

P052 Differential use of calcium stores in Reactive Oxygen Species (ROS)- dependent pancreatic beta-cell death
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Beta cells' susceptibility to oxidant stress contributes to chronic glucose toxicity and beta cell loss in diabetes mellitus. We are currently investigating how reactive oxidant species (ROS) modulate Ca^{2+} movements, since intracellular Ca^{2+} increases promote cell death. The aim of this study was therefore to investigate the functional role of oxidant-induced mitochondrial Ca^{2+} uptake and intracellular Ca^{2+} movements in response to both hydrogen peroxide (H_2O_2) and superoxide, the latter generated by the hypoxanthine/xanthine oxidase (HX/XO) system, in RINm5F cells. The dependency of oxidant-induced cell death on intracellular Ca^{2+} was confirmed using the intracellular Ca^{2+} chelator BAPTA, which completely attenuated cell death in response to both ROS. Blockade of mitochondrial Ca^{2+} uptake by the mitochondrial calcium uniporter inhibitor, ruthenium red (RR) attenuated H_2O_2 's effect by 34% suggesting mitochondrial Ca^{2+} uptake is a necessary step in H_2O_2 -induced cell death. By contrast, superoxide's cytotoxicity was found not to be dependent on mitochondrial Ca^{2+} uptake since inhibition with RR exaggerated the effect of superoxide. We then investigated the role of extracellular influx on superoxide's effect using the extracellular Ca^{2+} chelator, EDTA and the L-type channel blocker, nifedipine. Both agents completely attenuated superoxide's effect although no effect was seen on H_2O_2 -induced cell death. It is tempting to speculate that H_2O_2 facilitates store-operated Ca^{2+} release allowing uptake through the low affinity mitochondrial uniporter whereas superoxide participates predominantly in extracellular Ca^{2+} influx.