

## Reactive oxygen species generated from the mitochondria and not NAD(P)H-oxidase regulate L-type $\text{Ca}^{2+}$ channel function during acute hypoxia in ventricular myocytes

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

Hypoxia and the thiol-reducing agent dithiothreitol increase the sensitivity of the L-type  $\text{Ca}^{2+}$  channel ( $I_{\text{Ca-L}}$ ) to  $\beta$ -adrenergic receptor stimulation. We examined whether NAD(P)H-oxidase regulates cellular production of reactive oxygen species (ROS) and the function of  $I_{\text{Ca-L}}$  during hypoxia. 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 patch-clamped and current was recorded during exposure to the classic NAD(P)H-oxidase inhibitors apocynin or diphenyleneiodonium (DPI) and increasing concentrations of isoproterenol (Iso). DPI caused an increase in the sensitivity of the channel to Iso similar to that of hypoxia, but apocynin did not. In contrast, the  $\text{K}_{0.5}$  for activation of the channel by Iso in the presence of AngII, a potent agonist of NAD(P)H-oxidase during hypoxia was  $1.7 \pm 0.4$  nM which was similar to the  $\text{K}_{0.5}$  determined during hypoxia alone ( $1.6 \pm 1.1$  nM; NS). We measured cellular production of superoxide anion ( $\text{O}_2^-$ ) using the fluorescent indicator dihydroethidium. Hypoxia was associated with a  $41.2 \pm 5.2$  % decrease in  $\text{O}_2^-$  ( $n=21$ ;  $P<0.05$ ). In addition, DPI caused a  $21.3 \pm 4.7$  % decrease in  $\text{O}_2^-$  ( $n=16$ ;  $P<0.05$ ). However,  $\text{O}_2^-$  did not increase when cells were exposed to AngII during hypoxia ( $n=24$ ) or in room oxygen ( $n=6$ ). When mitochondria were partially uncoupled with FCCP, there was a  $31.3 \pm 4.5$  % decrease in  $\text{O}_2^-$  ( $n=23$ ;  $P<0.05$ ) and a significant increase in the sensitivity of  $I_{\text{Ca-L}}$  to Iso similar to that of hypoxia ( $n=7$ ). Accounting for the effect of DPI on  $I_{\text{Ca-L}}$ ,  $10 \mu\text{M}$  DPI caused a  $67.2 \pm 7.3$  % decrease in oxygen consumption ( $n=6$ ;  $P<0.01$ ) and  $28.8 \pm 2.1$  % decrease in  $\text{O}_2^-$  in isolated mitochondria ( $n=4$ ;  $P<0.05$ ) indicating that DPI is not a specific inhibitor of NAD(P)H-oxidase function. Hypoxia decreased  $\text{O}_2^-$  by  $69.3 \pm 0.8$  % in isolated mitochondria ( $n=4$ ;  $P<0.01$ ). We conclude that a decrease in ROS generated from the mitochondria and not NAD(P)H-oxidase regulates channel function during hypoxia.