Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol11Issue3abst045P.pdf

A comparison of protection against oxidative stress and endothelial function in female and male porcine coronary arteries.

PS Wong, MD Randall, RE Roberts. University of Nottingham, Nottingham, UK

A previous study reported that healthy young men have greater oxidative stress compared to premenopausal women (Ide *et al., Arterioscler Thromb Vasc Biol* **22**(3)**:** 438-442, 2002) and a different study in mitochondria from rats found that females expressed a higher level of antioxidant gene compared to male rats (Borras et al., *Free Radic Biol Med* **34**(5)**:** 546-552, 2003). Oxidative stress in endothelial cells could be contributed by reactive oxygen species catalysed by NADPH oxidases (Nox) (Altenhofer e*t al., Cell Mol Life Sci* **69**(14)**:** 2327-2343, 2012). We have previously reported sex differences in endothelial function specifically in the Endothelium-Derived Hyperpolarisation (EDH)-mediated vasorelaxation in porcine isolated coronary arteries (PCAs) (Wong *et al.*, http://www.pa2online.org/abstract/abstract.jsp?abid=30846, 2012). EDH is defined as the proportion of vasorelaxation which is insensitive to NO synthase inhibition and cyclooxygenase inhibition. Therefore, in this study, we compared the effects of Nox inhibitors on endothelium-dependent vasorelaxation in PCAs from male and female pigs (Large white hybrids pig, 4-6 months old, weighing ~ 50kg).

Distal PCAs were mounted in a wire myograph and pre-contracted with U46619 (5nM-65nM), a thromboxane A_2 mimetic. Concentration-response curves to bradykinin (0.01nM-1µM), an endothelium dependent relaxant, or forskolin (0.1nM-1µM), a cell permeable adenylyl cyclase activator were constructed in the presence of various inhibitors. L-NAME (300µM) and indomethacin (10µM) were used to inhibit the synthesis of NO and prostanoids respectively. Relaxation responses were carried out in the absence or presence of diphenyleneiodomium chloride (DPI) (10µM), a non-selective Nox inhibitor or 2-Acetylphenothiazine (ML-171) (10µM or 100µM), a selective Nox1 inhibitor. R_{max} (maximum relaxation) and pEC_{50} were analysed using 2-tailed, paired Student's t-test to compare differences between 2 groups. In 3 or more groups, one-way ANOVA was used and significant differences between groups were detected by Bonferroni's *post hoc* test.

The presence of ML-171 (10µM or 100µM) or DPI had no effect on the bradykinin-induced vasorelaxation in PCAs from female pigs, whereas in males, DPI significantly shifted the EC_{50} 2.8-fold to the right from pEC_{50}=8.00±0.07 (n=6) to pEC_{50}=7.55±0.08 (n=6) ($p<0.05$, one-way ANOVA followed by Bonferroni's *post hoc* test). Similarly, in the presence of L-NAME and indomethacin, DPI and ML-171 had no effect on the bradykinin-induced vasorelaxation in PCAs from female pigs. Conversely, in PCAs from male pigs, presence of L-NAME and indomethacin with DPI or ML-171 significantly shifted the EC_{50} 2.5-fold and 3.2-fold to the left respectively (n=5, $p<0.05$, one-way ANOVA followed by Bonferroni's *post hoc* test). In the presence of L-NAME and indomethacin, ML-171 had no effect on the forskolin-induced vasorelaxation. In summary, inhibition of NADPH oxidases enhances the EDH-mediated response in PCAs from male but not female pigs. This

could indicate that there is an increased Nox activity in males leading to reduced endothelium-dependent vasorelaxation, and this may underlie the greater oxidative stress observed in men.