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A Role For The Sodium Pump In Hydrogen Peroxide-Induced Relaxation In The Porcine Isolated Coronary Artery

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Hydrogen peroxide (H_2O_2) has been proposed to act as an endothelium-derived hyperpolarising factor (EDHF) in human, rat, mouse, porcine and canine arteries (Shimokawa, 2010 *Pflugers Arch* **459**(6)**:**915-922, Wheal *et. al.*, 2012 *Eur J Pharmacol* **674**(2-3)**:**384-390). In disease states, EDHF may act as a 'back up' system to compensate the loss of the nitric oxide pathway (Luksha *et al.*, 2009 *Atherosclerosis* **202**(2)**:**330-344). Here, the mechanism of action of exogenous H_2O_2 in porcine coronary arteries (PCAs) isolated from female pigs and the role of H_2O_2 as an EDHF in the cardiovascular system were investigated. Distal PCAs were mounted in a wire myograph, pre-contracted with U46619 (2nM-50µM), a thromboxane A_2 mimetic or KCl (60mM) to examine the role of H_2O_2 as an EDHF. Concentration-response curves to H_2O_2 (10nM-1mM), bradykinin (0.01nM-1µM), sodium nitroprusside (SNP) (10nM-10µM) or verapamil (1nM-10µM) were constructed. L-NAME (300µM) and indomethacin (10µM) were used to inhibit the synthesis of NO and prostanoids respectively. PEG-catalase (300Uml^{-1}) was used to breakdown H_2O_2 and carbenoxolone (100µM), a gap junction inhibitor was used to study the role of gap junctions. Tetraethylammonium (TEA) (10mM) was used as a non-selective potassium (K^+) channel inhibitor and glibenclamide (1µM) was used as an ATP-sensitive K^+ channel inhibitor. Apamin (500nM), TRAM-34 (10µM) and iberiotoxin (100nM), small, intermediate and large-calcium activated K^+ channels inhibitors respectively were used to examine the role of K^+ channels in the H_2O_2–induced relaxation. Ouabain (500nM) was used to inhibit the sodium-potassium pump activity and 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10µM) was used to selectively block guanylyl cyclase activity. Data were analysed using 2-tailed, paired Student's t-test or one-way ANOVA, followed by Bonferroni's *post hoc* test. H_2O_2 caused concentration-dependent relaxations with a maximum relaxation (R_{max}) of 99.8±11.5% (mean±SEM), pEC_{50}=4.17±0.15, n=4) and removal of endothelium (R_{max}=97.8±3.5%,pEC_{50}=3.96± 0.03,n=5) produced a comparable curve to the control supporting previous report that H_2O_2–induced relaxation is endothelium-independent. Addition of L-NAME and indomethacin (R_{max}=87.5±2.7%, pEC_{50}=7.6±0.05,n=5), in the absence or presence of carbenoxolone (R_{max}=94.4±8.0%,pEC_{50}=4.08± 0.10,n=4), ODQ (R_{max}=127±25%,pEC_{50}=3.7±0.2,n=4), glibenclamide (Relaxation at 1mM H_2O_2 (R)=97.7±2.1%,pEC_{50}=4.1±0.04,n=6), barium (R=103.9± 3.8%,n=5), iberiotoxin (R_{max} =119±16%, pEC_{50}=3.8±0.2,n=4), TRAM-34 (R_{max}=99.7±6.8%,pEC_{50} =3.8±0.07,n=6) or apamin (R_{max}=105±6.9%, pEC_{50}=3.9±0.06,n=6) had no effect on the H_2O_2–induced relaxation. TEA caused a 2.5-fold shift in the H_2O_2-induced relaxation ($P<0.05$) while high extracellular K^+ (R=40.8±8.5%,n=5) or ouabain (R=47.5±8.6%,n=6) significantly inhibited the H_2O_2-induced relaxation ($P<0.05$). PEG-catalase significantly inhibited the relaxation at higher concentration of H_2O_2 (0.1–1.0mM) ($P<0.05$). Ouabain (Relaxation at 1µM of bradykinin R=8.88±3.31%,n=7) significantly inhibited the bradykinin-induced relaxation ($P<0.05$) in the presence (R=70.1±10.8%,n=7) and absence (R=100.7±0.5%, n=7) of L-NAME and indomethacin. Ouabain (n=6) caused a significant rightward shift (20-fold) in the concentration-response curve to SNP. Ouabain had no effect in the verapamil-induced relaxation (n=6). In conclusion, the relaxation induced by H_2O_2 is primarily mediated via ouabain-sensitive sodium-potassium pumps in the porcine coronary artery.