Angiotensin-Converting Enzyme 2 and Angiotensin-(1-7)
An Evolving Story in Cardiovascular Regulation

Carlos M. Ferrario

Abstract—This lecture summarizes the chronology and rationale that led to the discovery of angiotensin-(1-7) as a hormone that, in its own right, opposes the vasoconstrictor and proliferative actions of angiotensin II. The work discussed here additionally analyzes the newest findings on angiotensin-converting enzyme 2, the angiotensin-converting enzyme homologue that efficiently hydrolyzes angiotensin II into angiotensin-(1-7). Both components of this system may significantly influence our future perspective of the role of the renin–angiotensin system, not just in terms of its role in the regulation of cardiovascular and renal function but, moreover, as regulators of a vast array of disease processes in which inflammation and immune mechanisms play a role. (Hypertension. 2006;47[part 2]:1-7.)

Key Words: hypertension ■ receptors, angiotensin ■ angiotensin converting enzyme ■ angiotensin II

As recounted by my mentor, Irvine H. Page, MD, Arthur Corcoran, better known as “Corc,” was born in Waterlo, Quebec in 1909. A 1934 graduate of McGill University School of Medicine, he joined Dr Page at the Rockefeller Institute in New York in 1936 and followed him to the Cleveland Clinic in 1944. Dr Corcoran was a unique character in the world of hypertension. Steeped in classical education in the Oslerian tradition, he was demanding of both himself and all of his associates. His book, A Mirror Up To Medicine, published in 1961, is a testimony to his enthusiasm, to his devotion to clinical medicine, and to his knowledge of the disease that we call “essential hypertension.”1 As a clinician–scientist, Dr Corcoran’s major achievement was the early application of clearance methods in both hypertensive patients and animals.2–5 His expert knowledge of renal physiology matched, at that time, that of the Smith–New York University Group. His premature death in 1965 at the age of 56 during a visit to Czechoslovakia interrupted a luminous career and the completion of projects that sought to explain the relationship of salt to hypertension and the anti-hypertensive influence of the kidneys. It is an undeserved honor for me to memorialize his contribution to the field of hypertension as I summarize the results of the work that my associates and I undertook in exploring the antihypertensive effects of the heptapeptide angiotensin-(1-7) [Ang-(1-7)] in the regulation of blood pressure and the pathogenesis of arterial hypertension.

The Beginning
My training in hypertension research began under the mentoring and caring roles of Irvine H. Page, MD, and James W. McCubbin, MD, who introduced me to the field of neurogenic hypertension. Among many things, they taught me that hypertension should be viewed as a process in which the neurogenic, humoral, and endocrine mechanisms that regulate tissue perfusion become dysregulated so that the harmony of Page’s mosaic proposed in 1949 was no longer in balance.6 Page envisioned the mosaic theory as a kaleidoscope, made of small pieces of inlaid glass or diverse elements, with each piece of glass or element being movable so that it could continuously change in composition. Fifty-six years later, the concepts embedded in the mosaic theory remain most appropriate, because research has revealed that the renin–angiotensin system (RAS) is fascinatingly more complex biochemically than anticipated, with intriguing phylogenetic and evolutionary aspects yet to be fully understood. In the human species alone, there now appears to be very diverse physiological and pathophysiological involvement and, hence, important therapeutic implications.

The study of Ang-(1-7) began with the fortuitous observation that the addition of angiotensin (Ang) I to canine brain stem homogenates resulted in the appearance of a peak in the high-performance liquid chromatography chromatogram that corresponded to the NH2-terminal truncated peptide Ang-(1-7).7 Both Dr Robson Santos, at that time a postdoctoral fellow in our laboratory, and I were surprised by this finding and indeed discouraged to pursue this observation further by the more experienced faculty of the Research Division of the Cleveland Clinic, primarily because the prevailing dogma at that time was that angiotensin peptides lacking the phenylalanine amino acid in the eighth position of the angiotensin II (Ang II) molecule would be biologically inactive. Although additional research into this subject seem doomed, Santos and I decided not to heed the advice of our colleagues and...
Formation and Functions of Ang-(1-7)

Research on Ang-(1-7) has expanded knowledge of the enzymes and the complexity of the biochemical mechanisms responsible for the formation of biologically active peptides in the RAS. The accepted tenet that the formation of Ang II results from the linear and sequential actions of renin and angiotensin-converting enzyme (ACE) on angiotensinogen and Ang I, respectively, was revised through our demonstration that Ang I was also a substrate for the formation of Ang-(1-7). Between 1991 and 1992, our group showed that 3 separate tissue-specific endopeptidases cleaved Ang I into Ang-(1-7), and their location in the tissues and their access to the substrate dictated which enzyme was predominantly responsible for peptide formation. Initial experiments showed that prolyl endopeptidase (E.C. 3.4.24.26) formed Ang-(1-7) from Ang I in canine brain homogenates and vascular endothelial cells obtained from bovine, human aorta, and umbilical veins. Neutral endopeptidase E.C.3.4.24.11 (neprilysin) was then characterized to process Ang I into Ang-(1-7) in the circulation, whereas, thimet oligo peptidase (E.C. 3.4.24.15) generated Ang-(1-7) from the Ang I substrate in vascular smooth muscle cells. Ten years elapsed before the independent discovery of ACE2 provided a more complete understanding of the biochemical physiology of the enzymes involved in the formation of angiotensin peptides.

A thorough analysis of the biological actions of Ang-(1-7) is outside the scope of this presentation; several review articles have rigorously examined the evidence. Suffice it to say that the actions of Ang-(1-7) are composed of both activation of peripheral vasodilator mechanisms and antitrophic effects mediated by inhibition of protein synthesis. In addition, Ang-(1-7) amplifies the vasodilator actions of bradykinin (BK). Additional evidence suggests that Ang-(1-7) reduces the release of norepinephrine acting through a BK/nitric oxide–mediated mechanism that stimulates cGMP/protein kinase G signaling. Other experiments also point to an interrelationship between Ang-(1-7) and the prostaglandin–BK–nitric oxide system. In the spontaneously hypertensive rat (SHR), the vasodilator effects of Ang-(1-7) are blocked in the presence of the cyclooxygenase I inhibitor, indomethacin, confirming previous findings in which the vasorelaxing response of canine coronary artery ring induced by Ang-(1-7) was abolished by the BK antagonist icatibant, whereas the peptide also inhibited the degradation of 125I-[Tyr5]-BK and the appearance of BK-(1-7) and BK-(1-5) metabolites in the effluent from the preparation. Cross-talk between the prostaglandin–BK–nitric oxide systems and Ang-(1-7) suggests that a multilayered interaction between these systems is important for a wide array of physiological functions.

Similar mechanisms participate in the antiproliferative actions of Ang-(1-7) first demonstrated in vascular smooth muscle cells (VSMCs). In those experiments, exposure of cultured VSMCs to Ang-(1-7) inhibited the trophic actions of Ang II and reduced the expression of the mitogenic effects of both normal serum and platelet-derived growth factor. The growth-inhibitory actions of Ang-(1-7) were blocked by the selective D-alanine7-Ang-(1-7) [D-Ala7-Ang-(1-7)] antagonist and the nonselective angiotensin receptor blocker sarcosine1-threonine2-Ang II. In contrast, subtype-selective antagonists for the angiotensin II type 1 (AT1) and type 2 (AT2) receptors had no effect on the inhibitory actions of Ang-(1-7) on DNA synthesis. Antiproliferative effects of Ang-(1-7) were additionally confirmed in vivo. Chronic administration of the heptapeptide in rats prevented the growth of the neointima produced by balloon injury of their carotid artery. The molecular mechanisms of the antiproliferative effects of Ang-(1-7) in cultured rat aortic VSMCs were recently investigated by Tallant and Clark, who found that the Ang-(1-7) dose-dependent release of prostacyclin and the antiproliferative response to Ang-(1-7) from VSMCs was inhibited by indomethacin. In contrast, neither a lipoxygenase inhibitor nor a cytochrome p450 epoxygenase inhibitor prevented the antiproliferative effects of Ang-(1-7). Furthermore, Ang-(1-7) stimulated release of cAMP from VSMCs which might result from prostacyclin-mediated activation of adenylate cyclase. The cAMP-dependent protein kinase inhibitor Rp-adenosine-3’,5’-cyclic monophosphorothioate attenuated the Ang-(1-7)–mediated inhibition of serum-stimulated thymidine incorporation. Finally, Ang-(1-7) inhibited Ang II stimulation of mitogen-activated protein kinase activities [extracellular signal–regulated kinase (ERK)1/2], whereas Ang-(1-7) itself had no effect on ERK1/2 activation. However, preincubation with increasing concentrations of Ang-(1-7) caused a dose-dependent reduction in Ang II-stimulated ERK1/2 activities. These results suggest that Ang-(1-7) inhibits vascular growth through the release of prostacyclin,
the prostacyclin-mediated production of cAMP, activation of cAMP-dependent protein kinase, and the attenuation of mitogen-activated protein kinase activation. The data obtained in these experiments expanded on the earlier demonstration by Zhu et al33 that Ang-(1-7) was a partial antagonist of Ang II–induced contraction of aortic rings, VSMC DNA, and protein synthesis and activation of protein kinase C and ERK1/2 in Wistar rats.

Santos et al34 identified the orphan mas receptor as a functional binding site for Ang-(1-7). Genetic deletion of the G protein–coupled receptor encoded by the mas protooncogene abolished the binding of Ang-(1-7) in mouse kidneys, the antidiuretic action of Ang-(1-7) after an acute water load, and the Ang-(1-7)–induced relaxation response in aorta. In comparative studies, they showed that Ang-(1-7) binds to mas-transfected cells in which it elicits arachidonic acid release. Recently, Tallant et al35 showed that the Ang-(1-7)–dependent reduction in cardiomyocyte growth was mediated by the mas receptor.

**ACE2 and Ang-(1-7)**

The period before the discovery of ACE2 could be characterized both as a time of limited interest in exploring the potential for Ang-(1-7) to act as an endogenous inhibitor of the pathophysiological actions of Ang II and of limited acceptance of its role in mammalian regulation of homeostasis. Any doubts that remained regarding its relative importance and contribution to cardiovascular regulation were dissipated by the independent cloning of ACE2 by Donoghue et al17 and Turner et al18 using genome-based strategies to probe for either proteins with functions similar to that of ACE or proteins involved in cardiac function. Their studies generated enormous interest and provocative hypotheses about its function.36–38 Indeed, Yagil and Yagil37 suggested that characterization of the functional activity of this ACE homologue may change altogether our perception of the role of the RAS and its importance in both health and disease processes.

Our interest in ACE2 arose from a collaborative effort of Mark Chappell and myself with Drs Michael Crackower and Josef Penninger, who, then at Amgen Laboratories in Canada, were investigating the potential role of ACE2 in the regulation of cardiac function. Our first publication showing that the ACE2 gene maps to a defined quantitative trait locus on the X chromosome in 3 different rat models of hypertension and that, in all hypertensive rat strains, ACE2 messenger RNA and protein expression were markedly reduced provided a first clue to its potential role.39 Furthermore, in the same study, Crackower et al39 found that targeted disruption of ACE2 in mice resulted in a severe cardiac contractility defect, increased Ang II levels, and upregulation of hypoxia–induced genes in the heart. Genetic ablation of ACE on an ACE2 mutant background completely rescued the cardiac phenotype, whereas disruption of ACER (Drosophila ACE homologue) resulted in a severe defect of heart morphogenesis.39

The stage for an intensive, still ongoing investigation of the interactions between ACE2 and Ang-(1-7) was set by the independent demonstration that ACE2 had a preferential ability for cleaving Ang II into Ang-(1-7).40,41 In the studies reported by Rice et al,40 Ang II was cleaved efficiently by ACE2 to Ang-(1-7) [catalytic constant/Michaelis constant of $2.2 \times 10^6$ M$^{-1}$s$^{-1}$] and was cleaved by neutral endopeptidase 24.11 (NEP) [catalytic constant/Michaelis constant of $2.2 \times 10^5$ M$^{-1}$s$^{-1}$] to several other degradation products. The fact that ACE2 was insensitive to blockade with ACE inhibitors46 and that we had shown before that inhibition of ACE resulted in increases in Ang-(1-7),42 in part because of our finding that Ang-(1-7) was cleaved with similar efficiency by both the N-domain and C-domain of ACE, suggested that ACE2 plays a critical role in regulating the balance between vasoconstrictor and vasodilator effects within the RAS cascade.

The heart was our first target in assessing the interactions of ACE2 and Ang-(1-7), because myocytes expressed the components of the system,43 the ACE2 gene is abundant in the rat heart (Figure 1), and the heptapeptide reversed arrhythmia reperfusion injury.44 In addition, Loot et al45 found that chronic infusion of Ang-(1-7) restored cardiac contractility after myocardial infarction (MI). Knowledge that the density of Ang-(1-7) immunostaining increased in the penumbra region surrounding the rat ischemic myocardium43 led us first to evaluate the expression of ACE2 28-days after MI in rats with an associated blockade of AT1 receptors.46 A 3-fold increase in ACE2 mRNA within the viable myocardium was found in these experiments, correlating with increases in plasma concentrations of Ang-(1-7). Because in animals not exposed to Ang II blockade, coronary artery ligation had no effect on cardiac ACE2 mRNA, we additionally investigated whether increased ACE2 expression was determined by the binding of Ang II to AT1 receptors. This was not the case, because increased ACE2 mRNA was still present in infarcted rats with dual AT1/AT2 receptor blockade.46 The potential for ACE2 to have a significant influence on the regulation of cardiac function and remodeling is additionally suggested by the demonstration that lentiviral vector encoding mouse ACE2 (lenti-mACE2) or green fluorescent protein injected intracardially in 5-day–old Sprague-Dawley rats resulted in significant attenuation of cardiac hypertrophy and myocardial fibrosis induced by Ang II infusion.47
The first demonstration of a modulatory role of the AT1 receptor on cardiac ACE2 mRNA led us to investigate the signaling mechanism responsible for increased gene expression. The question we asked ourselves was whether the ACE2 gene responded to the compensatory increase in circulating or tissue levels of either Ang II or Ang-(1-7) brought about by the loss of myocardial contractility after MI. The nature of the signaling stimulus was addressed in experiments in which intact rats were medicated for 12 days with either an ACE inhibitor (lisinopril), an AT1 receptor blocker (losartan), or both drugs. As shown in Figure 2A, both treatments given separately increased cardiac ACE2 mRNA, whereas ACE2 activity measured as the rate of Ang-(1-7) formation from Ang II in their cardiac membranes increased only in rats medicated with losartan or the combination therapy (Figure 2B). In rats medicated with lisinopril, plasma levels of Ang II fell, whereas plasma Ang-(1-7) increased. On the other hand, losartan treatment was accompanied by increases in the plasma levels of both Ang II and Ang-(1-7). These data suggested that Ang II may be a stimulus determining cardiac ACE2 gene expression, because either reduction in its levels or prevention of Ang II binding to the AT1 receptor increased ACE2 mRNA.

It should not be assumed that regulation of ACE2 gene expression is under a similar control in all tissues. This possibility was explored by our recent experiments in which ACE2 gene expression and activity was measured in the renal cortex of rats submitted to the same treatment protocols described above (Figure 2C and 2D). In these studies, renal cortex ACE2 mRNA was not changed by 12-day administration of lisinopril, losartan, or both drugs combined, although ACE2 activity was significantly increased by either treatment alone. The demonstration of a tissue-specific regulation of ACE2 gene expression and ACE2 enzyme activity is additionally underscored by our finding of a differential effect of AT1 receptor blockade on ACE2 mRNA in the aorta and carotid arteries of SHRs. In this study, we showed increased ACE2 gene expression in the aorta but not the carotid arteries of SHRs given olmesartan for 2 weeks. Additional evidence for a transcriptional regulation of the ACE2 enzyme is found in the data obtained by Gallagher et al in neonatal rat cerebellar or medullary astrocytes. In these studies, we showed that Ang II–mediated reduction in ACE2 mRNA was blocked by losartan or valsartan, whereas PD123319 was ineffective. The reduction in ACE2 mRNA by Ang II also was associated with a 50% decrease in cerebellar and medullary ACE2 protein, which was blocked by losartan. Treatment of medullary astrocytes with Ang-(1-7) did not change ACE2 mRNA; however, Ang-(1-7) prevented the Ang II–mediated reduction in ACE2 mRNA. Addition of D-Ala7-Ang-(1-7) blocked the inhibitory actions of Ang-(1-7). Altogether, these studies suggest that ACE2 is highly regulated at transcription, a characteristic not unexpected for a rate-limiting enzyme that maintains the equilibrium between opposing arms of a biochemical pathway. It would not be surprising that multiple layers of regulation, including translational and posttranslational modifications, are involved in determining the tissue-specific activity of the enzyme.

Exploring the Significance of the ACE, ACE2, and Ang-(1-7) Axis

The data summarized here suggest that the levels of ACE and ACE2 dictate whether vasoconstriction or vasodilation will predominate. Research on the RAS has remained focused on Ang II, with ACE considered as the central modulator of the system. But, as discussed above, this arm of the system represents only 1 of the 2 principal components of the RAS, when we consider that Ang I and Ang II can be processed into Ang-(1-7) through a separate arm formed by the Ang-(1-7) forming enzymes (neprilysin, prolyl endopeptidase, and thimet oligopeptidase). In illustrating the 2 arms of the system as shown in Figure 3, some notable new information is revealed, the specifics of which have been described recently in another publication. Primarily, the data suggest the existence of a feed-forward mechanism in which both ACE and ACE2 play a critical role in determining the net concent-

![Figure 2](http://hyper.ahajournals.org/content/58/3/333/F2.large.jpg)
tration of Ang II and Ang-(1-7) with obvious outcomes in terms of blood pressure regulation.\(^{22}\)

Through the catalytic action of ACE, Ang II is formed through hydrolytic removal of 2 amino acids (His\(^{9}\)-Leu\(^{10}\)) of the Ang I substrate (Figure 3, step 1). ACE2, acting as a monopeptidase, can then act on Ang II cleaving the Pro\(^{7}\)-Phe\(^{8}\) amino acid bond of the molecule to produce Ang-(1-7) (Figure 3, step 2). In turn, ACE then hydrolyzes Ang-(1-7) by cleaving 2 amino acids from the molecule (His\(^{6}\)-Pro\(^{7}\)) to form Ang-(1-5) (Figure 3, step 3). Thus, this overlooked characteristic of the system has profound consequences in determining the balance of the actions between both the pressor-proliferative and the depressor-antiproliferative components of the system. Both clinical and physiological experiments support this view. Inhibition of ACE decreases Ang II production and increases Ang-(1-7) because of the following: (1) increased Ang-(1-7) formation from elevated levels of Ang I; and (2) inhibition of Ang-(1-7) metabolism by ACE.\(^{19,42,52–56}\) On the other hand, blockade of AT\(_{1}\) receptors with its attendant elevation in both Ang I and Ang II stimulates ACE2 activity while facilitating an increase in Ang-(1-7) formation through the other Ang-(1-7)-forming enzymes.\(^{20,48,49}\)

**An Additional Perspective**

In the present brief review, we have considered the current understanding of the RAS from studies conducted by me and my colleagues, addressing the more notable recent developments in our knowledge thereof. It is important, however, to discuss what is likely to comprise some important avenues of future investigation, because emerging studies on ACE2 and Ang-(1-7) suggest that this system may play a critical role in inflammation and as an acute-phase reactant system.

Within the physiological mechanisms that regulate embryogenesis and fetal maturation, Ang-(1-7) and ACE2 play a critical role, because recent studies showed that pregnancy increases the expression of these factors. In human placenta, we showed pronounced immunocytochemical expression of ACE2 and Ang-(1-7) and specific staining in cytotrophoblast, syncytiotrophoblast, and the endothelium of the blood vessels of the primary and secondary villi.\(^{35}\) In addition, ACE2 and Ang-(1-7) are expressed in the maternal stromal in the invading and intravascular trophoblast and the decidual cells. The similar localization of Ang-(1-7) and ACE2 in the placenta strongly suggests that ACE2 is involved in the processing of Ang-(1-7) in local tissue expression of the peptide. A recent study by our colleagues Neves and Brosnihan (unpublished observations, 2005) showed the upregulation of ACE2 mRNA in a normal pregnant uterus and downregulation of ACE2 mRNA in a model of preeclampsia in the rat (reduced uterine perfusion pressure) with no change in the level of ACE2 mRNA in the placenta of normal and preeclamptic pregnant rats. Taken together, these studies suggest an important role for ACE2 in the uteroplacenta unit, which can be regulated with placenta ischemia.

Antiangiogenic properties of Ang-(1-7), first described by Machado et al.,\(^{58,59}\) and those reported by us on protein synthesis\(^{35,60}\) endow this peptide with antitumoral properties since Gallagher and Tallant\(^{61}\) showed that Ang-(1-7) inhibits growth in 3 human lung cancer cell lines (human adenocarcinoma SK-LU-1, A549 cells, and non-small lung cancer SK-MES-1 cells). Treatment with Ang-(1-7) resulted in both a dose- and time-dependent reduction in serum-stimulated DNA synthesis in all 3 of the cell lines, with an IC\(_{50}\) in the subnanomolar range. The Ang-(1-7) receptor antagonist D-Ala\(^{7}\)-Ang-(1-7) blocked the attenuation of the serum-stimulated DNA synthesis of SK-LU-1 cells by Ang-(1-7), whereas neither AT\(_{1}\) nor AT\(_{2}\) angiotensin receptor subtype antagonists prevented the response to the heptapeptide. Importantly, other angiotensin products, including Ang I, Ang II, Ang-(2-8), Ang-(3-8), and Ang-(3-7) did not attenuate mitogen-stimulated DNA synthesis of SK-LU-1 cells, demonstrating the specificity of Ang-(1-7) to inhibit SK-LU-1 cancer cell growth selectively. Pretreatment of SK-LU-1 cells with Ang-(1-7) reduced serum-stimulated phosphorylation of ERK1/2, indicating that the antiproliferative effects may occur, at least in part, through inhibition of the ERK signal transduction pathway. The results of this study suggest that
Ang-(1-7) inhibition of lung cancer cell growth may represent a novel chemotherapeutic and chemopreventive treatment for lung cancer.

A role for ACE2 in other organ system diseases was revealed by the demonstration that ACE2 also serves as the cellular entry point for the severe acute respiratory syndrome (SARS) virus. In the studies of Kuba et al.,32 SARS-CoV infections and the Spike protein of the SARS-CoV reduced ACE2 expression, whereas blockade of the RAS attenuated acute lung failure produced by injection of SARS-CoV Spike into mice. Both ACE2 and blockade of AT1 receptors protect mice from severe lung injury induced by acid aspiration or sepsis, whereas administration of recombinant ACE2 to mice protects the rodent from the lethal effects of acute lung injury.63

Acknowledgments

As this period of our research is documented, I need to reiterate that whatever progress was made in this field is to be credited to the concerted effort of all of those who worked in our laboratories, first at the Cleveland Clinic Foundation and then at the Wake Forest University School of Medicine. The contributions made by my colleagues Drs. David B. Averill, K. Bridget Brosnihan, Mark C. Chappell, Debra I. Diz, Patricia E. Gallagher, and E. Ann Tallant should be recognized as having a decisive influence on the progress we made. By acting as a unit, both intellectually and materially, they facilitated our understanding of the problem and carved their own niche in the pages of hypertension research.

I will be remiss if I do not express my deepest appreciation to the National Heart, Lung, and Blood Institute and the reviewers of our grants (whomever they may be), because it was their wisdom that allowed us to attain these accomplishments. A 30-year history of continuing research funding (Program Project Grants HL-6835 and HL-51952) allowed our program the stability and material resources to carry out the research as summarized here. I am also enormously grateful to Richard H. Dean (President, Wake Forest University Health Science Center) who, in 1992 and thereafter, provided us with intellectual guidance and sustained support of our research program. I also gratefully acknowledge the edge grant support provided by Unifi, Inc, Greensboro, NC, and the sustained support of our research program. I also gratefully acknowledge the support of Richard H. Dean (President, Wake Forest University Health Science Center) who, in 1992 and thereafter, provided us with intellectual guidance and sustained support of our research program. I also gratefully acknowledge the edge grant support provided by Unifi, Inc, Greensboro, NC, and the sustained support of our research program.

References

Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A. 2003;100:8258–8263.


Angiotensin-Converting Enzyme 2 and Angiotensin-(1-7). An Evolving Story in Cardiovascular Regulation
Carlos M. Ferrario

Hypertension. published online December 19, 2005;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 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/2005/12/19/01.HYP.0000196268.08909.fb.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/