Vascular Matrix Metalloproteinase-2–Dependent Cleavage of Calcitonin Gene-Related Peptide Promotes Vasoconstriction

Carlos Fernandez-Patron, Ken G. Stewart, Yunlong Zhang, Erkki Koivunen, Marek W. Radomski, Sandra T. Davidge

Abstract—Matrix metalloproteinase (MMP)-2 has been historically associated with the process of vascular remodeling through the cleavage of extracellular matrix proteins. However, we recently found that MMP-2 also cleaves the endothelium-derived peptide big endothelin-1, ET-1[1–38] and yields the novel vasoconstrictor ET-1[1–32]. We therefore investigated the effects of MMP-2 inhibitors as potential vasodilators. MMP inhibition with ortho-phenanthroline (0.3 to 30 μmol/L) induced vasorelaxation of isolated rat mesenteric arteries (maximum of relaxation=74.5±27.6% at 30 μmol/L). However, phosphoramidon (0.3 to 30 μmol/L), which inhibits some metalloenzymes, but not MMP-2, did not dilate the arteries. Selective inhibition of endogenous MMP-2 with the novel tissue-permeable cyclic peptide CTTHWGFTLC (CTT, 10 μmol/L) also caused vasorelaxation (by 85±6%), whereas STTHWGFTLS (10 μmol/L), an inactive CTT analogue, did not dilate the arteries. Interestingly, the vasorelaxation that results from MMP-2 inhibition was endothelium-independent. Thus, we examined whether MMP-2 acted on peptides derived from the smooth muscle or the perivascular nerves. Recombinant human MMP-2 cleaved calcitonin gene-related peptide (CGRP) specifically at the Gly14-Leu15 peptide bond and reduced the vasodilatory potency of CGRP by 20-fold. Inhibition of MMP-2 increased the amount of intact CGRP in arteries and enhanced vasorelaxation induced by anandamide, which stimulates CGRP release. Vasorelaxation in response to MMP-2 inhibition was abolished by CGRP[8–37], a selective CGRP receptor antagonist, and by capsaicin, which depletes arterial perivascular nerves of CGRP. We conclude that vascular MMP-2 cleaves endogenous CGRP and promotes vasoconstriction. These data suggest a novel mechanism of regulating the vasoactive and, possibly, the neurohormonal actions of CGRP and establish MMP-2 as a modulator of vascular function. (Circ Res. 2000;87:670-676.)

Key Words: vascular • matrix metalloproteinase • calcitonin gene-related peptide

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases expressed in many cells and tissues including vascular smooth muscle and endothelium.1 The breakdown of extracellular matrix proteins (collagens, gelatin, fibronectin, elastins, and laminins) at specific peptide bonds (Gly-Leu/Ile/Val, Ala-Leu/Ile, Gly-Phe) is thought to be a primary mechanism of the biological effects of MMPs. Thus, MMP enzymes have been historically associated with vascular remodeling, a long-term process of cumulative structural changes of the vessel wall.1 The remodeling of the vasculature during hypertension, atherosclerosis, pregnancy, development, and aging may account for some of the alterations in the passive properties of blood vessels in these conditions.1–5

We hypothesized that MMPs could also affect vascular function through the cleavage of vasoactive peptides or their inactive precursors thereby modifying the vasoactivity of these peptides. In support of this general hypothesis, we previously found that vascular MMP-2, the major gelatinase in the vessel wall, cleaves the (Gly32-Leu33) peptide bond of the endothelium-derived peptide big endothelin-1 (ET-1) (ET-1[1–38]) and generates the novel vasoconstrictor ET-1[1–32].6

In this investigation, we used a novel and highly selective MMP-2 inhibitor, CTTHWGFTLC (CTT),7 to examine the contribution of MMP-2 to vascular function, and we expected that this compound would promote vasodilation by blocking the conversion of big ET-1 to ET-1[1–32].6 Interestingly, selective inhibition of MMP-2 was found to induce endothelium-independent vasodilation. Because the vascular endothelium is the main source of endothelin peptides under physiological conditions, we reasoned that MMP-2 may be inducing vasoconstriction primarily by cleaving other yet-undefined vasoactive peptides. The results of our experiments show that vascular MMP-2 specifically cleaves a potent vasodilatory neuropeptide, calcitonin gene-related peptide.
(CGRP), to less vasoactive metabolites and thus promotes vasoconstriction.

Materials and Methods
Recombinant human MMP-2 and MMP-9 were obtained from Chemicon International (Mississauga, Ontario, Canada). CGRP antibodies and the radioimmunoassay (RIA) kit for CGRP were obtained from Phoenix Pharmaceuticals, Inc (Belmont, Calif). Anandamide, capsaicin, and vasoactive peptides (human α-CGRP, human β-CGRP, rat CGRP, adrenomedullin [ADM], substance P, and atrial natriuretic peptide [ANP]) were purchased from Sigma. The selective MMP-2 inhibitor CTT and its inactive noncyclic analogue STTHWGFTLS (STT) were synthesized as previously described.7

Cleavage of Vasoactive Peptides by MMP-2
The general strategy has been described previously.6 Briefly, synthetic peptides (ANP, ADM, substance P, human α-CGRP, human β-CGRP, and rat CGRP; 500 pmol each) were incubated with MMP-2 (140 nmol/L) at 37°C for varying times, from 30 minutes to 72 hours, in HEPES-phosphate saline solution (PSS), pH 7.4. MMP-2 (140 nmol/L) at 37°C for varying times, from 30 minutes to 72 hours, in HEPES-phosphate saline solution (PSS), pH 7.4. Composition of HEPES-PSS was as follows (in mmol/L): NaCl 142, KCl 4.7, MgSO₄ 1.17, CaCl₂ 1.56, HEPES 10, and KH₂PO₄ 1.18. The products of these incubations were analyzed by high-performance liquid chromatography (HPLC), mass spectrometry analysis, and bioassay.

Bioassays for Vascular Effects of MMP-2
Animal protocols were conducted in accordance with institutional guidelines issued by the Canada Council on Animal Care. Male Sprague-Dawley rats (450 to 550 g; BioSciences Animal Services, University of Alberta, Edmonton, Canada) were anesthetized with methohexital sodium (50 mg/kg) and were killed by exsanguination. Small rat mesenteric arteries (~210 μm for inner diameter; 1 to 1.5 mm in length) were dissected from fat tissue and adventitia. Vascular reactivity was studied using vascular diameter (perfusion apparatus; Living Systems Instrumentation) and isometric force (wire myograph; Kent Scientific Corporation) measuring systems. These systems complement each other. Thus, we could measure the actions of specific drugs on arterial lumen, adventitia, or both and correlate them with changes in arterial diameter or isometric force. In the perfusion system, the arteries were cannulated and superfused (at 37°C) with HEPES-PSS, pH 7.4, supplemented with glucose (5.5 mmol/L) while perfused at a flow rate of 10 μL/min.6 Perfused arteries were preconstricted by adding phenylephrine (1.6 mmol/L) to the bath (adventitial side). After 30 minutes, this concentration of phenylephrine caused a 40% to 50% reduction of the resting arterial diameter. Drugs of interest were then added to the bath (adventitial side) or injected into the perfusion line toward the artery to test for luminal effects. The changes in arterial diameter were studied with the aid of a micrometer coupled to a microscope (Olympus SZH10).6 When testing whether the effects of drugs were endothelium-dependent, the arteries were mechanically denuded of endothelium using a human hair threaded through the lumen of the artery and rubbed back and forth. To confirm the effectiveness of deendothelialization, arteries were preconstricted (40% to 50%) with phenylephrine and tested for the absence of relaxation to methacholine (1 μmol/L).6 When measuring the luminal effects of CTT, CGRP, MMP-2 cleavage products of CGRF, or specified drugs on arterial diameter, small volumes (5 to 10 μL) of these substances (0.01 to 10 nmol) were injected into the perfusion line using an HPLC injection valve (Rhodyne Model 9725L, Mandel Scientific Co) provided with a 20-μL loop.6 In the wire myograph, the force developed was used to measure arterial function. The arteries were preconstricted (50%) with phenylephrine (EC₅₀ = 3.9 ± 0.1 μmol/L), and relaxation responses to the specified drugs were measured. Depletion of CGRP in peripheral vessels of the arteries was done by treating arteries for 1 hour with capsaicin (10 μmol/L) followed by washout of capsaicin for 15 minutes (3 times).8–10

Measurement of CGRP
Segments of dissected rat mesenteric arteries (3 mm in length) were equilibrated for 1 hour in HEPES-PSS buffer (room temperature) and subsequently incubated (37°C) for 1 hour in 100 μL capsaicin (10 μmol/L in HEPES-PSS); anandamide (100 μmol/L); and phenylephrine (10 μmol/L), in the absence and presence of CTT (10 μmol/L) or control HEPES-PSS. The solutions were transferred to polypropylene tubes and assayed for immunoreactive CGRP using a rat 125I-CGRP RIA kit (sensitivity: 1 pg CGRP).

Statistics
Results obtained on independent experiments (or animals) were analyzed using Student’s t test or one-way ANOVA. Values of P<0.05 were considered statistically significant.

Results
Pharmacological Inhibition of MMP-2 Induces Vasorelaxation of Rat Mesenteric Arteries
A novel, tissue-permeable, and highly selective gelatinase inhibitor,7 the cyclic peptide CTT, was used to examine whether activity of endogenous MMP-2 modulated vascular function. Although CTT is known to inhibit both MMP-2 and MMP-9, rat mesenteric arteries expressed only the former enzyme (Figure 1A). The gelatinolytic activity of MMP-2 was inhibited by CTT as shown by zymography (Figure 1A). The gelatinolytic activity is MMP-2–dependent.

Figure 1. MMP-2 in rat mesenteric arteries is inhibited by CTT. A, Control, representative gelatin zymography showing the presence of MMP-2 in the homogenate of rat mesenteric arteries. + CTT, Inhibition of MMP-2–dependent gelatinolysis upon exposure (1 hour) of arteries to CTT (10 μmol/L), B, In situ zymography revealing a net gelatinolytic activity in the wall of a rat mesenteric artery. Bar = 50 μm. C, Inhibition of in situ gelatinolytic activity by CTT. The representative images correspond to frozen sections supplemented with 1 μL HEPES-PSS (Control) or CTT (10 μmol/L in HEPES-PSS) buffer to inhibit MMP-2. Similar inhibition was observed when CTT was replaced by tissue inhibitor of metalloproteinases (TIMP)-1 as well as a monoclonal MMP-2 antibody, but not BSA, showing that most of the detected gelatinolytic activity is MMP-2–dependent.

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not MMP-2,11 induced a small constriction (maximum of constriction= 25.6±11.2% at 30 μmol/L, n=4) rather than a dilation of the arteries.

We also studied the vasoactive effects of luminal infusions of CTT. Again, dose- and time-dependent vasorelaxation resulted when CTT (0.1 to 10 nmol) was injected into the line toward the arteries, whereas phosphoramidon induced vasoconstriction (online Figure 1 [see online data supplement available at http://www.circresaha.org] and data not shown, respectively).

These data taken together demonstrate that the activity of endogenous MMP-2 promotes vasoconstriction.

MMP-2 Cleaves the Neuropeptide CGRP but Not Other Vasoactive Peptides

Inhibition of MMP-2 resulted in concentration-dependent vasodilation of both endothelium-intact and denuded arteries (Figure 3). Therefore, we examined the possibility that MMP-2 promoted vasoconstriction through the cleavage of peptide mediators derived from the vascular smooth muscle and/or perivascular nerves. A number of vasopeptides, such as angiotensin I, vasoactive intestinal peptide, and vasopressin, do not contain any known MMP-2–sensitive bonds. However, 4 vasodilatory peptides (ANP, ADM, and the neuropeptides substance P and CGRP) were found to contain Gly-Leu bonds. We investigated whether these peptides were cleaved by MMP-2 and whether this cleavage affected their vasoactivity. Substance P, ADM, and ANP were not cleaved by MMP-2 (Figure 4A). In contrast, CGRP was readily cleaved by MMP-2 (Figure 4A); cleavage was evident after 30 minutes and complete after 6 hours of exposure to MMP-2 (Figures 4A and 4B).

Although MMP-9 was not detected in rat mesenteric arteries6 (Figure 1A), this enzyme is expressed during pathological reactions of the vasculature and cleaves some substrates of MMP-2 (eg, collagen type IV).1 Therefore, we tested whether MMP-9 could cleave CGRP. Interestingly, recombinant human MMP-9 did not cleave CGRP under conditions in which MMP-2 did (Figure 4B).

**Figure 2.** Inhibition of MMP-2 promotes vasorelaxation in endothelium-intact arteries. A, Solid bars, Maximum of vasorelaxation of phenylephrine-preconstricted, perfused arteries to cumulative amounts of CTT added to the bath to yield the indicated concentrations (n=4). The arteries were exposed to each concentration of CTT for 30 minutes. Open bars, STT, an inactive CTT analogue, did not relax the arteries (n=3); the arteries were exposed to each concentration of STT for 30 minutes. B, Representative time course of vasorelaxation in response to CTT (10 μmol/L). Results are mean±SEM from 3 to 4 independent experiments.

**Figure 3.** Inhibition of MMP-2 promotes endothelium-independent vasorelaxation. Vasorelaxation of endothelium-denuded (−) and endothelium-intact (+) arteries upon incubation with CTT (10 μmol/L). NS indicates not significantly different (P=0.26). Results are mean±SEM from 4 independent experiments.

**Figure 4.** MMP-2 specifically cleaves CGRP. A, Representative HPLC traces of synthetic peptides incubated in HEPES-PSS buffer in the absence (top) and presence (bottom) of MMP-2 for the indicated times. β-CGRP was cleaved within 6 hours, yielding 2 peptide fragments (1: CGRP[1–14]; 2: CGRP[15–37]), whereas substance P, ADM, and ANP were not cleaved, even after exposure to MMP-2 for 72 hours. B, MMP-2, but not MMP-9, time dependently cleaved CGRP.
Mass spectrometry and automated NH₂-terminal sequence analysis showed that MMP-2 cleaved CGRP at the Gly₁⁴-Leu₁⁵ peptide bond and yielded two novel peptides: CGRP[1–14], experimental molecular mass 11415.7 Da (oxidized form), and CGRP[15–37], experimental molecular mass 2280 Da, NH₂-terminus, LLSRS.

Cleavage of CGRP by MMP-2 Reduces the Vasodilatory Activity of CGRP

To examine the significance of CGRP cleavage by MMP-2 for vascular reactivity, synthetic CGRP was first incubated in vitro with recombinant human MMP-2. The vasodilatory potency of ADM was not affected when ADM was exposed to MMP-2. Concentration-response curves to intact CGRP or ADM and CGRP or ADM in the presence of MMP-2 as measured in the wire myograph. Bars on the right, Statistical analysis of EC₅₀ values of CGRP and ADM peptides. *P<0.05. Results are mean±SEM from 3 independent experiments.

These results show that cleavage of CGRP by MMP-2 decreases the vasodilatory action of CGRP.

Inhibition of Vascular MMP-2 Results in CGRP-Dependent Vasorelaxation

We investigated whether MMP-2 inhibition resulted in vasorelaxation by preventing cleavage of endogenous CGRP, which is released from perivascular nerves of rat mesenteric arteries. Incubation of arteries with CTT significantly increased the amount of intact CGRP present in the media compared with controls without CTT (Figure 6A). In contrast, pretreatment of the arteries with capsaicin (10 µmol/L), to deplete perivascular nerves of CGRP, abolished the vasodilation induced by CTT (Figure 6B), whereas a subsequent application of CGRP (10 nmol/L) elicited vasorelaxation (not shown). Moreover, the specific CGRP antagonist CGRP[8–37] (1.2 µmol/L) also inhibited the vasodilation induced by CTT (10 µmol/L) (Figure 6B). These data show that inhibition of MMP-2 activity increases the bioavailability of endogenous CGRP, thus promoting vasorelaxation.

We also tested whether vascular MMP-2 could regulate the vasoactivity of anandamide, an endogenous ligand of vanilloid receptors, which dilates arteries by releasing neuropeptides such as CGRP. Exposure of arteries to anandamide resulted in a significant release of CGRP (Figure 7A). Anandamide also induced concentration-dependent vasorelaxation (EC₅₀=43±10 µmol/L, n=3; Figure 7B), although less potent than capsaicin (EC₅₀=0.10±0.05 µmol/L, n=3). Importantly, anandamide-induced vasodilation was inhibited in the presence of the CGRP antagonist CGRP[8–37] (1.2 µmol/L) (data not shown). More-
Preincubation of the arteries for 1 hour with Evasorelaxation (adventitial side) of preconstricted, perfused arteries resulted in

Inset, EC50 values for vasodilatory effects of AEA in the presence or absence of CTT (1 μmol/L). *P<0.05. Results are mean±SEM from 3 independent experiments.

Figure 7. MMP-2 modulates the vasodilatory effects of anandamide. A, Exposure (1 hour) of the arteries to anandamide (AEA, 100 μmol/L) resulted in a significant release of CGRP from the arteries. B, Addition of AEA (0.1 to 100 μmol/L) to the bath (adventitial side) of preconstricted, perfused arteries resulted in vasorelaxation (○). Preincubation of the arteries for 1 hour with CTT (1 μmol/L) enhanced the vasodilatory action of AEA (●). Inset, EC50 values for vasodilatory effects of AEA in the presence or absence of CTT (1 μmol/L). *P<0.05. Results are mean±SEM from 3 independent experiments.

over, pretreatment of the arteries with CTT, at a subthreshold concentration (1 μmol/L), which did not result in vasorelaxation, potentiated the vasodilator effects of anandamide (Figure 7B). These data indicate modulation of the CGRP-dependent vasodilator effects of anandamide by MMP-2.

In further support of regulation of endogenous CGRP metabolism by MMP-2, the incubation with subthreshold concentrations of CTT not only increased the levels of intact CGRP in the arteries but also reduced arterial responsiveness to the vasoconstrictor phenylephrine (data not shown).

**Discussion**

The present results establish that MMP-2, an enzyme historically associated with vascular remodeling, is a modulator of vascular reactivity. Selective pharmacological inhibition of vascular MMP-2 induced vasodilation, showing that this enzyme promotes vasoconstriction.

Recently, a novel class of synthetic, low-molecular-weight, tissue-permeable selective inhibitors of MMP-2 was discovered from phage display peptide libraries. In the present study, we examined the effects of CTT, the prototype peptide of this new class of inhibitors, on the reactivity of rat mesenteric arteries. Because a cyclic structure of CTT is necessary for enzyme inhibition,7 the noncyclic analogue peptide STT effectively served as a negative control when testing the specificity of the vasoactive actions of CTT. Treatment of preconstricted rat mesenteric arteries with CTT, but not with STT, was found to induce vasorelaxation. Similar to CTT, a structurally unrelated MMP-2 inhibitor, ortho-phenantroline, also induced vasorelaxation, whereas phosphoramidon, which inhibits some metalloproteinases, but not MMP-2, did not dilate the arteries. Collectively, these data clearly demonstrate that MMP-2 promotes vasoconstriction.

To elucidate the mechanism of MMP-2-dependent vasoconstriction, we tested the hypothesis that MMP-2 acts through the cleavage and thereby activation/inactivation of vasoactive peptides. We recently found that vascular MMP-2 cleaves big ET-1 (ET-1[1–38]) yielding the novel vasoconstrictor ET-1[1–32].6 In the course of the present investigation, we observed that pharmacological inhibitors of MMP-2 relaxed both endothelium-intact and denuded arteries with similar concentration dependence and time course profiles. Because the vascular endothelium is the main source of endothelin peptides, we reasoned that MMP-2 might be inducing vasoconstriction of endothelium-denuded vessels by cleaving other vasoactive peptides derived from the smooth muscle and/or perivascular nerves. Examination of the amino acid sequence of various vasoactive peptides for the presence of MMP-2–sensitive peptide bonds (Gly-Leu/Ile/Phe/Val) revealed that several vasoactive peptides (ANP, ADM, substance P, and CGRP) contain these bonds and, therefore, could be susceptible to cleavage by MMP-2. These peptides were incubated with MMP-2 and the resultant products examined by HPLC. Interestingly, MMP-2 readily cleaved only CGRP at the Gly14-Leu15 peptide bond. It is not clear yet why MMP-2 cleaved only CGRP and not the other vasoactive peptides. A possible explanation could be that the latter peptides lack the required docking domain that allows MMP-2 to bind and subsequently cleave its substrates.1 Interestingly, ADM, which is structurally related to CGRP, was not cleaved by MMP-2. Our preliminary data suggest that ADM may act rather as an inhibitor of MMP-2 (unpublished observation, 2000).

We then studied the significance of MMP-2–dependent cleavage of CGRP for vascular reactivity and found that CGRP metabolism resulted in reduced vasodilator potency of this peptide. Experimental data suggest that MMP-2–dependent cleavage of endogenous CGRP modulates vasoreactivity of rat mesenteric arteries: (1) incubation with CTT increased the amount of intact CGRP in the media surrounding the arteries; (2) vasorelaxation induced by MMP-2 inhibition was abolished by depleting perivascular nerves of CGRP with capsaicin; (3) blockade of the CGRP receptors with the specific antagonist CGRP[8–37] also abolished vasorelaxation caused by MMP-2 inhibition; (4) inhibition of MMP-2 potentiated the vasodilator effects of anandamide, an endogenous agonist of CGRP release; and (5) MMP-2 inhibition also blunted the vasoconstrictor effects of phenylephrine, and this effect was associated with an increase in intact CGRP levels in arteries. Therefore, we suggest that MMP-2 promotes vasoconstriction through the cleavage of CGRP, which reduces CGRP-dependent vasorelaxation.

CGRP exerts its biological activity through binding to its receptor, an event effectively controlled by a receptor-affinity modulating protein.12,13 Our data also suggest MMP-2–dependent regulation of CGRP action on the vessel wall.
These effects of MMP-2 are mediated via the generation of novel peptides CGRP[1–14] and CGRP[15–37]. We have found that these peptides exert weaker vasodilatory effects on arteries than the parent CGRP. Interestingly, CGRP[15–37] blunted the vasorelaxation to a subsequent challenge of the arteries with intact CGRP (data not shown). Moreover, the synthetic commercially available peptide CGRP[8–37] also acts as an antagonist of the vasorelaxant effects of CGRP.9,10 Future studies will evaluate the physiological and pharmacological significance of the interactions between CGRP and the peptides formed by MMP-2.

In the present study, we used anandamide as a pharmacological tool to induce the release of CGRP from pervascular sensory nerves.10 The anandamide-CGRP pathway is thought to be involved in the regulation of various afferent and efferent neuronal functions, including nociception, visceral reflexes, local vasodilation, and neurogenic inflammation.10 We suggest that vascular MMP-2 is also involved in the regulation of these functions through the cleavage of CGRP. In our preparations, anandamide was less potent as a vasodilator than reported earlier.10,14 The cause for this difference is unclear. It may reflect a lower sensitivity of these preparations to vanilloid receptor agonists, because capsaicin was also less potent than documented.10 Alternatively, it may also be due to differences in potency of anandamide in various tissue preparations and bioassay systems (References 10 and 14 through 17 and citations therein).

Our previous6,16–21 and present findings indicate that MMPs are major modulators of hemostatic and vasoactive pathways under physiological and pathological conditions. Indeed, MMP-2 and MMP-9 were identified in human platelets and shown to differentially regulate platelet aggregation.18,19 Whereas release of MMP-2 promoted aggregation, MMP-9 inhibited aggregation.19 More recently we showed that thrombin, a key enzyme in the response to tissue injury and the clotting cascade,22 induces a rapid release of MMP-2 from arteries20,21 as well as platelets.18,19 MMP-2 was also found to contribute to the vasoactivity of thrombin, through pathways that may complement the proteinase-activated receptor-1 and involve the generation of ET-1[1–32].21 MMP-2–dependent cleavage of CGRP and generation of ET-1[1–32]6 may be relevant at sites of active remodeling (eg, the settings of an atherosclerotic plaque) and inflammation, as well as in vasospastic and thrombotic disorders such as hypertension, preeclampsia, and thrombosis, for which the concurrent upregulation of MMP-2, big ET-1, and CGRP has been documented.1,23–26 MMP-9, which is a closely related gelatinase MMP-2, is upregulated under these conditions and may contribute to the pathophysiology.1,19,26 Interestingly, only MMP-2, but not MMP-9, cleaved CGRP, whereas MMP-9, but not MMP-2, cleaves substance P.27 Therefore, the biological activity of vasopetides and neuromediators may be differentially regulated by different MMPs, which likely contributes to a complex modulation of vascular contractility in pathophysiology.

In conclusion, vascular MMP-2 promotes vasoconstriction by cleaving a potent vasodilatory neuropeptide, CGRP, thereby inhibiting CGRP-dependent vasodilation. We suggest that MMP-2 is a novel, major modulator of vascular function.

MMP inhibitors are now being tested in the clinic for their ability to stop progression of cancer and inflammatory diseases.1,28 The ability of MMP-2 inhibitors to facilitate vasodilation, induce regression of vascular disease,2 reduce ischemia/reperfusion-induced damage,11 and inhibit platelet aggregation18 may prove valuable in the treatment of vasospastic and thrombotic conditions.

Acknowledgments

This work was supported by grants of the Medical Research Council of Canada (MRC) and the Heart and Stroke Foundation of Canada (HSFC) to S.T. Davidge and the MRC to M.W. Radomski. E. Koivunen was supported by the Academy and the Cancer Society of Finland. S.T. Davidge is a scholar of the Alberta Heritage Foundation for Medical Research (AHFMR) and the HSFC. M.W. Radomski is a scientist of the MRC. C. Fernandez-Patron is a postdoctoral fellow of the MRC, the Canadian Hypertension Society, and the AHFMR.

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Circ Res. 2000;87:670-676
doi: 10.1161/01.RES.87.8.670

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Legend to Figure: Dose and time-dependent vasorelaxation of reperfused arteries to luminal infusions of CTT (0.1-10 nmol). Arrows indicate the moment of application of the indicated amounts of CTT. Results are means ± SEM of 3 independent experiments.

Online Figure 1 MS # 1333 R1 Permeability Partition of the Vasomotor Effects of MMP-2
Legend to Figure: Vascular effects of intact CRP (CRP[1-14]) and the novel peptides resulting from MMP-2 cleavage (CRP[1-14] and CRP[1-37]) after inclusion into rat mesenteric arteries. Results are means ± sem of 3 independent experiments for each peptide.

Online Figure 7: MS # 1333. R1, Remanadan-Potenz et al. Vascocontractor effects of MMP-2.

ON-LINE SUPPLEMENT