Identifying the site of the source of reactive oxygen species within the mitochondria after transient exposure of cardiac myocytes to hydrogen peroxide

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Oxidative stress is a feature of cardiovascular disease and hydrogen peroxide (H_2O_2) can act as a signaling molecule to mediate cardiovascular pathology. We have previously demonstrated that transient exposure of ventricular myocytes to H_2O_2 leads to a further increase in reactive oxygen species (ROS) from the mitochondria (supporting the "ROS-induced ROS-release" hypothesis). Exposure of cardiac myocytes to 30μM H_2O_2 for 5 min followed by 10U/ml catalase for 5 min to degrade the H_2O_2 caused a 65.4 ± 8.4% further increase in superoxide by the mitochondria (n = 47) (Viola *et al.*, 2007). NADPH-oxidase, xanthine oxidase and nitric oxide did not contribute to the increase in superoxide. We tested whether a transient exposure to H_2O_2 altered protein synthesis in the myocytes. Ventricular myocytes were isolated from hearts excised from anesthetised guinea pigs. We found that 5 min exposure to 30μM H_2O_2 followed by 10U/ml catalase for 5 min caused a two fold increase in protein synthesis measured as ^{3}H-Leucine incorporation (n = 10). This suggests that a transient exposure to H_2O_2 may be sufficient to induce cardiac hypertrophy. We wished to identify the site of the source of ROS in the mitochondria. Previous studies have shown the main source of ROS production by the mitochondria occurs via complex III although complex I may also be a source of ROS production (Turrens, 1997; St-Pierre *et al.*, 2002; Muller *et al.*, 2003; Turrens, 2003). We exposed myocytes to 1μM DPI, which binds just prior to the ROS generation site of complex I, followed by 30μM H_2O_2 for 5 min and 10U/ml catalase for 5 min. Superoxide was assessed with the fluorescent indicator dihydroethidium (DHE). The presence of DPI completely attenuated the increase in DHE after exposure to H_2O_2. We also exposed guinea pig cardiac myocytes to 1μM rotenone, which binds just after the ROS generation site of complex I, followed by 30μM H_2O_2 for 5 min and 10U/ml catalase for 5 min. The presence of rotenone attenuated the increase in DHE after exposure to H_2O_2 by 45%. These data suggest that the source of production of ROS is distal to complex I. Identifying the site of production of ROS may represent a possible therapeutic target to prevent the development of cardiac hypertrophy associated with a transient exposure to H_2O_2.

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