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# Daily treatment with a novel long-lasting α-CGRP analogue protects against angiotensin-II induced hypertension

Sensory nerves contain and release the highly potent vasodilator α-calcitonin gene-related peptide (α-CGRP)[1]. α-CGRP KO mice exhibit enhanced hypertension compared to WT mice, suggesting that α-CGRP may protect against the onset of hypertension and related pathologies[2]. We have determined the protective effects of a novel stabilised α-CGRP analogue NN0174-0000-0308 (Novo Nordisk, patent number WO 2011/051312 A1) in angiotensin II (AngII)-induced hypertension *in vivo*.

Male wild-type (WT) mice, aged 12-16 weeks were used. CD1 mice were anaesthetised *i.p.* with ketamine (75mg/kg) and medetomidine (25mg/kg)[3]. Blood flow was measured at baseline and following intradermal injection of the α-CGRP analogue (100pmol/site) or vehicle (0.219M Mannitol, 5% HPCD, 1.6% ammonium acetate, pH6.5) in the ears of mice pre-treated with CGRP receptor antagonist BIBN4096 (0.3mg/kg, *i.v.*) or saline, using Full-field Laser Perfusion Imager (FLPI, Moor)[3]. Blood pressure was recorded at baseline and following implantation of osmotic mini-pumps containing either AngII (1.1mg/kg/day for 14 days) or saline (control) in conscious C57BL/6 mice implanted with a radiotelemetry transmitter (PA-C10, DSI)[2,3]. Mice received vehicle or α-CGRP analogue (50nmol/kg/day, *s.c.*) daily for 14 days of AngII infusion. As CGRP is associated with flushing in humans[1], we monitored the systemic effects of the α-CGRP analogue on blood flow using FLPI and behavioural responses with a light aversion assay[4]. At day 14, organs were harvested and weighed. Heart hypertrophy was assessed via molecular techniques (immunoblotting and qRT-PCR). Data shown as mean $\pm$ SEM, and analysed by two-way ANOVA + Bonferroni *post hoc* test.

Local administration of the α-CGRP analogue causes cutaneous vasodilatation, which was blocked by BIBN4096 in mice (131.4 $\pm$ 18.6 for vehicle vs 83.3 $\pm$ 9.5 for BIBN4096 ($x10^3$ flux units), n=6, *p<0.05). Daily systemic treatment with the α-CGRP analogue in saline-infused mice did not significantly affect blood pressure or physical activity compared to vehicle (n=4-6). In parallel, we observed no significant change in behavioural responses by the light aversion assay or in peripheral blood flow. The α-CGRP analogue (50nmol/kg/day for 14 days) reduced AngII-induced changes in blood pressure, heart weight: bodyweight ratio and cardiac remodeling (n=4-7). We observed a significant decrease in mRNA or protein expression of markers of fibrosis (TGF-β1,fibronectin), remodelling (α-smooth muscle actin, collagen, ANP, BNP, MMP2), inflammation (IL-6, RANTES), oxidative stress (GPX-1, HIF-1α, HO-1, nitrotyrosine) by the α-CGRP analogue in AngII-infused hearts (*p<0.05).

We conclude that the α-CGRP analogue is able to increase vascular blood flow by acting on the CGRP receptors. Our study further highlights that the delivery of a stabilised α-CGRP analogue can protect against the onset of AngII-induced hypertension, in terms of reducing blood pressure and protecting against cardiac hypertrophy. We provide evidence for a potential novel therapeutic strategy, with the concept that CGRP agonists mediate anti-hypertensive and anti-cardiac remodelling effects when treatment starts early onset of hypertension.

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

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