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Calcitonin Gene-Related Peptide (CGRP) Does Not Promote An Adverse Cardiovascular Phenotype In Aged Mice But Inhibits Monocyte Recruitment *In Vitro*

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Calcitonin gene-related peptide (CGRP) is a sensory nerve-derived vasodilator neuropeptide that has been implicated as being protective in a wide range of cardiovascular disease models (1). We have recently shown that in an angiotensin II model of hypertension, CGRP knockout (KO) mice suffer from exacerbated hypertension, vascular remodelling and inflammation when compared to wild type (WT) mice (2). With these findings in mind, we hypothesised that CGRP might also be protective in another model of cardiovascular stress, namely ageing.

Ageing is associated with impaired cardiovascular homeostasis, including hypertension and vascular remodelling. Much of the phenotype is driven by vascular inflammation. To study this phenomenon, we used male αCGRP WT and KO mice at fifteen months of age and compared them with juvenile animals aged three months. All *in vivo* experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986. Assessment of blood pressure by tail cuff volume pressure recording revealed that ageing WT and KO mice were normotensive (125.1 ± 3.9 mm Hg *vs* 123.6 ± 1.91 mm Hg, respectively, n=6-8) when compared to juvenile WT and KO animals (124.6 ± 4.2 mm Hg *vs* 132.2 ± 5.9 mm Hg, respectively, n=6-8). There was no contribution from αCGRP gene deletion. Further to this, markers of vascular remodelling such as aortic smooth muscle hyperplasia and collagen deposition were unchanged between groups.

As ageing can be associated with dysregulated gene expression in the absence of overt phenotypes, we characterised aortic mRNA expression of pro-inflammatory markers from ageing CGRP WT and KO mice by RTqPCR. We have shown a significant elevation in expression of pro-oxidant enzymes Nox2 and Nox4 with age, accompanied by an elevation in VCAM-1 and F4/80 expression, all unaffected by αCGRP gene deletion.

Despite an absence of vascular protection *in vivo, in vitro* studies have uncovered the ability of CGRP to regulate monocyte adhesion to endothelial cells in a parallel plate flow chamber assay. Pre-incubating HUVEC with 300nM CGRP for 6 hours was found to significantly reduce THP-1 adhesion to TNFα-activated HUVEC, in comparison to control (44.70 ± 8.57 *vs* 22.20 ± 7.54 cells per field of view, n=4, $p<0.001$). This was found to be independent of expression of the cell adhesion molecules VCAM-1, JAM-A, JAM-C and PECAM.

In summary, these results suggest that CGRP does not confer cardiovascular protection in a mouse model of ageing. However, we have novel evidence to suggest that CGRP has intrinsic anti-inflammatory properties working to reduce leukocyte recruitment, although the mechanism for this at this stage is unclear.

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(1) Russell FA *et al* (2014). *Phys. Rev.* (in press)

(2) Smillie SJ *et al* (2014). *J. Hypertension* **63(5)**: 1056-62.