The in vivo and in vitro cardiovascular effects of C9, a C-terminal fragment of chemerin, in rats

Introduction: Chemerin is best known for its roles in chemotaxis, adipogenesis and inflammation through its receptor CMKLR1. Recently chemerin has emerged as a modulator of vascular tone in animal models. In rats, one study showed chemerin caused contraction of aorta, and in a further study, chemerin caused a potentiation of the response from other modulators of vascular tone, phenylephrine and endothelin-1. It was also found that chemerin reduced vasodilatation of acetylcholine on phenylephrine constricted vessels. In mice, chemerin was found to increase systolic blood pressure.

Aim: The aim of this study was to investigate the effect of chemerin in rats in vivo and identify the signalling pathways that contribute to the vascular response.

Methods: Male Sprague Dawley rats (220-265g) were anaesthetised (1.5% isofluorane, 1l/min oxygen) and a pressure/volume catheter was inserted into the left ventricle of the heart. A stable baseline pressure was measured prior to intravenous injection, through the jugular vein, of either chemerin peptide C9 (chemerin(145—157)) (1 mM) or saline control. Data were recorded using Powerlab, Chart 5 software and the difference in pressure was calculated between baseline and peak response. The cellular distribution of CMKLR1 was studied in tissue sections of rat aorta by immunohistochemistry. cAMP and β-arrestin recruitment assays were performed on CMKLR1 transfected CHO-K1 cells to investigate downstream signalling of the receptor in vitro. In both assays, concentration response curves (CRCs) were constructed for chemerin peptides: full length chemerin (21-157) and C9 (3nM-3μM). Finally, pharmacological experiments were carried out on rings of rat aorta (4mm) set up in organ baths to test the signalling mechanism on ex vivo tissue for comparison. CRCs to C9 were constructed to test the ability of C9 to contract. In further experiments, vessels were pre-constricted with U46619 (100nM) and relaxed back to baseline by stimulating cAMP production with adenylyl cyclase activator NKH477 (300nM) before adding CRCs to C9, to test the involvement of the G_i pathway in this response. The C9 response was expressed as a percentage of the maximum KCl (100mM) response of the vessel.

Results: C9 caused an increase in left ventricular systolic pressure of 16.4±3.34 mmHg (n=7). CMKLR1 was localised to the smooth muscle cells of rat aorta. In β-Arrestin recruitment assays full length chemerin was more potent (pD_2=9.3±0.1, n=6) than C9 (pD_2=7.2±0.1, n=8). In cAMP assays full length chemerin was less potent (pD_2=8.4±0.1, n=6) than C9 (pD_2=9.3±0.1, n=3). C9 caused contraction of rat aorta, pD_2=6.7±0.2, E_{max}=60.9±6.6. In vessels which had been pre-constricted and relaxed back to baseline through activation of adenylyl cyclase, C9 caused a potentiated response, pD_2=6.5±0.1, E_{max}=85.7±4.1.

Conclusions: These data support the emerging role of chemerin as a modulator of blood pressure in rat. This study identified for the first time that the shorter C-terminal fragment of chemerin caused a potent increase in blood pressure through the G_i signalling pathway. Intriguingly this peptide was relatively more potent towards the G-protein signalling pathway than full length chemerin, suggesting that there could be biased signalling, which warrants further investigation as it could be exploited in future drug design.