A hydrogen peroxide insult causes sustained alteration in the sensitivity of the L-type Ca^{2+} channel to β-adrenergic receptor stimulation in ventricular myocytes

L.C. Hool, H.M. Viola and P.G. Arthur, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley, WA 6009, Australia.

We have shown previously that mitochondrial-derived hydrogen peroxide (H_2O_2) regulates the function of the L-type Ca^{2+} channel. A decrease in mitochondrial-derived H_2O_2 is associated with an increase in the sensitivity of the channel to the beta-adrenergic receptor agonist isoproterenol (Iso) and exposing myocytes to H_2O_2 attenuates the response. Here we examine the effect of a hydrogen peroxide insult on the function of the L-type Ca^{2+} channel. Ventricular myocytes were isolated from hearts excised from anaesthetised guinea-pigs as approved by the Animal Ethics Committee of The University of Western Australia and in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC). The cells 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, and then the response of the channel to Iso was examined. In the absence of a peroxide insult, 10 nM Iso elicited a current that was 72.1±8.0% of the current elicited by 1 μM Iso (a maximally stimulating concentration of the agonist) within the same cell (n=6). However, after exposure of cells to peroxide 10 nM Iso elicited a current that was just 18.6 ± 10.0 % of the response elicited by 1 μM Iso within the same cell (n=6; $P<0.05$) suggesting that the peroxide insult significantly decreased the sensitivity of the channel to Iso. More importantly this effect persisted for several hours after the peroxide insult. We examined whether the effect was a result of enhanced production of reactive oxygen species by the cell. Cellular production of superoxide was measured using the fluorescent indicator dihydroethidium. Exposing cells to 30 μM H_2O_2 for 5 min followed by 10U/ml catalase for 5 min caused a 61.1±14.0% increase in superoxide production (n=13; $P<0.05$) compared to controls exposed to catalase only (n=8). The increase in superoxide was completely attenuated when cells were exposed to the mitochondrial inhibitor myxothiazol (6-10 nM; n=14; $P<0.05$). The NAD(P)H oxidase inhibitor apocynin (300 μM, n=5) did not alter the increase in superoxide associated with a peroxide insult nor did 100 μM of the xanthine oxidase inhibitor, allopurinol (n=5). We propose that a hydrogen peroxide insult causes a further increase in hydrogen peroxide production from the mitochondria and the increase in peroxide results in a sustained decrease in sensitivity of the channel to β-adrenergic receptor stimulation.