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No role for hydrogen sulphide in the anticontractile effect of perivascular adipose tissue in porcine coronary artery.

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Introduction: Hydrogen sulphide (H_2S) has been described as a novel candidate for an adipose derived relaxing factor (ADRF) in rats and mice[1]. However, there are controversial findings in the expression and function of H_2S synthesizing enzymes in different species[2]. We have previously demonstrated that perivascular adipose tissue (PVAT) inhibits thromboxane receptor-mediated contractions in the porcine coronary artery (PCA)[3]. Therefore, the aim of this study was to determine the role of H_2S in PVAT-induced vasorelaxation of the porcine coronary arterial (PCA) tone.

Method: Western immunoblotting was performed to investigate the expression of the different H_2S synthesizing enzymes (CSE, CBS and 3-MPST) in PVAT. Contractile responses to the thromboxane mimetic U46619 in segments of porcine coronary artery with and without PVAT were recorded in an isometric tension recording system. H_2S synthesizing enzymes activity in PVAT was estimated using SF7-AM fluorescence assay. A Student's 2-tailed paired t-test was used to analyse the data. Data are expressed as mean±S.E.M.

Results: Western immunoblotting showed that CBS and 3-MPST are expressed in PVAT whilst no CSE expression was identified (n=8). Contractions to the thromboxane mimetic U46619 were reduced in the presence of PVAT (Rmax = 82± 5% with PVAT vs Rmax = 106± 8% of vessels lacking PVAT ($P<0.01$)). Besides, log EC50% was also significantly reduced in the rings with fat attached (pEC50 = 7.9 ± 0.06) compared to control (pEC50 =8.2± 0.04) ($P<0.005$) (n=12). Pre-incubation with the hydrogen sulphide enzyme inhibitors 4-propargylglycine (PPG) (10µM) and 2-(aminooxy)-acetic acid (AOAA) (100µM) did not prevent this anticontractile response to PVAT (n=6). Assessment of H_2S synthesis using the fluorescent tag SF7 demonstrated CBS activity in PVAT, which was inhibited by AOAA (5.6±0.15 fluorescence units in control vs 5.2±0.1 with AOAA, n=5). 3-MPST activity was also detected in PVAT (61.6±5.7 fluorescence units, n=5). However, the activities of these enzymes in PVAT were much lower in comparison with the activities of the same enzymes in rat liver (CBS, 26.5 control vs 5.0 with AOAA; 3-MPST, 138.6).

Conclusion: H_2S can be synthesised in PVAT in the PCA through CBS and 3-MPST, but has no role in the anti-contractile effect of PVAT.

References:

1. Fang *et al.* (2009). *J Hypertens* **27**: 2174-85.
2. Kohn *et al.* (2012). *PLoS One* **7**: e41951.
3. Ahmad *et al.* (2017). *Br J Pharmacol* **16**:2773-2783.