Calcitonin Gene-Related Peptide Protects Against Hypertension-Induced Heart and Kidney Damage

Scott C. Supowit, Arundhati Rao, Mark C. Bowers, Huawei Zhao, Gregory Fink, Barbara Steficék, Parag Patel, Khurshed A. Katki, Donald J. DiPette

Abstract—Calcitonin gene-related peptide is a potent vasodilator neuropeptide that is localized in perivascular sensory nerves. To determine whether α-calcitonin gene-related peptide possesses protective activity against hypertension-induced end organ damage, hypertension was induced in α-calcitonin gene-related/calcitonin peptide knockout and wild-type mice by uninephrectomy, deoxycorticosteroid administration, and 0.9% saline drinking water. These mice were instrumented previously for long-term telemetric blood pressure recording. Control groups were sham-operated and given tap water. Mean arterial pressures were determined, and 3 weeks after initiation of each protocol, tissues were taken for histopathologic studies. The deoxycorticosteroid-salt protocol produced a significant 35% mean arterial pressure increase in both mouse strains. No pathological changes were observed in sections of aortas and femoral arteries from any of the groups studied. Likewise, heart and kidney sections from the hypertensive wild-type mice showed no pathological changes compared with their normotensive counterparts. In contrast, marked vasculitis was seen in the heart sections from the deoxycorticosteroid-salt–treated α-calcitonin gene-related peptide knockout mice with thickening and inflammation of the vessel walls. In addition, myocarditis and focal epicarditis with areas of myocardial necrosis were present. Kidneys of these mice exhibited prominent glomerular changes including congestion of the capillary loops, focal mesangial and crescent proliferation, and focal histocytic infiltration. Urinary microalbumin was significantly higher in the hypertensive α-calcitonin gene-related peptide knockout compared with hypertensive wild-type mice. These data suggest that deletion of the α-calcitonin gene-related peptide gene makes the heart and kidneys more vulnerable to hypertension-induced end organ damage. (Hypertension. 2005;45:1-6.)

Key Words: hypertension, experimental mice calcitonin gene-related peptide blood pressure

Calcitonin gene-related peptide (CGRP), a 37-aa neuropeptide, is derived from tissue-specific splicing of the primary transcript of the calcitonin (CT)/CGRP gene.1–4 Whereas CT is produced mainly in the C cells of the thyroid, CGRP synthesis is limited almost exclusively to specific regions of the central and peripheral nervous systems. There is a second CGRP gene (β-CGRP) that does not produce CT and is also synthesized primarily in neuronal tissues.4 The 2 CGRP genes, α-CGRP and β-CGRP in the rat and I and II in humans, differ in their protein sequences by 1 and 3 amino acids, respectively, and the biological activities of the 2 peptides are quite similar in most vascular beds.1,2

Immunoreactive CGRP and its receptors are widely distributed in the nervous and cardiovascular systems.1–3 In the peripheral nervous system, prominent sites of CGRP synthesis are the dorsal root ganglia (DRG). These structures contain the cell bodies of sensory nerves that terminate peripherally on blood vessels and centrally in laminae I/II of the dorsal horn of the spinal cord.2 A dense perivascular CGRP neural network is seen around the blood vessels in all vascular beds. In these vessels, CGRP-containing nerves are found at the junction of the adventitia and the media passing into the muscle layer.4 Receptors for CGRP have been identified in the media, intima, and endothelial layer of resistance vessels.1,4

CGRP is the most potent vasodilator discovered to date, and it has positive chronotropic and inotropic effects.5,6 CGRP has been shown to selectively dilate multiple vascular beds, with the coronary vasculature being a particularly sensitive target.6,7 Systemic administration of CGRP decreases blood pressure (BP) in a dose-dependent manner in normotensive and hypertensive animals and humans.1,5–7 The primary mechanism responsible for this reduction in BP is peripheral arterial dilation.

A direct role for CGRP in experimental hypertension has been established. CGRP can significantly attenuate the patho-
logical effects of chronic hypoxic pulmonary hypertension.8 Furthermore, we have reported that CGRP plays a compensatory vasodilator role to attenuate the BP increase in 3 models of experimental hypertension: deoxycorticosterone-salt (DOC-salt),9,10 subtotal nephrectomy (SN-salt),11 and t-NAME induced hypertension during pregnancy.12 This antihypertensive activity appears to be mediated by an up-regulation of neuronal (DRG) CGRP synthesis and release or through an enhanced sensitivity of the vasculature to the dilator effects of this neuropeptide.9–13

These studies point to a key role for CGRP in the regulation of peripheral vascular tone and regional organ blood flow under normal physiological and pathophysiological conditions. To better understand the long-term effects of CGRP on cardiovascular function, an α-CGRP/CT knockout (KO) mouse model has been generated using a gene targeting approach.14 We demonstrated previously that the α-CGRP/CT–deficient mice display a significant increase in basal BP and a significant decrease in basal coronary flow rates.15 Because CGRP has such potent biological effects on the heart and kidneys, and in light of several lines of indirect evidence suggesting that CGRP is an endogenous organ-protective agent,1,2,6,7,17 the purpose of this study was to determine whether end organ damage is enhanced in hypertensive α-CGRP KO mice compared with their hypertensive wild-type (WT) counterparts.

Methods

Animals

Experiments were approved by the university animal care and use committee and were consistent with the ethical guidelines of the National Institutes of Health. The mouse model lacking the α-CGRP/CT gene was created by replacing exons 2 through 5 of the mouse CT gene with PGK neoBPA.14 Homologous recombination was confirmed by Southern analysis with 5′ and 3′ probes. The homozygous α-CGRP (−/−) breeding pairs were derived from an inbred strain on a 129/C57 genetic background. KO mice were generated and kindly provided by Robert F. Gagel, MD (University of Texas, M.D. Anderson Cancer Center, Houston). α-CGRP KO mice were subsequently backcrossed into C57BL/6 mice. This strain of mice was then used for the WT controls. Characterization of these KO mice with regard to phenotype and α-CGRP and β-CGRP expression has been published previously.14,15

Induction of DOC-Salt Hypertension and Mean Arterial Pressure Determination

Although we confirmed previously the elevated basal BP in the α-CGRP KO mice compared with their WT counterparts by long-term radiotelemetric measurement,18 it was necessary to establish the BP phenotype of the DOC-salt–treated α-CGRP and WT mice. Ten-week-old male mice were anesthetized with ketamine (80 mg/kg body weight) and xylazine (4 mg/kg body weight), the radiotelemetric catheter was surgically implanted into the left carotid artery, and the transmitter body was placed subcutaneously in the lower right side of the abdomen.19 After a 1-week recovery period, radiotelemetric recording data of mean arterial pressure (MAP) and heart rate were collected for 6 days (10 seconds/10 minutes per day). At this time, the DOC-salt protocol was initiated. The 2 control groups, consisting of α-CGRP KO and WT mice without DOC-salt treatment, no significant gross postmortem and microscopic examination was done using 15- to 16-week-old male α-CGRP KO (n=4) and WT (n=3) mice. After being weighed, mice were anesthetized with ketamine/xylazine and then euthanized. The body cavities, and integumentary, alimentary, respiratory, circulatory, urogenital, endocrine, hematopoietic, musculoskeletal, and nervous systems were examined for the presence of any gross postmortem alterations. In addition, the heart, kidneys, brain, lungs, liver, stomach, small and large intestine, adrenal gland, spleen, testicles, epididymis, and skin were removed and fixed in PBS containing 10% neutral buffered formalin, dehydrated, embedded in paraffin, and cut into 5-μm-thick sections. These were stained with hematoxylin and eosin.

After the BP and heart rate determinations in the DOC-salt and control α-CGRP KO and WT mice, animals were anesthetized with ketamine/xylazine and then euthanized. Hearts, kidneys, aortas, and femoral arteries were removed and fixed in PBS containing 4% paraformaldehyde, dehydrated, embedded in paraffin, and cut into 4-μm-thick sections. These were stained with hematoxylin and eosin or trichrome. The grading schemes for the histopathologic analyses are arbitrary. Because these analyses are primarily qualitative and subjective, 2 pathologists blinded with regard to the identity of the tissues samples, performed these studies to minimize individual bias. Because the lesions in the kidneys are almost exclusively seen in the glomeruli, the Glomerular Activity Index (an adapted biopsy index for lupus nephritis) was used. This index is as follows: glomerular proliferation 0 to 3, polymorph nucleocyte (PMN) 0 to 3, necrosis (0 to 3)/x2, cellular crescents (0 to 3)/x2, monocytes 1 to 2. We observed a glomerular proliferation of 2, crescents (2 to 3)/x2, monocytes 1 to 2. The total was 8 of 24, which would be a combined grade of 2, which is considered moderate. In the heart and vessels, the inflammation is graded on a scale of 1 to 4 with 1 being minimal, 2 being focal and mild, 3 being focal and moderate or diffuse and mild, and 4 being diffuse and severe with necrosis.

Urinary Microalbumin Analysis

Twenty-four-hour urine samples were collected from the mice in metabolic cages 21 days after initiation of the DOC-salt protocol. Microalbumin was quantified using a murine microalbuminuria ELISA (Exocell Inc). Assays were performed as recommended by the supplier.

Statistical Analysis

Statistical significance was determined by the Student t test or where appropriate by ANOVA followed by the Tukey–Kramer multiple comparisons test. The acceptable level of significance was P<0.05.

Results

Gross Postmortem and Histopathologic Examination

Although the α-CGRP KO mice appear to have a normal phenotype,14 with the exception of an elevated basal MAP,15,18 before using these animals for histopathologic studies after induction of hypertension with the DOC-salt protocol, a comprehensive pathological evaluation was performed to determine whether there were any significant developmental or pathological changes in the absence of treatment. No significant gross postmortem or histopathologic alterations were detected in the body cavities, or the integumentary, alimentary, respiratory, circulatory, urogenital, endocrine, hematopoietic, musculoskeletal, and nervous systems of the α-CGRP KO mice compared with their WT counterparts. In addition, there was no microscopic evidence of vascular alterations or vascular variations among the mice.
examined. The exception to the results described above was that the heart-to-body weight ratio was increased \( \approx 10\% \) in the \( \alpha \)-CGRP KO mice compared with the WT mice as reported previously.\(^{18} \)

MAP Measurements
Figure 1 shows the results of the long-term telemetric recording of MAP. In agreement with our previous study,\(^{18} \) basal average 24-hour MAP (days 7 to 12) was significantly higher in the \( \alpha \)-CGRP KO (120±3 mm Hg) compared with WT (107±3 mm Hg) controls. After initiation of the DOC-salt protocol (day 12), the BP increased rapidly in both groups to final values (days 28 to 34) of 166±5 mm Hg for the \( \alpha \)-CGRP KO and 147±4 mm Hg for the WT mice. When normalized to basal BP, this represents an \( \approx 35\% \) (and equal) increase in MAP for the 2 groups. The MAP was not significantly changed from basal levels in the 2 control groups during the course of the treatment period. The \( \alpha \)-CGRP KO and WT mice displayed a normal 24-hour circadian rhythm before and after DOC-salt treatment. The average heart rate tended to be higher in the \( \alpha \)-CGRP KO mice but was not statistically significant.\(^{18} \)

Histopathologic Study of the Hypertensive and Control \( \alpha \)-CGRP KO and WT Mice
At the conclusion of the BP measurement studies, mice were euthanized and the hearts, kidneys, aortas, and femoral arteries were removed for histopathologic examination. In agreement with previous results, determination of the heart-to-body weight ratios from each of the 4 groups showed a modest but significant increase in the control \( \alpha \)-CGRP KO (0.51±0.02) compared with control WT (0.45±0.01) mice. As expected, there was a significant increase in heart-to-body weight ratios in the 2 DOC-salt–treated groups (DOC-salt \( \alpha \)-CGRP KO [0.68±0.02] versus DOC-salt WT [0.67±0.02]) when compared with their matching control group. However, there were no differences seen between the 2 DOC-salt–

\[ \text{WT-C} \]
\[ \text{KO-C} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
\[ \text{K} \]

\[ \text{WT-DOC} \]
\[ \text{KO-DOC} \]

\[ \text{H} \]
Marked (3+, scale of 1+ to 4+) vasculitis was seen in the heart sections from DOC-salt–treated α-CGRP mice, with thickening and inflammation of the vessel walls. Perivascular inflammation was also noted and the endothelial cells were prominent, which is consistent with inflammation of this critical cell layer. In the myocardium, there was prominent 3+ thickening and inflammation of the vessel walls. Perivascular inflammation that extended to the epicardium. The kidneys of these mice showed moderate 2+ inflammation, crescentic proliferation, and focal histocytic infiltration. The arrow in the bottom left panel points to a normal glomerulus, whereas the top arrow in the bottom right panel demonstrates histocytic infiltration, and the bottom arrow crescent formation.

Figure 4. Histopathology of a trichrome-stained heart (H) and kidney (K) sections from DOC-salt hypertensive WT (WT-DOC) and α-CGRP KO (KO-DOC) mice. The top arrow in the top right panel points to an area of myocardial necrosis, and the bottom arrow points to a blood vessel demonstrating vasculitis. The arrow in the bottom left panel points to a normal glomerulus, whereas the top arrow in the bottom right panel demonstrates histocytic infiltration, and the bottom arrow crescent formation.

Urinary Microalbumin in the DOC-Salt Hypertensive α-CGRP KO and WT Mice

The DOC-salt protocol produced an approximate 10-fold increase in urinary output in α-CGRP KO and WT mice (Table). DOC-salt treatment also produced a 2.5- and 7.0-fold increase in microalbumin excretion in the DOC-salt–treated WT and α-CGRP KO mice, respectively, with the excretion in the α-CGRP KO mice being significantly greater than the WT mice (34.2±3.3 versus 15.9±0.5 ug/24 h; *P*<0.01).

Discussion

The most significant finding of this study was that DOC-salt hypertensive α-CGRP KO mice displayed markedly enhanced cardiac and renal damage compared with the DOC-salt hypertensive WT mice, as determined by histopathologic techniques. In contrast, there were no detectable differences in the aortas or femoral arteries from any of the groups tested. Consistent with the histopathologic changes, the DOC-salt α-CGRP KO mice exhibited significantly greater microalbumin levels in the urine compared with the DOC-salt WT mice. It is important to note that the BP increase in the DOC-salt α-CGRP KO and WT mice, when normalized to baseline, was not different between the 2 strains. This result was not expected because we demonstrated previously that CGRP plays a counter-regulatory role to attenuate the BP increase in DOC-salt hypertension. There are several possible explanations for why the percent increase in MAP during DOC-salt treatment was not higher in the α-CGRP KO mice compared with the WT controls. First, it may be that the α-CGRP mice do not display a greater sensitivity to challenges to BP homeostasis because their basal BP is already significantly increased. Second, permanent deletion of this neuropeptide during development may activate other compensatory mechanisms that buffer the BP elevation in this setting.

Our interpretation of these results must be qualified by the possibility that the higher (≈20 mm Hg) absolute MAP seen in the DOC-salt α-CGRP KO mice compared with the DOC-salt WT mice could play a significant role in the pathological differences observed in this study. We do not think that this is likely for the following reasons. (1) Basal BP is 10 to 15 mm Hg higher in the α-CGRP KO compared with WT controls in the absence of any pathological changes in the heart and kidneys between the 2 groups; 2) The DOC-salt protocol produces an ≈40 mm Hg increase in the MAP of the WT mice, again without any significant histopathologic damage to cardiac and renal tissues even in the presence of an increase in microalbuminuria; and 3) The dramatic difference in the degree of heart and kidney damage between the DOC-salt–treated α-CGRP KO and WT mice suggest that the lack of this neuropeptide in perivascular sensory nerves and not the BP differential is primarily responsible for the pathological changes observed, although a role for the later mechanism cannot be ruled out. Experiments could be proposed in the attempt to distinguish between the difference in BP versus the absence of CGRP in the present data. Such experiments would involve the use of additional agents to either increase the BP in the WT mice or decrease the BP in the α-CGRP KO mice. Either modality would influence the effect of the added drug or counter-regulatory systems into the interpretation of the results obtained. Although we plan to pursue this avenue, it is beyond the scope of this initial study.

This study also demonstrated that there were no significant gross postmortem or histopathologic alterations in any major organ systems between untreated 16-week-old α-CGRP KO
and WT mice except for mild cardiac hypertrophy in the KO mice, in accordance with their elevated BP. Importantly, there was no microscopic evidence of vascular alterations or abnormalities among the mice studied that might confound the interpretation of the results obtained.

The absence of histopathologic changes in heart and kidney sections from the DOC-salt hypertensive WT mice, as determined by the staining techniques used, was striking. However, there was a significant increase in urinary microalbumin excretion in the DOC-salt–treated WT mice compared with normotensive controls that is indicative of early renal damage. The most likely reason that we did not observe any changes using light microscopy is that the earliest pathological changes in this setting are foot process fusion and microvillous transformation of glomerular epithelial cells. Although these alterations do affect glomerular function, they are only detectable by electron microscopic analysis. Our findings in the DOC-salt WT mice are in agreement with previous studies that demonstrated significantly reduced end organ damage in angiotensin II–salt hypertensive mice (C57/BL6), compared with rats, probably through the overproduction of superoxide dismutase.17

The results from the WT mouse strain presented herein are similar to those reported by Peng et al,21 who also used DOC-salt–treated C57/BL6 mice. Four weeks after initiation of the DOC-salt protocol, the experimental group displayed a 17% increase in systolic BP using the tail cuff method. This result is different from the data we obtained (35% increase in MAP) and is most likely attributable to the different methodologies used. These investigators reported DOC-salt induced left ventricle hypertrophy that is consistent with our findings. They also observed renal damage as manifested by proteinuria, which is in line with our results.

Because of the dramatic differences in cardiac and renal damage, in the absence of changes in the aortas and femoral arteries produced by the DOC-salt protocol in the α-CGRP KO compared with the WT mice, our results suggest that CGRP possesses a protective action against hypertension-induced heart and kidney damage. This activity could be mediated directly by CGRP or indirectly through subsequent neurohormonal or other physiological changes caused by CGRP gene deletion. As described previously, this neuropeptide is 100 to 1000× more potent than other vasodilators such as adenosine, acetylcholine, bradykinin, and substance P, and the coronary vasculature is particularly sensitive to the dilator effects of CGRP. We and others have reported that CGRP appears to be responsible for ∼30% of basal coronary blood flow in rats and mice.16,22 In addition, using isolated heart preparations from the α-CGRP KO and WT mice, we observed that CGRP protects against ischemia/reperfusion injury (S.C.S., H.Z., K.A.K., P.S.D., unpublished observations, 2000). Similar results have been obtained in the rat.1 In the kidney, it is known that CGRP decreases renal resistance and increases renal perfusion. Sensory nerve endings containing CGRP have been identified on the renal vasculature, especially in the vicinity of the glomerulus.23 Indeed, it is the glomeruli that are the kidney structures most vulnerable to DOC-salt hypertension-induced damage in the α-CGRP KO mice. Thus, deletion of α-CGRP may significantly reduce heart and renal perfusion, thereby exacerbating the damage subsequent to increased generation of reactive oxygen species and the inflammatory response.

In addition to the loss of a neuropeptide that acts as a direct vasodilator in multiple vascular beds, α-CGRP KO mice display a significant increase in activity of the sympathetic nervous compared with WT controls.24 We reported recently that the renin-angiotensin system (RAS) is activated in α-CGRP null mice, perhaps as a result of the upregulation of sympathetic nerve activity.25 The proinflammatory activity of angiotensin II is well documented, and although DOC-salt hypertension is characterized by a depression of the RAS, in this model, circulating angiotensin II has been shown to increase monocyte/macrophage infiltration and vasculopathy in the kidneys and heart.26 Therefore, deletion of the α-CGRP gene may indirectly augment hypertension-induced end organ damage through enhanced activity of the sympathetic nervous system and RAS.

There is also indirect evidence that CGRP can counteract hypertension-induced heart and kidney damage via inhibition of oxidative stress. Studies in mice that carry only a single copy of the adrenomedullin (AM) gene (the homozygous AM−/− deletion is embryonic lethal) have a greater susceptibility to heart and kidney damage after angiotensin II/salt loading than the WT controls.17 This protective activity of AM appears to be mediated via attenuation of reactive oxygen species formation. AM, also a potent vasodilator, is a member of the CT/CGRP gene family and binds to the same ligand-binding component of the receptor (CT receptor-like receptor [CRLR]) as CGRP.27 The pharmacological specificity of the CRLR is determined by the coexpression of receptor activity-modifying protein 1 (RAMP1; CGRP) and RAMP2 (AM).

**Perspectives**

These data indicate that deletion of the α-CGRP gene enhances hypertension-induced end organ damage in the heart and kidney. The mechanism of this increased tissue damage may be through the loss of CGRP-mediated vasodilator activity resulting in higher BP, reduced renal and cardiac blood flows, or an increase in local tissue production of oxidative and inflammatory mediators. This is the first report of a sensory nerve-mediated cardioprotective and renal protective effect against hypertension-induced end organ damage. Traditionally, sensory nerves were defined as purely afferent neurons that monitor changes in their chemical and physical environment and convey this information to the central nervous system. They also have the capacity to act in an efferent manner. This efferent function is mediated by the release of neuropeptides, including CGRP, from their peripheral terminals that regulate vasodilation and other tissue activities independently of sensation. Thus, this potential organ-protective activity of CGRP may reflect another significant function of the efferent arm of the sensory nervous system.

**Acknowledgments**

This study was supported by US Public Health Service grant HL44277.
References


Calcitonin Gene-Related Peptide Protects Against Hypertension-Induced Heart and Kidney Damage

Scott C. Supowit, Arundhati Rao, Mark C. Bowers, Huawei Zhao, Gregory Fink, Barbara Steficek, Parag Patel, Khursheed A. Katki and Donald J. DiPette

Hypertension. published online December 6, 2004;

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/early/2004/12/06/01.HYP.0000151130.34874.fa.citation

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/