoptosis

H.M. Viola, P.G. Arthur and L.C. Hool, School of Biomedical Biomolecular and Chemical Sciences, The University of Western Australia, WA, Australia.

We examined the effects of a transient hydrogen peroxide (H_2O_2) exposure on myocyte function at a concentration insufficient to cause apoptosis or necrosis. Myocytes were exposed to 30 μM H_2O_2 for 5 min followed by 10U/ml catalase for 5 min to degrade the H_2O_2. Cellular superoxide was measured using dihydroethidium (DHE). Exposure to H_2O_2 caused a 66.1% increase in DHE signal (n=45, $p<0.05$) compared to controls exposed to catalase only (n=8) without activation of caspase 3 or evidence of necrosis. The increase in DHE was attenuated when cells were exposed to the mitochondrial inhibitor myxothiazol (7nM, n=14) and when calcium uptake by the mitochondria was inhibited with 2μM Ru360 (n=5). We investigated the L-type Ca^{2+} channel ($I_{Ca\text{-}L}$) as a source of calcium influx. The increase in superoxide could be attenuated when $I_{Ca\text{-}L}$ was inhibited with 2 μM nisoldipine (n=9). Basal channel activity was significantly increased from 5.4pA/pF (n=7) to 8.9pA/pF (n=25) after H_2O_2. The response of the channel to β-adrenergic receptor stimulation was used as a functional reporter for changes in cellular production of reactive oxygen species since a decrease in cellular H_2O_2 is associated with altered sensitivity of the channel to β-adrenergic receptor stimulation. After H_2O_2, the $K_{0.5}$ for activation of the channel by isoproterenol was significantly increased from 5.8 to 27.8nM. This effect and the increase in basal activity persisted for several hours after H_2O_2. In addition, intracellular calcium was persistently elevated with a two fold increase from a resting calcium of 24nM (n=5, $p<0.05$). We propose that extracellular H_2O_2 is associated with an increase in mitochondrial-derived superoxide via calcium influx from $I_{Ca\text{-}L}$. The effect persists because a positive feedback exists between increased basal channel activity, elevated intracellular calcium and superoxide production by the mitochondria. This may represent a mechanism for cardiovascular pathology that involves elevated calcium and reactive oxygen species.