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Effects of perivascular fat on endothelium-dependent relaxation in young healthy rats

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Vasoactive properties of many endogenous and exogenous substances are often tested in isolated arteries that are cleared of adherent tissues including fat. However, recent evidence suggests that perivascular adipose tissues (PVAT) regulate vascular tone by releasing relaxant factors (1) and changes in PVAT function might be linked to endothelial dysfunction in disease states (2). In this study, we investigated the effects of PVAT on endothelium-dependent relaxation in aortic rings from young healthy rats.

Male Wistar rats (10-12 weeks) were killed by cervical dislocation and thoracic aortae were isolated and mounted in a 15ml organ bath for isometric tension recording. For each aorta, segments with (+PVAT) and without (-PVAT) periadventitial fat were obtained. Unless otherwise stated, vessels were contracted by 10μM methoxamine before cumulative additions of carbachol (1nM-10μM), sodium nitroprusside (0.1nM-1μM), GSK1016790A (1-100nM) or $CaCl_2$ (1.5-5mM). Rmax represents % relaxation of precontracted tone attained at the maximum concentration used, and pEC50 values were determined from log concentration-response curves. Data are shown as mean±sem (n=4-7) and analysed by two-way analysis of variance, followed by a Bonferroni post-hoc test or Student's t-test, where appropriate.

PVAT had no significant effects on contractions induced by 10μM methoxamine (-PVAT: 11±2mN; +PVAT: 12±1mN) or 60mM KCl (-PVAT: 16±2mN; +PVAT: 14±2mN), but greatly reduced endothelium-dependent relaxations to the muscarinic receptor agonist, carbachol (-PVAT: pEC50=6.8±0.2, Rmax=92±9%; +PVAT: pEC50=6.0±0.3, Rmax=52±15%; P<0.01). Inhibition of nitric oxide synthase by L-NAME (300μM for 30min) similarly reduced the carbachol response, but the additional presence of PVAT abolished any residual relaxation (+L-NAME-PVAT: pEC50=5.8±0.2, Rmax=42±9%; +L-NAME+PVAT: Rmax=-14±6%; P<0.01). PVAT also attenuated relaxation to the selective agonist of TRPV4 receptors, GSK1016790A (GSK) (-PVAT: pEC50=8.0±0.1, Rmax=56±10%; +PVAT: pEC50=7.7±0.2, Rmax=44±14%; P<0.01). This response was abolished by endothelium removal (-PVAT-endothelium, at 10nM GSK: 2±8%; P<0.01), L-NAME (-PVAT+L-NAME, at 10nM GSK: 4±11%; P<0.01) or TRPV4 antagonists (at 10nM GSK, -PVAT+1μM HC067047 for 30min: 9±10%; -PVAT+10μM ruthenium red for 30min: -17±10%, P<0.01 for both). Moreover, endothelium-dependent relaxation to increasing extracellular Ca^{2+} was greatly inhibited by PVAT (-PVAT: pEC50=2.50±0.1, Rmax=75±8%; +PVAT: pEC50=2.4±0.1, Rmax=27±13%; P<0.01). A similar extent of inhibition was also seen with endothelium removal or the antagonist for extracellular Ca^{2+}-sensing G-protein-coupled receptors, calhex231 (3μM for 30min) (data not shown). Importantly, PVAT had no significant effect on endothelium-independent relaxations to sodium nitroprusside (data not shown).

These data suggest that PVAT impairs endothelium-dependent relaxation elicited by distinct receptors. Further investigations are needed to elucidate the mechanisms underlying this phenomenon.

(1) Gollasch M (2012). *Br J Pharmacol* **165**: 633-642.

(2) Ma L *et al* (2010). *Hypertens Res* **35**: 446-453.