Impaired vascular responses to calcitonin gene-related peptide in the ageing αCGRP knockout mouse.

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The sensory neuropeptide calcitonin gene-related peptide (CGRP) is a potent microvascular vasodilator (Brain *et al*., 1985). CGRP receptors are comprised of the seven transmembrane Class B GPCR, termed the calcitonin-like receptor (CLR), a receptor activity-modifying protein 1 (RAMP1) molecule and a small intracellular protein, known as the receptor component protein (RCP). It has been shown in rats that vascular reactivity to CGRP diminishes with age (Chan and Fiscus, 2002) and that CGRP levels are reduced with advancing age (Yamaga *et al*., 2001). We sought to investigate how responsiveness to CGRP changes with age in αCGRP wild-type (WT) and knockout (KO) mice.

Male and female αCGRP WT and KO mice (15 months) were used for all experiments. Experiments were performed according to the Scientific Procedures Act 1986. Blood pressure measurements were taken *via* tail cuff plethysmography for one week. Mice were killed *via* induction of anaesthesia and subsequent cervical dislocation. Second-order mesenteric arteries were removed and mounted in a Mulvany-Halpern wire myograph containing Krebs buffer (37°C, gassed with 95% air / 5% CO_2). Cumulative concentration response curves were constructed to the α_1-adrenoceptor agonist, phenylephrine (100nM – 100μM). Following this, vessels were preconstricted with U46619 and increasing doses of CGRP were added (1nM – 100nM). mRNA expression of CGRP receptor constituents were analysed in aortic tissue. Briefly, mRNA was extracted and reverse-transcribed into cDNA using commercially-available kits. Specific primers were designed against CLR, RAMP1 and RCP and RTqPCR was performed on aortic samples using the SybrGreen assay. Raw data were normalised to reference genes and data were expressed as copies per microlitre. All data are expressed as mean ± SEM.

There were no differences in baseline haemodynamics in aged αCGRP WT and KO mice (MAP 105.1 ± 4.1 and 104.9 ± 3.0 mmHg, respectively, n=6). Resistance vessels from both aged αCGRP WT and KO mice produced a dose-dependent vasoconstriction to phenylephrine that was not significantly different between groups. WT vessels produced a 75.3 ± 7.5 % relaxation to the highest dose of CGRP whilst KO vessels were only capable of producing 31.3 ± 8.7 % relaxation to the same dose (2-way ANOVA, $P < 0.01$, n=3-5). We also showed a trend towards an increased mRNA expression of RAMP1 and a significant increase in CLR mRNA in aortic tissue (two-tailed unpaired t-test, $P < 0.05$, n=4) was also observed. RCP expression appeared to be unchanged between groups.

In conclusion, we provide new evidence to suggest that vascular responsiveness to exogenous CGRP is weak in the aged αCGRP KO mouse. This effect appears to occur in spite of an upregulated mRNA expression of CGRP receptor subunits located within the vasculature. The results provide further evidence for a lack of involvement of CGRP in basal blood pressure regulation, as blood pressure was similar between both groups.

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Brain *et al.* (1985) Nature 313 (5997) 54 - 56

Chan and Fiscus (2002) Eur. J. Pharm. 434 (3) 133 -139

Yamaga *et al.* (2001) Japan J. Pharm. 86 (4) 448 - 450