Calcitoningene-Related Peptide Protects Against Cardiovascular Dysfunction Independently of Nitric Oxide In Vivo

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ABSTRACT: The neuropeptide CGRP (calcitonin gene-related peptide) is a potent vasodilator, with a cardioprotective role, although the precise mechanisms are unclear. Here we show the ability of endogenous and exogenous CGRP to restore blood pressure, when nitric oxide synthesis is blocked, in a model of cardiovascular disease associated with endothelial dysfunction and impaired nitric oxide production. Male wild-type and αCGRP knockout mice received L-nitro-arginine methyl ester (150 mg/kg in drinking water) to induce a sustained hypertension with evidence of cardiovascular remodeling. The hypertensive response was exacerbated in L-nitro-arginine methyl ester-treated αCGRP knockouts, indicating that endogenous αCGRP acts in a protective manner, when nitric oxide production is diminished. Exogenous CGRP rescued αCGRP knockout mice from both hypertension and cardiovascular remodeling. Further studies using a non-recovery protocol with a CGRP receptor antagonist (BIBN4096 BS) revealed that CGRP acts via the canonical CGRP receptor (CLR [calcitonin-like receptor]/RAMP1 [receptor activity-modifying protein]); with no effect of an antagonist (AC187) of a second CGRP-responsive receptor (the amylin-1 receptor, CTR [calcitonin receptor]/RAMP1). Blood flow, in resistance vessels of the exteriorised mesentery, was investigated. Noradrenaline–induced vasoconstriction with recovery, in L-nitro-arginine methyl ester-treated wild-type mice. However, αCGRP knockout, or BIBN4096 BS-treated wild-type mice demonstrated a similar constrictor response to noradrenaline, but significantly impaired blood flow recovery. The combined findings highlight that αCGRP protects against cardiovascular dysfunction, signaling via the canonical CGRP receptor and acting when nitric oxide production is lost, such as in endothelial dysfunction associated with vascular disease. These in vivo results support the proposal that CGRP provides a novel treatment for cardiovascular disease. (Hypertension. 2021;77:1178-1190. DOI: 10.1161/HYPERTENSIONAHA.120.14851.) • Data Supplement

Key Words: atherosclerosis ■ blood pressure ■ fibrosis ■ nitric oxide ■ vasodilation

Nitric oxide (NO) is essential for the maintenance of low blood pressure (BP) and a healthy cardiovascular system.1 Endothelial dysfunction is directly linked to a loss of NO and onset of hypertension and vascular disease including atherosclerosis in humans2 and in mice lacking the eNOS (endothelial NO synthase) gene.3

The sensory neuropeptide CGRP (calcitonin gene-related peptide) is a potent vasodilator, especially in the microvasculature4 and the α isoform of CGRP is considered the major cardiovascular form.5-7 CGRP signals via 3',5'-cyclic adenosine monophosphate to mediate vasodilation directly in vascular smooth muscle cells and via a NO-dependent vascular relaxation in endothelial cells. Both mechanisms are implicated in emerging evidence of its cardiovascular protective properties.7-8 CGRP antagonists and antibodies, are beneficial in relieving migraine headache, without significant effects on cardiovascular regulation in humans, leading...
to questions about the functional relevance of CGRP in cardiovascular regulation. To date, relatively few studies of the CGRP blocking agents have been performed in patients with cardiovascular disease, with little evidence of whether CGRP has beneficial cardiovascular effects when NO synthesis is disrupted, such as in endothelial dysfunction.

Naive αCGRP knockout mice, as used here, have similar BP levels to wild-type (WT) mice; in keeping with CGRP antagonist and antibody data in healthy humans that CGRP is not involved in the physiological regulation of BP. However, the involvement of endogenous CGRP changes as cardiovascular stress impacts; with studies involving αCGRP knockout mice and CGRP antagonists indicating that CGRP is protective in cardiovascular dysfunction, facilitating recovery and re-vascularisation. Using an Ang II (angiotensin)–induced hypertension model, we have shown that αCGRP knockout mice exhibit exaggerated hypertension, loss of eNOS mRNA and protein, increased aortic oxidative stress and fibrosis, providing a link between the protection offered by CGRP and NO. Potentially, CGRP agonists are novel therapeutic agents for treating cardiovascular disease, as shown in our study with a stable CGRP agonist.

The canonical CGRP receptor is composed of a heterodimeric G-protein-coupled receptor (CLR [calcitonin-like receptor]/RAMP1 [receptor activity-modifying protein]) linked with a single transmembrane RAMP1 (receptor activity-modifying protein) at the cell membrane which is important for receptor trafficking and efficacy, with evidence that RAMP1 is upregulated in rodent models of heart failure. A second functional receptor for CGRP comprises the CTR (calcitonin receptor) and RAMP1; known as the amylin 1 receptor because it is also activated by amylin. The importance of this receptor in the vascular actions of CGRP is unknown.

There have been very few investigations into the ability of CGRP to act in a protective manner independently of NO. Here, the ability of CGRP to influence mouse BP, measured via 2 different techniques, vascular remodeling and the mechanisms involved were investigated under conditions where nitric oxide loss is compromised.

MATERIAL AND METHODS
All supporting data are available within the article and detailed methods, and supplementary results are in the Data Supplement.

Animals
Experiments comply with Animal Research: Reporting In Vivo Experiments guidelines, the UK Home Office Animals Nonstandard Abbreviation and Acronyms

| Abbreviation | Definition                  |
|--------------|-----------------------------|
| Ang II       | angiotensin II              |
| BP           | blood pressure              |
| CGRP         | calcitonin gene-related peptide |
| CLR          | calcitonin-like receptor    |
| CTR          | calcitonin receptor         |
| eNOS         | endothelial NO synthase     |
| L-NAME       | L-nitro-arginine methyl ester |
| NO           | nitric oxide                |
| RAMP1        | receptor activity-modifying protein |
| WT           | wild-type                   |

What Is Relevant?
• Blood flow can be compromised in cardiovascular disease, often due to endothelial dysfunction and ensuing loss of nitric oxide. CGRP administration compensates for this, potentially protecting against cardiovascular dysfunction; providing evidence for a novel therapeutic approach involving the CGRP pathway.

Summary
Patients with hypertension can possess dysfunctional nitric oxide production with associated endothelial dysfunction. Using genetically modified mice and pharmacological tools, we provide evidence for the therapeutic potential of the αCGRP pathway. Our results confirm that αCGRP can act via the canonical CGRP receptor to benefit cardiovascular regulation, under conditions where nitric oxide loss is compromised.

What Is New?
• Results support the feasibility of CGRP (calcitonin gene-related peptide) protecting against hypertension and cardiovascular remodeling when nitric oxide production is dysfunctional.
• The study indicates that CGRP acts via the CGRP receptor (CLR [calcitonin-like receptor]/RAMP1 [receptor activity-modifying protein]), rather than the amylin 1 receptor (CTR [calcitonin receptor]/RAMP1) to cardiovascular effects.
• Results show that αCGRP can promote the restoration of blood flow in mesenteric resistance vessels in vivo thereby decreasing vascular resistance.

Novelty and Significance

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Influences of L-NAME and αCGRP on BP in WT and αCGRP Knockout Mice

WT and αCGRP knockout naive mice possess comparable baseline BP (Figure 1A) measured by tail cuff. Addition of the L-NAME to the drinking water did not have a lasting effect on water intake, or influence food intake and body weight (Figure S2A through S2D in the Data Supplement). However, L-NAME treatment led to a sustained increase in BP that was further significantly enhanced in αCGRP knockout mice (Figure 1A). Although L-NAME treatment increased remodeling irrespective of genotype, there was no significant difference in heart weight (Figure 1B; Table S3), cardiac smooth muscle actin expression (Figure 1C), interstitial cardiac fibrosis (Figure 1E), or aortic remodeling (Figure 1F) between WT and αCGRP knockout mice. The inhibition of eNOS protein is supported by the finding that phosphorylated eNOS is reduced in aortic tissues of L-NAME-treated mice compared with vehicle-treated mice (Figure 1D); but with little influence on nitrosylated protein production (Figure 5E and 5F). Neither neuronal nitric oxide synthase or inducible nitric oxide synthase protein were localized to aortic tissue (Figure S2H and S2I).

Thus, although L-NAME–induced hypertension was enhanced in αCGRP knockout mice, this was not accompanied with increased cardiovascular remodeling in the αCGRP knockout mice (Figure 1B, 1C, and 1E). Investigation of the kidneys suggested that there was no significant change in renal nitrosylation (Figure SSA), but a significant increase in mesangial matrix expansion was enhanced in αCGRP knockout mice, this was not accompanied with increased cardiovascular remodeling in the αCGRP knockout mice (Figure 1B, 1C, and 1E). Investigation of the kidneys suggested that there was no significant change in renal nitrosylation (Figure S5A), but a significant increase in renal nitrosylation (Figure S5A), but a significant increase in mesangial matrix expansion was enhanced in αCGRP knockout mice. This was accompanied with increased cardiovascular remodeling in the αCGRP knockout mice (Figure 1B, 1C, and 1E).

Effect of Exogenous αCGRP on αCGRP Knockout Mice

To understand if exogenous αCGRP possesses similar protective cardiovascular characteristics, we examined the influence of exogenous αCGRP as a therapeutic treatment when mice were receiving L-NAME. The αCGRP dose chosen for this study was previously demonstrated to be protective in mice.29 Here, 2 weeks of αCGRP infusion administered via osmotic mini-pumps substantially reversed the L-NAME–induced BP increases and gain in heart weight, without affecting body weight (Figure 2A through 2C). In contrast to the

Statistical Analysis

All data presented are mean±SEM unless otherwise stated. Statistical analysis was by an unpaired 2-tailed Student t test for 2 data groups, 1-way ANOVA or 2-way ANOVA plus Bonferroni post hoc test for multiple comparison between 3 or more data groups. Repeated-measures 2-way ANOVA was used for data with multiple time points. P<0.05 was considered statistically significant.
Figure 1. Effect of L-nitro-arginine methyl ester (L-NAME) on blood pressure and cardiovascular remodeling in wild-type (WT) and αCGRP (calcitonin gene-related peptide) knockout (KO) mice over 6 wk. A, Tail cuff systolic, mean arterial, and diastolic blood pressure measurements obtained from conscious WT and αCGRP KO mice receiving drinking water or L-NAME treatment (150 mg/kg) in drinking water. B, Left ventricle (LV) normalized to body weight (BW). C, Immunoblot with densitometric analysis of α-SMA expression in left ventricular tissues. D, Immunoblot of aortic tissues for phosphorylated eNOS (Serine 1177; p-eNOS) normalized to total eNOS (t-eNOS). E, Brightfield images of Picro Sirius stained left ventricular tissue and histological quantification of fibrosis. Images taken ×20 magnification; scale bar 50 μm. F, Images of Masson’s trichrome stained aortic tissue and histological quantification of vascular remodeling. Images taken ×10 magnification; scale bar 100 μm. Mean±SEM, n=5–12. For A, ***P<0.001 for control WT vs L-NAME WT for all time points in systolic blood pressure (SBP) and mean arterial pressure (MAP), **P<0.01 for weeks 2–6 for diastolic blood pressure (DBP), $$$P<0.001 for control αCGRP KO vs L-NAME αCGRP KO for all time points in SBP, MAP, and DBP, and #P<0.05, ##P<0.01, ###P<0.001 for L-NAME WT vs L-NAME αCGRP KO. For B–F, *P<0.05, **P<0.01, and ***P<0.001 for between groups as indicated. Statistical analysis via repeated-measures 2-way ANOVA plus Bonferroni post hoc test for multiple comparison. α-SMA indicates α-smooth muscle actin.
**Figure 2.** Effect of αCGRP (calcitonin gene-related peptide) infusion for 2 wk on L-nitro-arginine methyl ester (L-NAME)–induced hypertension and cardiac remodeling in αCGRP knockout (KO) mice.

αCGRP KO mice received L-NAME treatment (150 mg/kg) in drinking water for 6 wk and from week 4 the mice additionally received 2 wk of saline or αCGRP (165 µg/kg per day) in osmotic minipumps. A, Systolic blood pressure (SBP), mean arterial pressure (MAP) and diastolic blood pressure (DBP) measurements obtained via tail cuff. The gray shaded indicates typical baseline levels. Mean±SEM, n=7–8. Statistical analysis via 2-way ANOVA plus Bonferroni post hoc test for multiple comparison. ***P<0.001 Saline vs αCGRP, ###P<0.001 wk 3 αCGRP vs week 5 or 6 αCGRP groups. B, Body weight and (C) left ventricle (LV) normalized to body weight (BW). D, Immunoblot with densitometric analysis of α-SMA expression in left ventricles. Positive control (cntl); VSMC. E, mRNA expression of Col IV (collagen type 4), SM22-alpha (smooth muscle actin), Col1a1 (collagen type 1 alpha 1), and BNP (brain natriuretic peptide) in left ventricular tissues. Mean±SEM, n=6–8. For B–E, *P<0.05, **P<0.01, ***P<0.001 between groups as indicated. Statistical analysis via unpaired 2-tailed Student t test.
Figure 3. L-nitro-arginine methyl ester (L-NAME)–induced hypertension in wild-type (WT) αCGRP (calcitonin gene-related peptide) knockout (KO) mice measured via carotid cannulation; evaluation of CGRP and amylin receptor involvement. **A**, Blood pressure was measured in WT and αCGRP KO mice after 14 days with and without L-NAME treatment. Statistical analysis via 2-way ANOVA plus Bonferroni post hoc test for multiple comparison. **B**, Mean arterial pressure (MAP) measurements before and after IV administration of vehicle (<5% DMSO+saline) or BIBN4096 BS (BIBN 0.3 mg/kg) in control WT mice or **C**, L-NAME-treated WT mice. **D**, Percentage change in MAP analysis of **B** and **C**. **E**, Percentage change from baseline to lowest MAP value after αCGRP (0.75 µg/kg, IV) and peak MAP value after BIBN4096 BS (0.3 mg/kg, IV) or AC-187 (0.15 mg/kg, IV) and then a combination of CGRP and AC-187 or BIBN 4096 BS. **F**, Percentage change in MAP after injection with CGRP receptor antagonists BIBN4096 BS and AC-187 in WT (CGRP [+/+]) and αCGRP KO (CGRP [−/−]) mice. Values are average of 3 peak measurements. Mean±SEM, n=5–7. *P<0.05, **P<0.01, ***P<0.001 between groups as indicated. For **B–C**, statistical analysis via unpaired 2-tailed Student t test. For **D**, statistical test via 2-way ANOVA plus Bonferroni post hoc test for multiple comparison. For **E** and **F**, statistical test via 1-way ANOVA plus Bonferroni post hoc test for multiple comparison.
Figure 4. CGRP (calcitonin gene-related peptide) receptor analysis in wild-type (WT) and αCGRP knockout (KO) mice treated with L-nitro-arginine methyl ester (L-NAME).

A. Immunoblots and densiometric analysis of CLR (calcitonin-like receptor) and RAMP1 (receptor activity-modifying protein) expression in aortic (B) cardiac and (C) renal tissues from untreated and L-NAME treated WT and αCGRP KO mice compared with GAPDH. Kidney tissues and human embryonic kidney (HEK) cell lines used for RAMP1 immunoblotting presented a nonspecific band at ≈35 kD which was excluded from analysis. Mean±SEM, n=5–7. **P<0.01 between groups as indicated. Statistical analysis via 2-way ANOVA plus Bonferroni post hoc test for multiple comparison.
effect of endogenous CGRP, exogenous αCGRP protected against cardiac hypertrophy (Figure 2C through 2E), in keeping with our previous results using the stable CGRP analogue/agonist.  

CGRP-Dependent Receptor Mechanisms

Central BP was measured in anaesthetized mice following cannulation of the carotid artery in a separate cohort of mice. Control WT and αCGRP knockout mice had similar central BP at baseline (Figure 3A). However, 2 weeks of L-NAME treatment led to increased BP in WT mice, which was exacerbated in L-NAME-αCGRP knockout mice (Figure 3A and Figure S4A); supporting that observed via tail cuff in conscious mice (Figure 1A). To investigate the involvement of the CGRP receptors, the selective CGRP receptor antagonist BIBN4096 BS (0.3 mg/kg, IV) was administered following 5 minutes of baseline BP recording (Figure S4B). BIBN4096 BS treatment enhanced BP in control WT mice (Figure 3B) and to a greater extent in L-NAME-WT mice (Figure 3C and 3D and Figure S4B). This enhancement of central BP in WT mice (Figure 3C) was at a time when peripheral blood flow in WT and αCGRP knockout mice had been previously shown to be not affected.  

As the role of the amylin-1 receptor mediating CGRP actions is unknown, we examined an amylin-1 receptor antagonist, AC187, 30 at the highest achievable non-vasoactive dose (0.15 mg/kg, IV). Interestingly, AC187 opposed BIBN4096 BS in that it produced a hypotensive, rather than a hypertensive effect at higher doses (Figure S4C). CGRP (0.75 µg/kg, IV) reduced BP, as expected. This response was inhibited by BIBN4096 BS (Figure 3D and 3F), but not AC187 (Figure 3E and 3F). Western blot analysis for CLR and for RAMP1 with a specific antibody (Figure S6) revealed evidence of stable levels of CLR (Figure 4A through 4C). RAMP1 expression was raised in aortic and heart tissues (Figure 4A and 4B) after L-NAME treatment. RAMP1 expression was stable in kidney tissues (Figure 4C). VSMCs isolated from WT and αCGRP knockout mice expressed RAMP1 and glycosylated CLR (Figure S7).

Effect of CGRP on the Microvasculature

To probe mechanisms further, we investigated if endogenous αCGRP could influence the cutaneous microvasculature.  

DISCUSSION

The primary finding from this research is that CGRP is able to protect against exacerbated BP when nitric oxide synthase is inhibited in mice, as determined by genetic and pharmacological modulation of the CGRP pathway. This research was achieved using WT and αCGRP knockout mice and a CGRP receptor antagonist BIBN4096 BS that is ≈150-fold more selective for the canonical CGRP receptor, than the amylin 1 receptor.
Figure 5. Mesenteric blood flow recovery after noradrenaline (NA, 23.5 µmol/L topical spray; 100 µL) in first, second, and third order arterioles in vivo.

Blood flow of first order (left), second order (middle), and third order (right) mesenteric vessels obtained from control wild-type (WT) and α-CGRP (calcitonin gene-related peptide) knockout (KO) mice receiving drinking water only. A, Blood flow traces of first, second, and third order vessels in response to topical saline and NA. Each response indicates mean±SEM for every second during the recording period. B, Mean baseline blood flow (0–5 min) of mesenteric vessels. C, Maximum percentage change of blood flow following NA. D, Area under the curve (AUC) for blood flow recovery in vessels. E, Slope gradient for blood flow recovery phase. F, Percentage of blood flow recovery from baseline to end of recording. Mean±SEM, n=5–7. *P<0.05, **P<0.01 between groups as indicated. Statistical analysis via unpaired 2-tailed Student t test.
Figure 6. Dysfunctional mesenteric blood flow recovery after topical application of NA (23.5 µmol/L) in first order mesenteric arterioles in vivo.

Blood flow measurements obtained from L-nitro-arginine methyl ester (L-NAME)-treated wild-type (WT) and αCGRP (calcitonin gene-related peptide) knockout (KO) mice (left) and from WT mice that have been pretreated with CGRP antagonist BIBN4096 BS (0.3 mg/kg, IV) or vehicle immediately before imaging (right). A, Blood flow traces of first order vessels in response to topical saline and NA. B, Mean baseline blood flow (0–5 min) values from mesenteric vessels. C, Maximum percentage change of mesenteric blood flow following NA. D, Area under the curve (AUC) for blood flow recovery in mesenteric vessels. E, Slope gradient for the blood flow recovery phase. F, Percentage of blood flow recovery from baseline to the end the blood flow recording. Mean±SEM, n=6–8. *P<0.05, **P<0.01, ***P<0.001 between groups as indicated. Statistical analysis via unpaired 2-tailed Student t test for 2 data groups and 2-way ANOVA plus Bonferroni post hoc test for multiple comparison for 4 data groups.
Independently, administration of a continuous infusion of exogenous CGRP rescued the raised BP and cardiovascular remodeling in L-NAME-treated mice. The results indicate that CGRP is able to act independently of NO to protect against the raised BP. This leads to the expectation that CGRP will be functionally active in cardiovascular disease when the NO system has become dysfunctional due to endothelial damage.

Surprisingly, there is limited in vivo research into the cardiovascular interactions of CGRP and NO as both are potential potent cardiovascular protective agents. Yallampalli et al.41 studied L-NAME-treated pregnant rodents to model preeclampsia and reported that co-administration of CGRP and L-NAME to pregnant rats prevented gestational hypertension caused by L-NAME treatment alone. Furthermore, Gangula et al.42 showed that infusion with a CGRP antagonist did not affect untreated rats but raised BP in L-NAME-treated rats. Both studies imply that CGRP can play a compensatory role to attenuate high BP, in keeping with this current study. CGRP has also been shown to cause relaxation of endothelium-absent uterine arteries from pregnant patients in ex vivo experiments.43 Mechanisms of action, however, are yet to be elucidated. We used the L-NAME hypertension model to produce sustained hypertension for up to 6 weeks. Compared with our previous studies (eg, Ang II model40), we did not find robust cardiac remodeling. This is in keeping with a study by Bartunek et al.44 investigating the effect of L-NAME on cardiac hypertrophy and is also likely due to the various mechanism by which Ang II induces growth of cardiac cells hence facilitates cardiac hypertrophy and if left uncontrolled, heart failure. L-NAME treatment in both WT and αCGRP knockout mice produced modest cardiac remodeling compared with the robust Ang II mediated effects reported by our group40 and others. Where exogenous αCGRP was able to rescue the modest increase in cardiac ventricular weight and expression of remodeling markers after L-NAME treatment, endogenous αCGRP did not seem to be able to. We, therefore, suggest that endogenous αCGRP may only be relevant in reversing cardiac remodeling in the presence of robust pathophysiological processes, such as in Ang II–induced hypertension, or that sensitivity to αCGRP may be altered in these circumstances via receptor expression or activity.

We hypothesize that L-NAME inhibited endothelial-derived NO, especially as constitutive eNOS (encoded by NOS3) deficient mice develop hypertension,45 and eNOS maintains a low vascular tone and BP.45 However, the nonselective nitric oxide synthase inhibitor L-NAME will also inhibit other isoforms.47 However, we did not detect protein expression of either neuronal nitric oxide synthase or inducible nitric oxide synthase in aortic tissues. Neuronal nitric oxide synthase has been implicated in CGRP release from sensory nerves.48 Potentially, this may contribute to the ability of L-NAME to mediate hypertension, but if so, only to a minor degree as the BP was significantly raised in L-NAME-αCGRP knockout compared with WT mice.

A major question is whether a second, recently proposed, CGRP receptor is involved in BP regulation. Protein expression of the CGRP receptor components CLR and RAMP1 were localized to cardiovascular tissues and CLR expression was stable in all tissues investigated. RAMP1 was raised in certain L-NAME treated tissues (eg, the aorta), in keeping with the concept that RAMP1 can be upregulated in some cardiovascular tissues, which in turn could lead to increased levels of the CGRP receptor and thus increased activity of CGRP.49 The evidence that BIBN4096 BS attenuates CGRP activity indicates that CGRP acts via the CLR/RAMP1 receptor in BP regulation. In support, an antagonist for a second CGRP-responsive receptor (the amyl 1 receptor, CTR/RAMP1) did not influence BP at the dose used. AC187 has been reported to possess 400-fold selectivity for the amyl 1 receptor compared with the CGRP receptor.50,51 This contrasts with studies in trigeminal cell studies, where CGRP could signal via both receptors.51 Thus, as classically recognized, we consider that the CGRP receptor CLR/RAMP1 is influential in CGRP-mediated cardiovascular regulation. There are substantial studies of the receptor in vitro, which includes receptor recycling and desensitization.51 It is also known to internalize after activation.52,53 However, the data is indicative that both endogenous and exogenous CGRP can play a functionally important protective role. Endogenous CGRP levels are reduced as sensory nerves become depleted, and there is evidence that this happens in cardiovascular disease.54 This is relevant to the knowledge that αCGRP does not appear to play a significant role in hypertension as the endogenous depletion of αCGRP would make it impossible for αCGRP to be influential.

As CGRP is a potent cutaneous vasodilator,4 we investigated its ability to influence skin blood flow. Our evidence in skin from WT and αCGRP knockout mice suggests that NO, rather than αCGRP is the more important endogenous cutaneous vasodilator, as L-NAME reduced paw skin blood flow and that this was not further influenced by αCGRP deletion. This is in keeping with the observation that peripheral vasoconstriction is associated with increased sympathetic tone in rats.53 L-NAME has been reported to influence a range of vascular beds, including the mesenteric microcirculation after its direct application.54 Here, we observed blood flow in vivo by direct laser speckle imaging. The results revealed that L-NAME-treated-αCGRP knockout first order mesenteric arterioles have a slower recovery from acute noradrenaline–induced vasoconstriction than those that those from L-NAME-treated-WT mice. These results were confirmed by use of a CGRP antagonist. This suggests that endogenous αCGRP supports the recovery from mesenteric vasoconstriction, when endothelial dysfunction, associated with reduced NO production, exists.
The findings provide new information on the extent of mechanisms by which CGRP may act in a protective manner; specifically, in situations where vascular NO production may be dysfunctional. Primarily, we have shown that endogenous and exogenous CGRP exerts cardiovascular protection when NO production is lost, such as occurs in a wide range of cardiovascular diseases as they impact. Mechanically, we show that CGRP is likely to act via the canonical CLR/RAMP1 receptor, facilitating mesenteric regulation of blood flow in vivo. The ability of exogenous CGRP, to combat hypertension and additionally vascular remodeling when NO production is disrupted leads us to suggest that stimulating the CGRP pathway may be an important approach to targeting cardiovascular disease known to be associated with NO dysfunction.

Perspectives
We show that αCGRP can act independently of NO to be protective against cardiovascular injury in vivo, in terms of hypertension and cardiovascular remodeling. This includes when exogenous αCGRP is administered to mice depleted of endogenous αCGRP and NO. The response requires αCGRP to act on CLR/RAMP1 receptors in the vascular wall and studies reveal that αCGRP acts in first order mesenteric arterioles to restore blood flow after vasoconstriction. Stimulation of the CGRP pathway could provide an important therapeutic target in cardiovascular disease associated with endothelial dysfunction.

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Disclosures
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