Evidence for Q_O site of mitochondrial complex III as the source of increased production of superoxide in cardiac myocytes after transient exposure to hydrogen peroxide

H.M. Viola,[1] E. Ingley[2] and L.C. Hool,[1] [1]School of Biomedical Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009, Australia and [2]The Western Australian Institute for Medical Research, Nedlands, WA 6009, Australia.

Oxidative stress is a feature of cardiovascular disease. We have previously shown that exposure of adult guinea-pig ventricular myocytes to 30μM hydrogen peroxide (H_2O_2) for 5min results in increased mitochondrial superoxide production. This causes a 2-fold increase in protein synthesis, suggesting transient exposure to H_2O_2 may be sufficient to induce cardiac hypertrophy in cardiac myocytes. Previous results suggested the source of superoxide was distal to complex I. We performed additional experiments to further explore the site of superoxide production. We exposed myocytes to 7nM myxothiazol that binds at complex III Q_O ROS generation site and examined superoxide generation assessed as changes in dihydroethidium (DHE) fluorescence after exposing myocytes to 30μM H_2O_2 for 5min then 10U/ml catalase for 5min. Myxothiazol completely attenuated the increase in DHE signal (n=16, $p<0.05$). In addition 7nM stigmatellin that also binds at complex III Q_O ROS generation site attenuated the DHE signal 63% (n=5, $p<0.05$). However, exposing myocytes to 7nM antimycin A that binds at complex III Q_i ROS generation site did not alter the DHE signal after exposure to 30μM H_2O_2. These data suggest the source of ROS production after transient exposure to H_2O_2 is the Q_O site of complex III. We have confirmed the results by assessing changes in DHE fluorescence in the myocytes in the presence of mitochondrial complex substrates administered *via* the patch-pipette. Complex III may represent a possible site to target in the prevention of the development of cardiac hypertrophy associated with oxidative stress.