Omapatrilat in Subtotal Nephrectomy-Salt Hypertension
Role of Calcitonin Gene-Related Peptide
Scott C. Supowit, Huawei Zhao, Donna H. Wang, Donald J. DiPette

Abstract—Calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, plays a counterregulatory role in subtotal nephrectomy–salt (SN-salt)–induced hypertension, reflecting a stimulation of the efferent vasodilator function of perivascular sensory nerves. To determine the effect of omapatrilat, a dual ACE and neutral endopeptidase inhibitor, on blood pressure and the potential antihypertensive role for CGRP, 24 male Sprague-Dawley rats were separated into 4 groups: (1) SN-salt, (2) SN-salt plus omapatrilat (80 mg · kg⁻¹ · d⁻¹ in the drinking water), (3) sham-operated plus salt, (4) sham-operated plus salt and omapatrilat. After 11 days the mean arterial pressure was higher in the SN-salt group (174±10 mm Hg) versus the sham-operated–salt (109±4 mm Hg) and sham-operated–salt plus omapatrilat (105±3 mm Hg) groups. Omapatrilat treatment of the SN-salt rats significantly decreased the mean arterial pressure to 123±7 mm Hg and significantly reduced the heart-to-body weight ratio. Intravenous administration of a specific CGRP receptor antagonist produced a significant 10±2 mm Hg mean arterial pressure increase in the untreated SN-salt hypertensive rats but was without effect in the other groups. This indicates that CGRP does not contribute to the antihypertensive actions of omapatrilat. In addition, CGRP mRNA and protein content in dorsal root ganglia were decreased ~25% in the SN-salt plus omapatrilat rats. Thus, omapatrilat not only markedly reduces the blood pressure in this model of renal failure–induced hypertension but may also prevent the abnormal compensatory stimulation of the vasodilator activity of the peripheral sensory nervous system. (Hypertension. 2001;38[part 2]:697-700.)

Key Words: nervous system ■ neuropeptides ■ hypertension, experimental ■ antihypertensive agents ■ vasodilator agents

Calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, participates in the regulation of vascular tone and regional organ blood flows, both under normal physiological conditions and in the pathophysiology of hypertension.¹,² A prominent site of CGRP synthesis is the dorsal root ganglia (DRG), which contain the cell bodies of sensory nerves that terminate peripherally on blood vessels and centrally in the spinal cord. A dense perivascular CGRP neural network is seen around the blood vessels in virtually every vascular bed.¹,² Several lines of evidence suggest that the vasodilator activity of CGRP is mediated by the release of this peptide from predominantly capsaicin-sensitive perivascular sensory nerve terminals.³

We have previously reported that in the deoxycorticosterone-salt⁴ and subtotal nephrectomy–salt (SN-salt)⁵ rat models of acquired hypertension, CGRP acts as a compensatory vasodilator to partially counteract the blood pressure (BP) increase. In deoxycorticosterone-salt rats, the antihypertensive activity of CGRP appears to be mediated through an upregulation in the neuronal (DRG) expression of this peptide. The SN-salt model did not exhibit this increase in CGRP production. We later demonstrated that in this setting, the counterregulatory effects of CGRP on BP were mediated via an enhanced sensitivity of the vasculature to the vasodilator activity of this peptide.⁷

Omapatrilat (OM), a drug currently under development for the treatment of hypertension and congestive heart failure, simultaneously inhibits the activities of ACE and neutral endopeptidase (NEP). NEP modulates the half-life of several potent vasoactive proteins, including the natriuretic peptides (ANP, BNP, CNP), bradykinin, substance P, adrenomedullin, and CGRP.⁸ Therefore, an increase in the vasodilator activity of CGRP, via inhibition of NEP, may be a third mechanism by which this peptide is able to exert its BP-lowering effects. Thus, this study was designed to test the hypothesis that CGRP contributes to the antihypertensive activity of OM in SN-salt hypertension.

Methods

Animal Protocols
A total of 24 male Sprague-Dawley rats (150 to 175 g. Harlan, Indianapolis, Ind) were used in this study, and all protocols were approved by our institution’s Animal Care and Use Committee. The OM was generously provided by Bristol-Meyers, Squibb (Princeton, NJ). For the surgical procedures, the rats were anesthetized with ketamine and xylazine (80 and 4 mg/kg IP, respectively). SN-salt hypertension was induced in 12 animals by the total removal of the right kidney and the upper and lower poles of the left kidney. These animals were given 1% saline to drink ad libitum. Twelve sham-operated rats that received 1%-saline drinking water were used as the controls. The animals were then divided into 4 groups: (1) SN-salt, (2) SN-salt+OM (80 mg · kg⁻¹ · d⁻¹ in the drinking water), (3) sham-salt, (4) sham-salt+OM. The studies were conducted 11 days after the surgical procedures.

Received March 26, 2001; first decision June 12, 2001; revision accepted July 2, 2001.
From the Department of Medicine, College of Human Medicine, Michigan State University, East Lansing, Mich.
Correspondence to Scott C. Supowit, PhD, Michigan State University, 138 Service Rd, B-338 Clinical Center, East Lansing, MI 48824. E-mail scott.supowit@ht.msu.edu
© 2001 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

© 2001 American Heart Association, Inc.
MAP Measurements and CGRP Receptor Antagonist Administration

For these studies, each rat was weighed then anesthetized as described above. The left carotid artery was cannulated for continuous measurement of MAP and heart rate with a pressure transducer coupled to a recorder (Gould Instruments). The right jugular vein was also cannulated for infusion of bolus doses of saline (200 μL) and the CGRP receptor antagonist CGRP8–37 (1 mg/kg, Phoenix Pharmaceuticals). We have previously demonstrated in vivo that this dose of CGRP8–37 completely blocks the BP-lowering effect of exogenous CGRP (intravenous administration) and has no agonist activity.5 Hemodynamic studies were performed ≈3 hours after surgery with the rats fully awake and unrestrained. After completion of these studies, the rats were deeply anesthetized by administration of ketamine and xylazine (100 and 5 mg/kg) through the jugular vein catheter. The rats were then killed, and the cervical, thoracic, and lumbar DRG were removed and then frozen at −80°C. The hearts from all the rats were removed and weighed.

Hybridization Probes and RNA Analysis

The α-CGRP hybridization probe was a 1.4-kb Sau 3A rat genomic restriction fragment containing CGRP exons 5 and 6.6 This probe hybridizes to both α- and β-CGRP mRNA species. The 18S rRNA hybridization probe was a 1.15-kb Bam HI–Eco RI restriction fragment of the mouse 18S rRNA gene.10 The DNA inserts were purified by agarose gel electrophoresis and subsequently labeled with [α-32P]dCTP by a random hexanucleotide DNA-labeling kit (Amersham). Total cellular RNA was isolated from the DRG by Trizol reagent (GIBCO-BRL). The RNA samples were subjected to electrophoresis on denaturing formaldehyde-agarose gels.11 The fractionated RNAs were then transferred to nylon membranes and hybridized with the labeled CGRP probe. As a control, the CGRP probe was removed from the membranes that were then hybridized with the 18S rRNA probe. The hybridization signals were quantified by scanning densitometry using the Imagequant program (Molecular Dynamics).

Radioimmunoassay

To determine immunoreactive CGRP (CGRP) content in the DRG from the 4 groups of rats, a commercially available rabbit anti-rat CGRP radioimmunoassay kit (Phoenix Pharmaceuticals) was used.6 This antibody has 100% reactivity with rat α-CGRP and 79% reactivity with rat β-CGRP. The assay was performed as recommended by the supplier, and the total protein content in each sample was determined by the method of Bradford (Bio Rad).

Statistical Analysis

Statistical significance was determined by ANOVA followed by the Tukey-Kramer multiple comparisons test. A value of P<0.05 was assumed to be significant. The data in the figures are expressed as mean±SEM.

Results

Effects of Omapatrilat Treatment and CGRP8–37 Administration on Blood Pressure

Eleven days after the initiation of the protocols, each rat had arterial (for continuous MAP recording) and intravenous (for drug administration) catheters surgically implanted and were studied in a fully awake and unrestrained state ≈3 hours after surgery. As shown in Figure 1, the MAP was significantly higher in the SN-salt group (174±10 mm Hg) compared with the sham-operated controls (109±4 mm Hg). Administration of OM in the drinking water had no significant effect on MAP in the control rats (105±3 mm Hg); however, the MAP in the SN-salt+OM group was decreased markedly to 123±7 mm Hg, which was not significantly different from the values of the 2 control groups.

To determine whether CGRP contributes to the OM-mediated reduction in MAP, each animal was treated acutely with bolus doses of saline (200 μL) and the CGRP receptor antagonist CGRP8–37 (1 mg/kg in 200 μL saline) (Figure 2). Injection of saline produced a negligible increase in MAP in each of the 4 groups. As expected, CGRP8–37 administration produced a significant 10±1 mm Hg MAP increase in the SN-salt hypertensive rats, and was without effect in the sham-operated groups. In the OM-treated SN-salt group, CGRP receptor antagonist administration resulted in a 4±1 mm Hg increase in the MAP; however, this was not significantly different from the values obtained from the 2 control groups. The pressor activity of CGRP8–37 observed in the SN-salt hypertensive rats was short-lived, lasting ≈120 seconds. This transient effect of the antagonist has been observed in our previous studies and in those from other investigators who have used CGRP8–37 to blockad the CGRP receptor in vivo and most likely reflects the rapid proteolysis of this peptide receptor antagonist in the circulation.1,2,5,6

Effect of Omapatrilat on Cardiac Hypertrophy

The SN-salt rats that received the OM had a significant reduction in total heart weight (0.36±0.01%, heart/body weight) compared with that of the untreated SN-salt group (0.43±0.01%). OM treatment, however, did not completely normalize the heart weight to the values observed in the sham-operated (0.31±0.01%) and the sham-operated+OM (0.32±0.01%) groups.

Analysis of CGRP mRNA and Peptide Levels in the SN-Salt and Control Rats

To determine whether neuronal CGRP expression was altered between any of the groups, CGRP mRNA and iCGRP levels
were quantified in the DRG taken from the rats used in the aforementioned blood pressure experiments. Figure 3 is a representative Northern blot showing both the 1.2-kb CGRP mRNA species (α- and β-CGRP) and 18S rRNA present in the DRG RNA samples. Scanning densitometry was then performed to quantify the hybridization signals (Figure 4A). Normalization of the values for CGRP mRNA to those for the 18S rRNA showed that CGRP mRNA accumulation was significantly reduced in the SN-salt hypertensive rats. SN-salt and control rats. Total cellular RNA samples isolated from DRG were analyzed as described in the text. With the rats fully awake and unrestrained, bolus doses of saline (300 μL) and CGRP8–37 (1 mg/kg in 300 μL saline) were given intravenously. 1P<0.05, SN-salt vs the other 3 groups.

**Discussion**

The results of this study show that administration of the vasopeptidase inhibitor OM decreases the BP and attenuates the cardiac hypertrophy in SN-salt hypertension, a low-renin salt-dependent model of experimental hypertension. However, CGRP does not appear to contribute to the BP-lowering effects of this drug. To the best of our knowledge, this is the first report describing the use of OM in this particular model of acquired hypertension. The marked reduction in BP in this setting was not unexpected because OM has been shown to lower BP in several animal models of hypertension regardless of sodium status or the degree of activation of the renin-angiotensin system.8 Furthermore, it appears that the BP-lowering effect of OM is greater than that seen with ACE or NEP inhibitors alone.8,12 However, one must be cautious when interpreting the results between different studies using this model. Several variables must be taken into account, including the severity of hypertension, which is dependent on the duration of the protocol and the degree of renal mass reduction; the route of drug administration; and whether the drug is given acutely (hours) or chronically (days to weeks).

The significant reduction in cardiac hypertrophy observed following OM treatment again is not surprising given the extensive clinical and experimental experience with ACE inhibitors.13 However, we cannot rule out the possibility that this effect resulted solely from the lowered blood pressure. The impact of NEP inhibitors on cardio- and vaso-protective mechanisms is not nearly as well defined.8,14 It should be noted that OM administration did not completely normalize the heart-to–body weight ratio. It may be that although OM treatment of the SN-salt rats reduced the BP by 50 mm Hg, the final MAP of this group was ≈20 mm Hg higher than the levels seen in the 2 control groups. Even though these values were not statistically different with the number of animals in each group studied, the cardiac hypertrophy observed in the SN-salt+OM group may suggest that these animals were in fact mildly hypertensive compared with the untreated rats.

Acute administration of the CGRP receptor antagonist significantly increased the MAP in the SN-salt rats but was without effect in the 2 control groups, confirming our previous results that this peptide is playing a compensatory vasodilator role to attenuate the BP increase. In the SN-salt+OM rats, CGRP8–37 caused a small but insignificant MAP increase, suggesting that CGRP does not contribute to the antihypertensive actions of this drug. Because it is not possible to chronically inhibit CGRP activity by blockade of its receptor with the intravenously administered peptide receptor antagonist, it cannot be determined whether CGRP participates in the cardioprotective effects of OM. Therefore, chronic blockade of the CGRP receptor may render different results in regards to blood pressure and cardiac hypertrophy.

One possible interpretation of the BP results is that inhibition of NEP does not sufficiently augment CGRP levels to impact systemic BP. Even though CGRP is a substrate for NEP, it has been suggested that other peptidases such as tryptase and/or matrix metalloproteinase-2 are the primary regulators of CGRP degradation.15 We were unable to determine circulating levels of CGRP in this study because the antagonist interferes with the RIA. A more likely explanation is that a marked reduction in BP, as seen in this study, abolishes the need for the sensory nervous system, via CGRP, to buffer the BP elevation. We have recently reported that the antihypertensive effects of CGRP in this model are mediated...
through a marked increase in vascular reactivity to this peptide. The mechanisms that initiate and inhibit this response remain to be determined. We also do not know whether other classes of antihypertensive drugs will be able to reverse the counterregulatory actions of CGRP and if this response is related to the effectiveness of these agents in reducing the BP.

The most surprising result of this study was the decrease in DRG CGRP mRNA and peptide content in the SN-salt 1 OM rats. This suggests that OM treatment not only prevented the compensatory depressor response to CGRP, perhaps by preventing the increase in vascular reactivity to CGRP, but also inhibited further the efferent vasodilator activity of the sensory nerves through a down-regulation of CGRP synthesis. The mechanism by which this occurs and whether this effect is specific for vasopeptidase inhibitors are not known. In summary, OM treatment not only reduces the BP and attenuates cardiac hypertrophy in this model of renal-failure–induced hypertension but may also block the compensatory stimulation of the vasodilator activity of the sensory neuropeptide CGRP.

Acknowledgment

These studies were supported by National Institutes of Health grant HL-44277.

References

Omapatrilat in Subtotal Nephrectomy-Salt Hypertension: Role of Calcitonin Gene-Related Peptide
Scott C. Supowit, Huawei Zhao, Donna H. Wang and Donald J. DiPette

Hypertension. 2001;38:697-700
doi: 10.1161/hy09t1.095759

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/38/3/697

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/