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Expression of chemerin receptors GPR1 and CMKLR1 in human vascular tissues and evidence for chemerin as a novel vasoconstrictor.

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Introduction: Targeting G-protein coupled receptors (GPCRs) has made significant advances in cardiovascular disease treatment, but there is still no cure. Orphan GPCRs with unknown functions could prove to be future drug targets. Hypertension is a risk factor for coronary artery disease and the single most important cause of stroke. Chemerin is a chemoattractant and the ligand for CMKLR1 via which it has roles in inflammation and vascular endothelial dysfunction. Chemerin has also been proposed as the ligand for orphan receptor GPR1, which is structurally homologous to CMKLR1. The BRIGHT study identified GPR1 as a candidate gene for essential hypertension and chemerin reportedly contracts rat aorta via CMKLR1. Therefore we hypothesise that chemerin has a role in human vascular reactivity through one or both of these receptors.

Aim: The aim of this study was to localise GPR1 and CMKLR1 in the human vasculature, and explore the function of these receptors.

Methods: Saturation binding of [^{125}I]-Chemerin(149-157) to GPR1-transfected CHO-K1 cells was carried out for 1 hour at RT. β-Arrestin recruitment assays (DiscoveRx) were performed with either GPR1- or CMKLR1-transfected CHO-K1 cells. Concentration response curves (CRCs) were constructed for chemerin peptides; full length (21-157); C13 (145-157) and C9 (149-157), to explore the structure activity relationship. CRCs were repeated in the presence of a small-molecule antagonist CCX832 (1) (3-3000nM). Vascular reactivity of C13 was investigated in human endothelium-denuded saphenous vein (hSV) in organ baths with increasing concentrations of chemerin (10^{-12}-10^{-6}M). The cellular distribution of both receptors was studied in tissue sections of human aorta, coronary and mammary artery, saphenous vein and cultured human aortic smooth muscle cells (SMCs) using immunofluourescence double staining. mRNA levels of each receptor were determined by qPCR analysis.

Results: [^{125}I]-C9 bound with a K_D=0.87±0.23nM and Hill slope of 1 (n=3). The chemerin peptides activated both receptors with nanomolar potency (Table 1) (n=3-15). CCX832 was confirmed to be CMKLR1-selective, pA_2=6.5. C13 contracted hSV, pD_2=10.69±0.18, n=18. In human vessels, (n=3) GPR1 and CMKLR1 localised to SMCs and endothelial cells. Both receptors were also visualised in cultured SMCs. qPCR analysis revealed that there was more CMKLR1 than GPR1 in whole vessels, but GPR1 was more abundant in cultured SMCs.

Table 1	**pD_2 values**	
	GPR1	**CMKLR1**
Full length	9.23±0.03	9.30±0.05
C13	9.11±0.14	7.18±0.05
C9	8.82±0.06	7.39±0.02

Conclusions: For the first time, this work reveals widespread expression of GPR1 and CMKLR1 in human vessels. Chemerin binds to both receptors, and is a novel potent constrictor of human saphenous vein. Finally confirmation that CCX832 is a selective CMKLR1 antagonist will allow further studies to delineate the relative role of each receptor in chemerin-mediated vasoconstriction.

(1) Graham, K. et al. (2013) WO 2013109543 A1