Thesis title: **Expression and roles of sympathetic and sensory nerves in perivascular adipose tissue**

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Abstract

There is a call to elucidate the poorly understood link between perivascular adipose tissue (PVAT) and nervous systems controlling the vasculature. The principle aims of this study were to investigate the expression of sympathetic and sensory nerves in PVAT of rat mesenteric arteries, to characterize their role(s) in vascular tone and to investigate the interaction between these nervous systems and PVAT-derived vasoactive compounds. Immunofluorescence staining was employed to investigate the localisation of sympathetic and sensory nerves in mesenteric arteries. Rat mesenteric arterial beds (MABs; with and without PVAT, removed by careful dissection) were perfused with Krebs'-Henseleit buffer and changes in pressure recorded, or different sized rat mesenteric arteries (MA), rat abdominal aortas, porcine splenic arteries (PSA) and porcine coronary arteries (PCA) (with and without PVAT, removed by careful dissection) were isolated and set up for isometric recording. Responses to electrical field stimulation (EFS) were obtained under basal and raised tone conditions induced by methoxamine, an α1-adrenoceptor agonist, in the presence and absence of agonists/antagonists. Enzyme immunoassay (EIA) was conducted to quantify capsaicin-evoked calcitonin gene-related peptide (CGRP) release of different mesenteric arterial segments and the dissected PVAT. A multiplex assay was carried out to investigate the link between sympathetic and sensory nerves and PVAT-derived vasoactive compounds.

Immunofluorescence showed the presence of both tyrosine hydroxylase (TH; the rate-limiting enzyme of catecholamine biosynthesis)-immunoreactive and CGRP (the principal neurotransmitter for sensory nerves)-immunoreactive nerves at the adventitia and within PVAT. EFS elicited frequency-dependent vasoconstriction of the mesenteric beds. These responses were abolished by guanethidine, a sympathetic nerve blocker, indicating an involvement of sympathetic nerves. In the absence of PVAT, neurogenic contractile responses were markedly attenuated. There was no significant difference in concentration-dependent contractions to methoxamine in preparations with and without PVAT, thus suggesting that vascular smooth muscle remains intact in PVAT-denuded preparations. Contractile responses to EFS were significantly decreased after Krebs'-Henseleit solution containing PVAT was transferred to preparation without PVAT, indicating PVAT contains transferrable factor/s. Incubation with candesartan, but not losartan, angiotensin II receptor inhibitors, in the presence of PVAT significantly attenuated EFS-evoked contraction, indicating a potential involvement of PVAT-derived angiotensin II in PVAT-enhanced neurogenic contractile response. In PSA, removal of PVAT significantly attenuated EFS-evoked contractile responses. Exogenous methyl palmitate had no effect on sympathetic neurogenic contractions while exogenous apelin-13 reduced sympathetic responses in both PVAT-intact and PVAT-denuded PSAs.

EFS of PVAT-intact MABs in the presence of guanethidine and methoxamine elicited frequency-dependent vasodilatation due to stimulation of sensory nerves. Neurogenic vasodilatation was abolished in preparations with PVAT removed. In contrast, dose-dependent vasodilator responses to capsaicin, an agonist at vanilloid receptor subtype 1 (TRPV1), and exogenous CGRP, were comparable between PVAT-intact and PVAT-denuded preparations. This suggests that the PVAT removal procedures did not damage vascular smooth muscle relaxation in PVAT-denuded preparations. Myography experiments revealed that EFS-evoked vasodilator responses were greater in PVAT-intact preparations than in PVAT-denuded preparations in both MA and second order MA (2OMA) segments. EIA indicated that CGRP release was greater in dissected PVAT with

capsaicin compared to dissected PVAT without capsaicin in 2OMA preparations, which further supports the concept of the presence of sensory nerves in PVAT of MABs.

Sodium sulfide (Na_2S), a hydrogen sulphide (H_2S) donor, caused concentration-dependent vasodilation and this effect was attenuated by incubation with HC030031, a TRPA1 antagonist, and pre-treatment with capsaicin. The vasodilator response was greatly attenuated in the second response curve, indicating the involvement of desensitization mechanism. EFS elicited frequency-dependent vasodilatation due to stimulation of sensory nerves but these responses were attenuated in the presence of Na_2S. Incubation with H_2S-synthesizing enzyme inhibitors, DL-propargylglycine, aminooxyacetic-acid and aspartate, had no significant effect on EFS-evoked neurogenic vasodilatation. The presence of PVAT enhanced leptin release under normal oxygenation (95 % O_2 and 5 % CO_2), while gassing with 95 % N_2 and 5 % CO_2 enhanced interleukin-6. Leptin release was enhanced during EFS of sympathetic nerves under low oxygen level and EFS of sensory nerves under normal oxygen level in the presence of PVAT.

In conclusion, the present study provides clear evidence for the expression of sympathetic and sensory nerves within PVAT of mesenteric arteries and shows these nerves contribute to the regulation of vascular tone. H_2S causes vasodilatation of MABs by activating sensory nerves through the TRPA1 signalling pathway, and subsequently impairs sensory nerve function, demonstrating a capsaicin-like action. H_2S-producing enzymes and endogenous H_2S are not involved in EFS-evoked neurogenic vasodilator responses under the conditions of the present study. Activation of sympathetic and sensory nerves in PVAT can modify PVAT-derived mediator(s) release. Collectively, these data show that sympathetic and sensory nerves are expressed in PVAT and have functional roles in modulation of vascular contractility and PVAT-derived mediator release.