Calcitonin gene–related peptide inhibits angiotensin II–mediated vasoconstriction in human radial arteries: Role of the Kir channel

Anthony Zulli, PhD,^{a,b} Bei Ye, MBBS,^b Peter J. Wookey, PhD,^{a,b} Brian F. Buxton, MBMS, FRACP,^c and David L. Hare, MDDs, DPM, FRACP^{a,b}

Objective: The radial artery is increasingly used for coronary artery bypass grafts, but its potential for spasm increases postoperative risk. Alpha-calcitonin gene–related peptide is a potent antihypertensive peptide.

Thus, we set out to determine whether calcitonin gene–related peptide can impair angiotensin II–mediated vasoconstriction in human radial arteries and, if so, to determine its mechanism of action.

Methods: Radial arteries were placed in organ bath chambers and preincubated with 10^{-9} to 10^{-7} mol/L alpha-calcitonin gene–related peptide for 20 minutes before initiating an angiotensin II dose response curve (10^{-10} – 10^{-6} mol/L).

Results: Calcitonin gene–related peptide, 10^{-7} , 10^{-8} , 3×10^{-9} , and 10^{-9} mol/L, reduced angiotensin II–mediated vasoconstriction to $30.5\% \pm 7.2\%$ ($P < .001$), $32.2\% \pm 11.7\%$ ($P < .001$), $62.6\% \pm 8.4\%$ ($P < .001$), and $77.6\% \pm 6.7\%$ ($P < .01$), respectively, compared with control (normalized to 100%). Calcitonin gene–related peptide also significantly decreased basal vascular tension in human radial arteries ($P < .05$ in all cases). *N*-nitro-L-arginine methyl ester, 4-aminopyridine, charybdoxin, and apamin had no effect on calcitonin gene–related peptide relaxation, but Ba^{2+} impaired the effects of alpha-calcitonin gene–related peptide.

Conclusions: Alpha-calcitonin gene–related peptide dose dependently impaired angiotensin II–mediated vasoconstriction in human radial arteries, independent of nitric oxide and all potassium channels except the barium-sensitive Kir channel. Thus, calcitonin gene–related peptide is an endogenous inhibitor of angiotensin II–mediated vasoconstriction in the human radial artery.

From the Department of Medicine,^a University of Melbourne, Austin Health, Heidelberg, and the Departments of Cardiology^b and Cardiac Surgery,^c Austin Health, Heidelberg, Victoria, Australia.

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Address for reprints: Dr Anthony Zulli, Vascular Biology Laboratory, Department of Cardiology, Austin Health, Heidelberg 3084, Australia (E-mail: azulli@unimelb.edu.au).

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Radial arteries are being used more frequently as coronary artery bypass grafts. However, radial arteries are prone to spasm and thus increase the risk of graft failure.¹ Angiotensin II (ang II) is an active component of the renin–angiotensin system, which, through its receptor (AT1R), plays an important role in the control of vasoconstriction, cell growth and apoptosis, cell migration, and extracellular matrix deposition, events that are involved in cardiovascular disease.² On the other hand, calcitonin gene–related peptide (CGRP) is an inhibitor of cell growth and a potent endothelium-independent vasodilator,^{3–5} thus suggesting that it could inhibit ang II–mediated effects. Indeed, long-term ang II infusion is accompanied by an increase in CGRP receptor expression in mesenteric arteries but not in CGRP levels in plasma.⁶ α CGRP is a 37-amino acid peptide, produced by tissue-specific alternative splicing of the primary transcript of the calcitonin/ α CGRP gene.⁷ A second CGRP isoform, β CGRP, is encoded by a different gene locus and is localized almost exclusively to specific neuronal sites.⁸ These two CGRP isoforms— α and β in rats and I and II in humans—exhibit overlapping biological activities in most vascular beds,⁹ but α CGRP appears to be regulated by nitric oxide (NO).¹⁰ CGRP has complex cardiovascular actions. For example, the intramuscular gene transfer of CGRP inhibits neointimal hyperplasia after balloon injury in the rat abdominal aorta,¹¹ possibly by

Abbreviations and Acronyms

ang II	= angiotension II
CGRP	= calcitonin gene-related peptide
EDHF	= endothelium-dependent hyperpolarization
L-NAME	= <i>N</i> -nitro-L-arginine methyl ester
NO	= nitric oxide

affecting the NO system^{10,11} and the proliferation of vascular smooth muscle cells induced by fetal bovine serum.¹² More important, CGRP can act directly on smooth muscle cells and stimulate relaxation independently of endothelial function in porcine coronary arteries.³ Inasmuch as CGRP can induce vasorelaxation independently of endothelial function and CGRP appears to counteract ang II vasoconstriction in animal models, we sought to determine whether such effects extend to human arteries. To this end, we have used human radial arteries with impaired endothelial function to test the direct effects of CGRP on ang II-mediated vasoconstriction.

Methods

Discarded distal segments of radial arteries¹³ were obtained from 25 patients undergoing coronary artery bypass grafting using the “open” technique for harvesting. Patients aged 48 to 70 years gave informed consent and were receiving 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, angiotensin-converting enzyme inhibitors, nitrates, and beta adreno-receptor blockers. Patients receiving angiotensin receptor blockers were excluded from this study. Only nontraumatized vessel segments were used in this study. This study was approved by the Austin Hospital Medical Research Ethics Committee and followed institutional guidelines. Harvesting was performed by techniques previously established in our operating theaters¹; in particular, the specimens were obtained after intraluminal hydrostatic dilatation with papaverine, which impairs endothelial function.

Isometric Tension Studies

Blood vessels were cleaned of connective tissue and fat and stored at 4°C overnight. Pilot studies in our laboratory show no difference in vessel function between freshly isolated radial artery rings and those kept in overnight 4°C storage, as also described elsewhere.¹⁴ Vessels were then cut into 3-mm rings and sequentially mounted between two metal hooks in organ baths attached to force displacement transducers (Grass FT03; Grass Instrument Co, Quincy, Mass). The baths were filled with Krebs solution, kept at a constant temperature of 37°C, and continuously bubbled with 95% oxygen/5% carbon dioxide. After a 2-hour equilibration period, the rings were stretched to their optimized tension. This technique allows normalization of vascular rings to a physiologic pressure in vitro compared with in vivo and is the accepted method for isometric tension studies.^{15,16} After another 2-hour equilibration period, Krebs buffer was replaced with high potassium solution (KPSS, 124 mmol/L K⁺) to induce maximal constriction. After the rings reached plateau (approximately 8 minutes), the rings were flushed with Krebs solution. After another 2-hour equilibration

period, rings were incubated with 10⁻⁹ to 10⁻⁷ mol/L αCGRP¹⁷ 20 minutes before the initiation of an ang II dose response curve (10⁻¹⁰–10⁻⁶). The vessel equilibration time also served to minimize the effect of patient therapy on vessels.¹⁸ All experimental data were normalized to control, which was set at 100%.

To determine the influence of potassium channels or NO on the inhibitory effects of CGRP, *N*-nitro-L-arginine methyl ester (L-NAME; 10⁻⁵ mol/L, to inhibit NO), 4-aminopyridine (3 mmol/L, to inhibit voltage-dependent K⁺ channels), charybdotoxin and apamin (50 × 10⁻⁹ mol/L and 3 × 10⁻⁷ mol/L, respectively), to inhibit endothelium-dependent hyperpolarization (EDHF), and calcium-activated K⁺ channels and 10⁻⁴ mol/L barium chloride (to inhibit inwardly rectifying K⁺ channels) where used. These concentrations have been shown to be specific for each potassium channel.^{19,20}

Data Analysis

Data are presented as mean ± standard error of the mean, where “n” represents the number of independent samples studied. Data from arterial samples are expressed as percent response to 124 mmol/L KCl. Statistical comparisons were undertaken with either the Student *t* test or a 1-way analysis of variance (where appropriate) and a *P* value of .05 using GraphPad Prism software (GraphPad Software, Inc, San Diego, Calif).

Results**Effects of αCGRP on Ang II-mediated Vasoconstriction**

αCGRP potently inhibited ang II-mediated vasoconstriction at all concentrations used. Both 10⁻⁷ mol/L and 10⁻⁸ mol/L αCGRP inhibited maximal contraction evoked by 10⁻⁶ mol/L ang II by 69% (*P* < .001) and 68% (*P* < .001), respectively. Indeed, 10⁻⁷ mol/L αCGRP inhibited ang II-mediated vasoconstriction from 3 × 10⁻¹⁰ mol/L [ang II], reducing constriction from 1.56% ± 0.45% to -0.62% ± 0.41% (*P* < .05). The negative value indicates continued relaxation rather than constriction as seen with the control. The inhibitory effect of 10⁻⁷ mol/L αCGRP continued to 10⁻⁹ mol/L ang II, reducing constriction from 4.4% ± 1% to 0.08% ± 0.65% (*P* < .05). Starting at the lowest concentration of 10⁻⁹ mol/L ang II, αCGRP concentrations of 3 × 10⁻⁹, 10⁻⁸, and 10⁻⁷ mol/L all significantly inhibited ang II-mediated vasoconstriction. At a concentration of 10⁻⁹ mol/L, αCGRP inhibited ang II-mediated vasoconstriction only at the concentration of 3 × 10⁻⁶ mol/L, by 21% (*P* < .05) and at the maximal ang II concentration used, 10⁻⁶ mol/L by 22% (*P* < .01) (Figure 1, A). There was no difference in maximum contraction evoked by 124 mmol/L potassium between groups (Figure 1, B).

Effects of αCGRP on Basal Tone

αCGRP also significantly decreased basal vascular tension in human radial arteries. After the 20-minute period following the addition of αCGRP or vehicle (control), 10⁻⁷ mol/L αCGRP showed the highest decrease in basal tension (expressed as force in grams, Figure 1, C) compared with

Figure 1. Graph showing the inhibitory effects of CGRP on angiotensin II-mediated vasoconstriction. **A**, CGRP concentrations of 10^{-8} mol/L ($n = 5$) and 10^{-7} mol/L ($n = 9$), inhibition of angiotensin II remained constant at 68% and 69%, respectively, and did not follow the dose-dependent increase observed for lower concentrations ($P < .001$ in both cases). **B**, Box plots showing the maximum potassium (K^+) constriction per CGRP dose tested. Each experimental dose (closed box) was normalized to its adjacent segment (control, open box), as to restrict variation between segments. There was no difference in the maximum contraction to 124 mmol/L potassium in any group. **C**, Exposing human radial arteries to increasing concentrations of CGRP has a dose-dependent, albeit slight, dilatory effect on the basal arterial tone of isolated human radial arteries over a 20-minute period. Results are mean $\pm$ SEM, * $P < .05$, ** $P < .01$, † $P < .001$.

control: -0.64 ± 0.2 g versus 0.11 ± 0.03 g ($P < .001$). This was followed by 10^{-8} mol/L α CGRP (-0.37 ± 0.03 g; $P < .05$), 3×10^{-9} mol/L (-0.19 ± 0.08 g; $P < .05$), and 10^{-9} mol/L α CGRP (-0.16 ± 0.12 g; $P < .05$).

Effects of L-NAME on α CGRP-induced Vasodilation

To determine whether NO release was involved in the effects of CGRP, we added L-NAME to the organ baths 20 minutes before the ang II dose response curve. Then, at 10^{-8} mol/L (ang II), α CGRP (10^{-8} mol/L) was added and α CGRP completely relaxed arteries after approximately 10 minutes. The actual trace is shown in Figure 2, A, and the results of 3 independent experiments are shown in Figure 2, B.

Effects of α CGRP on Potassium Channels

To determine whether potassium channels were involved in the effects of α CGRP, we added 4-aminopyridine to the radial artery and vasoconstriction occurred rapidly, an effect that was completely reversed by the addition of 10^{-8} mol/L α CGRP but restored by the addition of Ba^{2+} (actual trace shown, Figure 2, C); the results of 5 independent experiments are shown in Figure 2, D. As well, charybdotoxin plus apamin (Figure 2, E and F) was added to radial arteries to inhibit endothelium hyperpolarization via the release of EDHF and calcium-activated K^+ channels. This had no effect on 10^{-8} mol/L α CGRP-induced reduction in basal tension, but Ba^{2+} impaired CGRP effects and induced potent vasoconstriction (actual trace shown, Figure 2, E). The results of 3 in-

dependent experiments are shown in Figure 2, F. Moreover, the addition of Ba^{2+} to radial arteries per se induced vasoconstriction, but the addition of 10^{-8} mol/L α CGRP had no effect (actual trace shown, Figure 2, G). The results of 3 independent experiments are shown in Figure 2, H.

Discussion

This study clearly establishes the importance of α CGRP in influencing radial artery basal tone and desensitizing it to the vasoconstrictive effects of ang II. Furthermore, it was demonstrated that α CGRP-induced vasodilation is preserved in the presence of L-NAME, an NO synthase inhibitor, suggesting that α CGRP is not dependent on NO for its activity, but could be associated with opening the Kir potassium channel. We further propose that α CGRP at a concentration of 10 nmol/L could be used in preoperative radial artery bath preparations to impair vasospasm and improve postoperative risk.

Growing evidence is indicating that α CGRP and ang II may act reciprocally to help maintain circulatory homeostasis.²¹⁻²⁵ Our results support this hypothesis, indicating that CGRP acts as a functional antagonist of ang II-mediated vasoconstriction and attenuates vasoconstriction in a dose-dependent manner. This result is consistent with previous rat model investigations, which found that CGRP inhibits ang II-induced vasoconstriction.^{21,22} Indeed, the only other comparable study involving human radial arteries also yielded similar results.²⁶

Figure 2. A, Actual trace showing the effects of 10^{-8} mol/L CGRP on ang II-induced vasoconstriction in the presence of 10^{-5} mol/L L-NAME. CGRP potently restores normal basal tension after approximately 10 minutes, indicating no effect of nitric oxide on CGRP-induced vasodilation. B, Tabulated results of 3 independent experiments. C, 4-Aminopyridine (4-Ap) was used to block voltage-dependent K^+ channels, and vasoconstriction occurred immediately after the addition of the drug. CGRP was able to restore basal tension within minutes, but 10^{-4} mol/L Ba^{2+} (Kir inhibitor) caused marked vasoconstriction even in the presence of CGRP. D, Tabulated results of 5 independent experiments. E, Charybdotoxin plus apamin (Ch+Ap) was added concomitantly to radial arteries to inhibit EDHF and Ca^{2+} -activated K^+ channels. As shown, 10^{-8} mol/L CGRP was able to relax the artery below basal tension, but 10^{-4} mol/L Ba^{2+} (Kir inhibitor) caused marked vasoconstriction even in the presence of CGRP. F, Tabulated results of 3 independent experiments. G, The addition of 10^{-4} mol/L Ba^{2+} to radial arteries caused marked vasoconstriction that was not able to be returned to baseline by the addition of 10^{-8} mol/L CGRP, indicating CGRP could act via the Kir channel. H, Tabulated results of 4 independent experiments. Results are mean $\pm$ SEM, * $P < .05$.

Altered vascular K^+ channel function in cardiovascular disease could be either a cause or consequence of the disease. A current review by Sobey²⁰ clearly describes the role of K^+ channels in cardiovascular disease. Nevertheless, studies have indicated that current medications used in the treatment of hypertension may already inadvertently involve CGRP and thus open K^+ channels. Qin and associates²⁷ demonstrated that the depressor effects of losartan (ang II receptor antagonist) and perindopril (angiotensin-converting enzyme inhibitor) may be partially mediated via increased synthesis and release of CGRP. Furthermore, the antihypertensive effect of the novel drug rutaecarpine may also be mediated by stimulation of CGRP synthesis and release.²⁸ Rutaecarpine is a quinazolinocarboline alkaloid isolated from a widely known Chinese herbal drug Wu-chu-yu. In animal models, its mode of action is suggested to be via stimulating endogenous CGRP release via activation of vanilloid receptor subtype 1.^{28,29} Whether rutaecarpine causes similar effects as CGRP in human radial arteries is the focus of ongoing studies.

The precise mechanisms that regulate the expression and release of CGRP are unknown. However, several different classes of regulatory factors act to modulate CGRP synthesis and release by antagonizing or activating nerve growth factor.³⁰ As CGRP is released directly onto the smooth muscle cell layer from the innervation into the wall, the physiologic concentration of CGRP at the site of action is many times greater than the picomolar plasma concentration observed in healthy human volunteers.³¹ It is generally considered that the levels of CGRP detected in plasma are likely to be due to leakage after localized release rather than a specific systemic function. As well, although CGRP is widely established as a potent vasodilator, its exact mechanism of action remains unclear. There are currently two modes of action, endothelium dependent and endothelium independent.³² The endothelium-dependent pathway suggests that CGRP activity is mediated via the production of endothelial NO, which then relaxes vascular smooth muscle cells.³² The endothelium-independent pathway proposes that CGRP directly

binds to receptors on the smooth muscle cells and is coupled to production of cyclic adenosine monophosphate by adenylate cyclase. The increase in intracellular cyclic adenosine monophosphate concentration stimulates protein kinase A, and this opens K^+ channels and activates calcium sequestration mechanisms to cause smooth muscle cell relaxation.³² The majority of data from studies involving rat, human, and porcine tissues suggest that CGRP can cause vasodilation in the absence of an endothelium, thereby supporting the role of an endothelium-independent pathway.^{32,33} Conversely, studies have also shown that NO synthase inhibitor blocks CGRP activity in the rat aorta, rat pulmonary artery, and human internal thoracic artery, suggesting CGRP-mediated vasodilation is dependent on the presence of NO and therefore the endothelium.³⁴⁻³⁶ In our study, blocking NO synthesis with L-NAME, a NO synthase inhibitor, did not affect CGRP-induced vasodilation, suggesting that CGRP is able to act via a pathway independent of the endothelium or NO. Therefore, this result supports the notion that in human radial arteries, CGRP activity might be mediated by an endothelium-independent pathway. It is difficult to speculate as to why the internal thoracic artery and radial artery respond differently to CGRP, although it could be associated with the increased wall thickness and intimal thickening of radial arteries.¹ However, we show clear evidence that CGRP can induce vasodilation in the presence of calcium-activated K^+ channel blockade, voltage-dependent K^+ channel blockade, but not with inwardly rectifying K^+ channel blockade (Kir), suggesting that a possible mechanism of action for CGRP is by opening the Kir channel. Our data suggest that the development of potassium channel openers could be a new avenue for the treatment of radial arteries before bypass surgery to impair spasm and lessen postoperative risk. Furthermore, the radial arteries used in this study did not respond to acetylcholine-mediated vasodilation (results not shown), indicating that a functional endothelium is not necessary for CGRP function.

In conclusion, this study has demonstrated that CGRP decreased the basal arterial tone in human radial arteries and, when challenged with ang II, CGRP significantly inhibited ang II-induced vasoconstriction in a dose-dependent manner. Inhibition of the Kir channel completely impaired the effects of CGRP, indicating an endothelium-independent mechanism for the actions of CGRP and is thus possibly more effective in diseased human vessels with underlying endothelial dysfunction.

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