

Sex differences in the regulation of porcine coronary artery tone by perivascular adipose tissue: role of adiponectin

Perivascular adipose tissue (PVAT) exerts complex effects on vascular tone with more than one factor released from PVAT stimulating contraction or relaxation of blood vessels (1). We and others have demonstrated clear sex differences in the regulation of vascular tone through, for example, release of endothelium-derived factors (2). No comparisons have been made between males and females in the regulation of vascular tone by PVAT. Therefore, the aim of this present study was to determine whether there are sex differences in PVAT-mediated regulation of the porcine coronary artery (PCA) tone.

Changes in tone in isolated coronary arteries with or without PVAT were recorded in an isometric tension recording system in the absence and presence of a range of putative inhibitors. Western blot was performed to examine the expression of adiponectin in PVAT. The level of adiponectin release from PVAT was measured using ELISA. R_{max} and pEC_{50} values were analysed using a 2-tailed, paired or unpaired Student's t-test to compare differences between 2 groups. Differences between 3 or more groups were assessed using a one-way ANOVA or two-way ANOVA in conjunction with the Sidak's post-hoc test to assess possible difference at individual concentrations.

In the presence of adherent PVAT, contractions to the thromboxane mimetic (U46619) (1nM-300nM) and endothelin-1 (1nM-300nM) were significantly reduced in PCAs from females, but not males (For U46619: $R_{max} = 82 \pm 4.8\%$ of the KCl response in the presence of fat compared to $106 \pm 8\%$ in the absence of fat, $p < 0.01$; for endothelin-1: $R_{max} = 76.5 \pm 10.2\%$ in the presence of fat compared to controls $114 \pm 18\%$, $P < 0.05$). In PCAs pre-contracted with U46619, re-addition of (0.3g) PVAT caused relaxation in PCAs from females (with PVAT: $R = 25.3 \pm 5.1\%$ compared to time control $R = 5.8 \pm 1.4\%$, $P < 0.01$), but not males (time control: $R = 4.4 \pm 0.6\%$; with PVAT: $R = 10.1 \pm 4.6\%$). This relaxant response in females was inhibited by a combination of both NO synthase inhibitor (L-NAME) (300 μ M) and the cyclooxygenase inhibitor indomethacin (10 μ M). Incubation with either L-NAME or indomethacin alone did not inhibit PVAT-derived vasorelaxation in comparison to using both inhibitors. Pre-incubation with anti-adiponectin antibody abolished the relaxant effect of PVAT. There was no difference in either expression or release of adiponectin from PVAT between both sexes. On the other hand, the adiponectin receptor agonist adipoRon (1-100 μ M) produced a greater relaxation in PCAs from females compared to males (at 100 μ M, females: $106.3 \pm 8\%$ relaxation; males: $77.8 \pm 5.8\%$ relaxation).

In summary, these findings demonstrate a clear sex difference in the regulation of coronary artery tone by PVAT, with adiponectin underlying the anticontractile effects in PCAs from females.

(1) Szasz, T et al. (2013). *Vasc Health Risk Manag* **9**: 105-116.

(2) Wong, et al. (2014). *British Journal of Pharmacology* **171**:2751–2766