Vascular Hypertrophy in Angiotensin II–Induced Hypertension Is Mediated by Vascular Smooth Muscle Cell–Derived \( \text{H}_2\text{O}_2 \)

Yong Zhang, Kathy K. Griendling, Anna Dikalova, Gary K. Owens, W. Robert Taylor

**Abstract**—Angiotensin II induces the development of vascular hypertrophy and hypertension. An increasing number of studies have demonstrated that reactive oxygen species are involved in many of the vascular responses to angiotensin II. However, the role of specific cell types and the precise identity of the functionally relevant reactive oxygen species remain unclear. In this study, we established a line of transgenic mice with vascular smooth muscle cell (SMC)–specific overexpression of the human catalase gene to explicitly test the functional role of vascular smooth muscle–derived hydrogen peroxide in the hypertensive and hypertrophic responses to angiotensin II in vivo. Catalase overexpression was confirmed by increased expression of catalase mRNA and protein, as well as by an increase in catalase enzymatic activity. The catalase transgenic mice were viable, had no change in basal hydrogen peroxide release (0.36±0.03 versus 0.37±0.14 \( \mu \text{mol/L} \)), and showed no overt developmental abnormality. In response to angiotensin II treatment, catalase transgenic mice exhibited lower hydrogen peroxide release compared with control animals. There was no effect on the hypertensive response to angiotensin II (147±10 versus 148±12 mm Hg). However, angiotensin II–induced aortic wall hypertrophy was dramatically attenuated in the catalase transgenic mice (wall thickness 32.4±2.0 versus 43.2±7.6 \( \mu \text{m} \); \( P<0.001 \)). These results demonstrate that vascular SMC–derived hydrogen peroxide plays an important role in angiotensin II–induced hypertrophy of the arterial wall. *(Hypertension. 2005;46:732-737.)*

**Key Words:** hypertension, experimental \( \bullet \) angiotensin II \( \bullet \) vascular diseases \( \bullet \) oxidative stress \( \bullet \) antioxidants

Among other pathological factors, angiotensin II (Ang II) plays an important role in the development of vascular hypertrophy and hypertension.1–5 The immediate hypertensive effects of Ang II occur as a result of vasoconstriction and antinatriuresis.6 Chronically, Ang II causes remodeling of the arterial vasculature via cellular hypertrophy. In the spontaneously hypertensive rat and the 1-kidney, 1-clip model of hypertension, administration of either an angiotensin-converting enzyme inhibitor or an Ang II receptor antagonist results in significantly reduced vascular hypertrophy.7 Similarly, Ang II infusion, but not norepinephrine infusion, causes vascular hypertrophy.8 Together, these data clearly indicate the potential importance of Ang II in vascular hypertrophy.

Numerous studies have suggested that reactive oxygen species (ROS) such as superoxide anion and \( \text{H}_2\text{O}_2 \) are involved in the hypertensive and hypertrophic responses to Ang II.9–12 In vascular smooth muscle cells (SMCs), the NAD(P)H oxidase is one of the major sources of superoxide and \( \text{H}_2\text{O}_2 \).13,14 Ang II increases intracellular \( \text{H}_2\text{O}_2 \), which can be readily converted into \( \text{H}_2\text{O}_2 \) by superoxide dismutase (SOD). Ang II–mediated increases in intracellular \( \text{H}_2\text{O}_2 \) are inhibited by extracellular catalase, the NAD(P)H oxidase inhibitor diphenylene iodonium, and the Ang II type 1 receptor antagonist losartan, suggesting that in vascular SMCs (VSMCs), Ang II increases intracellular \( \text{H}_2\text{O}_2 \) via a mechanism involving activating the NAD(P)H oxidase via the Ang II type 1 receptor.10,11

ROS have been implicated in hypertension in human studies of essential hypertension and experimental animal models of hypertension.13,15–18 Superoxide anion has been clearly implicated in pathogenesis of hypertension, per se.11–13,17 However, the role of \( \text{H}_2\text{O}_2 \) remains obscure because previous studies have suggested a role for \( \text{H}_2\text{O}_2 \) in vasoconstriction and vasorelaxation.19–23 Elevated plasma concentrations of \( \text{H}_2\text{O}_2 \) were observed in salt-sensitive Dahl rats.15 Several in vitro studies have shown that \( \text{H}_2\text{O}_2 \) was able to elicit contractions in artery segments from various species and locations such as human umbilical artery,24,25 rabbit carotid artery,26 and rat aorta,27 as well as pulmonary artery.28 Conversely, there are reports that \( \text{H}_2\text{O}_2 \) induces relaxation of endothelium-demuded aortic rings from spontaneously hypertensive rats.29,30 In the same species, catalase, but not SOD, attenuated the K⁺–channel opener levromakalmin-induced relaxation.31 Thus, whereas the role of superoxide anion in hypertension is relatively clear, the role of \( \text{H}_2\text{O}_2 \) remains less well defined.

**Correspondence to W. Robert Taylor, MD, PhD, Division of Cardiology, Emory University School of Medicine, 1639 Pierce Dr, WMB Building, Suite 319, Atlanta, GA 30322. E-mail wtaylor@emory.edu**

**Hypertension** is available at [http://www.hypertensionaha.org](http://www.hypertensionaha.org)

© 2005 American Heart Association, Inc.

DOI: 10.1161/01.HYP.0000182660.74266.6d
As suggested by several in vitro studies, ROS may also be involved in Ang II–mediated vascular hypertrophy.\(^{9,10}\) Whereas superoxide anion has not been directly implicated in Ang II–induced vascular hypertrophy, several lines of evidence suggest that H\(_2\)O\(_2\) may be the principal ROS involved in this pathological process. Ang II increases intracellular H\(_2\)O\(_2\) in VSMCs, and overexpression of catalase results in an inhibition of the subsequent VSMC hypertrophy in vivo.\(^{10,32}\) Despite the fact that Ang II has acute (vasoconstriction) and chronic (vascular hypertrophy) effects on the arterial vasculature and that there are ample data to support a role for ROS in both of these effects, the specific role of H\(_2\)O\(_2\) in Ang II–mediated vasoconstriction and vascular hypertrophy remains unclear. To directly assess the role of H\(_2\)O\(_2\) in Ang II–mediated hypertension and hypertrophy in vivo, we generated a line of transgenic mice with VSMC-specific overexpression of catalase. Using this unique model, we show that in the setting of Ang II–induced hypertension, vascular hypertrophy, but not hypertension itself, is mediated by VSMC H\(_2\)O\(_2\).

### Materials and Methods

#### Generation and Genotyping of the Transgenic Mouse Overexpressing Human Catalase

To develop smooth muscle–specific overexpression of catalase, we used a Cre/LoxP system (for details, please see the supplement, available online at http://www.hypertensionaha.org). All mice used in this study, including the transgenic mice and the wild-type control mice (littermates of the transgenic mice), were on a C57/BL6 background. All procedures were approved by the Emory University institutional animal care and use committee and were in compliance with the standards for the care and use of laboratory animals of the Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md.

#### Measurement of the Expression of Catalase mRNA From the Transgene

Two micrograms of total mRNA isolated from each of the wild-type and Tg\(^{cat}\) mice aortas were reverse transcribed and polymerase chain reaction (PCR) amplified with primers that flanked a region of catalase cDNAs. In this region, there is an Sph\(_{I}\) site that is species specific to the human catalase gene and an Eco\(_{RV}\) site that is species specific for the native mouse catalase gene. Thus, Sph\(_{I}\) and Eco\(_{RV}\) digestion of the PCR products can be used to specifically identify the human catalase gene expression in murine tissues.

#### Measurement of Catalase Enzymatic Activity in Aortic Tissue

The total catalase enzymatic activity in aorta was measured for each of the control and transgenic mice (4 mice for each type) using a colorimetric assay. After perfusing the mouse aorta with cold 0.9% saline, the aorta was harvested and placed in cold isotonic buffer containing 0.01% digitonin. The adventitial tissues were quickly removed from aorta. The aorta was then homogenized in 200 µL cold isotonic buffer containing 0.01% digitonin. After spinning the homogenate at 700g for 10 minutes, the supernatant (~100 µL) was collected for the assay of enzymatic activity. The turnover rate of 10 µmol/L H\(_2\)O\(_2\) catalyzed by the supernatant was measured at 240 λ. The catalase activity of the samples was represented by the K value calculated with the formula K=(2.3/Δln(logA1/A2)), in which A1 and A2 were the absorbance of the samples at 0 and 60 seconds.\(^{31}\) The K value was normalized to the protein concentration of each sample.

### Immunostaining of Catalase in the Aorta Tissue Sections

Tissue sections from paraffin-embedded aortas were subjected to fluorescent immunostaining using an antibody raised against human catalase (Athenos Research and Technology). The tissue sections were blocked with 1% gelatin/PBS and treated with the primary antibody (1:100 dilution) at room temperature for 1 hour. After washing with 1×PBS, slides were treated with the secondary antibody (fluorescein isothiocyanate–labeled goat-anti rabbit antibody; Jackson Immunoresearch Laboratories, Inc.) at room temperature for 30 minutes. Sections were washed with 1×PBS and then counterstained for nuclei with Hoechst solution.

### Ang II Treatment

Eight transgenic mice and 10 control mice (12 weeks old) were anesthetized, and an osmotic mini-pump (Alzet model 2001 or 2002) filled with Ang II was implanted subcutaneously in the midscapular region. Ang II was infused at a rate of 0.75 mg/kg per day. This dose has been shown previously by us and others to induce a modest degree of systolic hypertension.\(^{34,35}\) Ten transgenic mice and 10 control mice were subjected to a sham operation for use as controls. Blood pressures were measured with a noninvasive computerized tail-cuff system (BP2000 Visitech) as described previously.\(^{34}\)

### Measurement of ROS

H\(_2\)O\(_2\) production of aortas was measured with an Amplex Red H\(_2\)O\(_2\)/Peroxidase Assay Kit (Molecular Probes). Aortas were harvested and the adventitial tissue was dissected free in cold buffer (145 mmol/L NaCl, 5.7 mmol/L Na\(_2\)HPO\(_4\), 4.83 mmol/L KCl, 0.54 mmol/L CaCl\(_2\), 5.5 mmol/L glucose, and 1.22 mmol/L MgSO\(_4\)). After opening the aorta with scissors and washing out the blood, the aorta was placed in buffer alone at 37°C. Ninety minutes later, duplicate 50-µL aliquots were taken from each of the tubes and the concentration of H\(_2\)O\(_2\) measured according to the protocol provided by the manufacturer. Superoxide anion was quantitatively assessed using an high-performance liquid chromatography–based method for quantification of dihydroethidium.\(^{36}\)

### Measurement of Arterial Wall Hypertrophy

For the analysis of vascular hypertrophy, all animals were perfused with saline and subsequently pressure fixed at 100 mm Hg with buffered formalin. Five-micrometer serial sections of paraffin-embedded aortas were stained with hematoxylin and eosin for histological evaluation. Digital photomicrographs of aortic sections were analyzed using NIH Image, with the wall thickness defined as the distance between the outer and inner elastic lamina. The thickness of each aortic wall was represented by the average of 8 measurements made 45°C apart around the circumference of the aortic ring.

### Statistical Analysis

All results are presented as the mean±SEM. Statistical significance was determined by ANOVA to evaluate the difference between individual treatment groups.

### Results

#### Detection of Mouse and Human Catalase Gene Expression

To discriminate between native mouse catalase gene expression and transgenic human catalase gene expression, we took advantage of differences in unique restriction sites in a 403-bp PCR product generated from mouse and human catalase cDNA. After reverse transcribing total mRNA isolated from mouse aortas and PCR amplifying this region of catalase cDNAs, the digestion of the PCR products with restriction enzyme Sph\(_{I}\) or Eco\(_{RV}\) was indicative of the relative amounts cDNA derived from the transgene and from
Using this strategy, we were able to demonstrate in Tgcat-SMC animals a significant amount of PCR product that was cut by SphI, which is indicative of robust expression of the human catalase gene (Figure 1B).

Phenotypic Characterization of Transgenic Mice

To confirm that the catalase overexpression indeed resulted in an increase in enzymatic activity, we assayed for catalase enzymatic activity in aortic tissue harvested from Tgcat-SMC mice and their wild-type littermates. The results showed that aortic catalase enzymatic activity of the Tgcat-SMC mice was 2-fold higher than that of their wild-type littermates (Figure 2A). Immunostaining indicated that the overexpression of the human catalase gene was indeed confined to VSMCs (Figure 2B). Other antioxidant enzyme systems were not altered in the Tgcat-SMC mice (Figure 2B).

H$_2$O$_2$ Production in Aortic Tissue Is Decreased in Tgcat-SMC Mice With Ang II–Induced Hypertension

To directly examine the effects of catalase overexpression, we measured aortic H$_2$O$_2$ production using the Amplex red assay. Under baseline conditions, there was no difference in H$_2$O$_2$ production by the aortas of the Tgcat-SMC mice compared with wild-type littermates. However, whereas Ang II treatment resulted in a 2-fold increase in aortic H$_2$O$_2$ production in the wild-type mice, Ang II–treated Tgcat-SMC mice had virtually undetectable levels of aortic H$_2$O$_2$ production (Figure 3). Conversely, there was no significant effect of catalase overexpression on superoxide production (Figure 4).

Figure 1. Human transgenic catalase mRNA expression in Tgcat-SMC aortas. Expression of the human catalase transgene was evaluated by taking advantage of the fact that there are unique restriction digestion sites in a specific region of the human and mouse catalase cDNAs. We detected the production of human catalase mRNA in aortas of Tgcat-SMC mice by reverse transcription of total catalase mRNA in mouse aorta, PCR amplification of that region, and SphI and EcoRV digestion of the resultant PCR product (Figure 2A). Figure 2B shows an agarose gel demonstrating that part of the PCR products is digested by SphI into 2 DNA fragments (335 and 88 bp) but not by EcoRV, indicating that a significant amount of additional catalase mRNA production is generated by the transgenes.

Figure 2. Catalase enzymatic activity and VSMC-specific catalase overexpression in Tgcat-SMC aortas. A shows the measurement of catalase activity in aortas from 4 Tgcat-SMC mice and 4 wild-type (WT) littermates (*$P<0.01$). B shows representative catalase immunostaining of aortic sections in the top panels. Note the prominent catalase staining (green) localized to VSMCs in the section from Tgcat-SMC animal. The bottom panels show cardiac sections from wild-type, Tgcat-SMC mice, and mice with nontissue-specific overexpression of catalase (Tgcat). C shows a representative Western blot of other antioxidant enzymes indicating no change in expression of these proteins in the Tgcat-SMC mice.

Figure 3. Ang II–induced H$_2$O$_2$ production in aortic segments from Tgcat-SMC and wild-type (WT) mice. H$_2$O$_2$ production in aortic segments with or without the Ang II treatment was measured using the Amplex Red assay. Note that there was no difference between Tgcat-SMC mice and their wild-type littermates in baseline H$_2$O$_2$ production. However, H$_2$O$_2$ released by Ang II–treated aortas of wild-type mice was dramatically increased over baseline, whereas there was no increase in H$_2$O$_2$ production in response to Ang II in the Tgcat-SMC mice. *$P<0.01$ vs vehicle-treated animals.
Catalase Overexpression Does Not Alter the Hypertensive Response to Ang II Infusion

To determine the effects of a reduction in H₂O₂ production on blood pressure, we measured the blood pressures of wild-type and Tg⁹⁺⁺/⁻⁻/-⁺⁺ mice under basal conditions and after treatment with Ang II (0.75 mg/kg per day) for 2 weeks (Figure 5). We found that there was no significant difference in baseline blood pressure between the Tg⁹⁺⁺/-⁺⁺ mice and their wild-type littermates (120±10 versus 106±6 mm Hg; P=NS). Similarly, there was no difference in the hypertensive response to Ang II between the Tg⁹⁺⁺/-⁺⁺ mice and their wild-type littermates (147±10 versus 148±12 mm Hg; P=NS).

Catalase Overexpression Significantly Inhibits Ang II–Induced Vascular Hypertrophy

To test the hypothesis that the reduction in H₂O₂ production by VSMCs leads to diminished Ang II–induced vascular hypertrophy, we measured aortic wall thickness of the Tg⁹⁺⁺/-⁺⁺ mice and their corresponding littermates with or without Ang II treatment. Comparisons were made using tissue sections from approximately the same location of aortas. The results showed that without Ang II treatment, there was no difference in the aortic wall thickness between the Tg⁹⁺⁺/-⁺⁺ mice and the wild-type mice. In wild-type mice, Ang II treatment resulted in a 84.1% increase (from 23.4±1.2 to 43.2±7.6 μm) in wall thickness, whereas in Tg⁹⁺⁺/-⁺⁺ mice, Ang II caused only a 32.3% increase (from 24.5±2.1 to 32.4±2.0 μm) in wall thickness (P=0.05; Figure 6). These findings demonstrate the importance of H₂O₂ in Ang II–induced vascular hypertrophy independent of any effect on blood pressure.

Discussion

The data presented here demonstrate that in the setting of Ang II–induced hypertension, H₂O₂ plays a central and critical role in the development of vascular hypertrophy. Conversely, we have also shown that H₂O₂ appears not to be an important mediator of Ang II–induced hypertension. Numerous studies have previously shown that Ang II is a potent mediator of several pathological processes involving the arterial wall including vascular hypertrophy. A common mediator of Ang II effects on many tissues is the generation of ROS (including superoxide anion and H₂O₂) via activation of the NADPH oxidase. Although there is considerable evidence from in vitro studies supporting a role for H₂O₂ in Ang II–induced vascular cell hypertrophy, to date, there have been no studies that have examined the functional importance of H₂O₂ in Ang II–induced hypertension and the subsequent hypertrophic response of the arterial wall in vivo. In this study, we approached this question by genetically manipulating the level of intracellular H₂O₂ in VSMCs by overexpressing the catalase gene specifically in SMCs. By doing so, we are able to examine the impact of the changes in H₂O₂ levels on Ang II–induced hypertrophy and hypertension. Thus, we have shown that although smooth VSMC H₂O₂ appears not to be involved in the hypertensive response to Ang II infusion, H₂O₂ is a critical mediator of Ang II–induced vascular hypertrophy in vivo.

For the purposes of this study, we generated a unique transgenic mouse (Tg⁹⁺⁺/-⁺⁺) that selectively overexpresses the human catalase gene in SMCs. Although there was no overt gross phenotypic difference in these mice compared with their wild-type littermates, we did document a significant increase in SMC-specific catalase protein expression and enzymatic activity within the arterial wall. Basal H₂O₂ production by the arterial wall was not different between Tg⁹⁺⁺/-⁺⁺ mice and their wild-type littermates suggesting that: (1) there was a compensatory change in the amount of H₂O₂ generated, (2) other cell types are the primary cellular source of basal H₂O₂ production, or (3) basal catalase enzymatic activity was not rate limiting. However, in the setting of Ang II infusion, H₂O₂ production was significantly reduced in the Tg⁹⁺⁺/-⁺⁺ mice. Thus, it appears that although overexpression of catalase in SMCs resulted in increased protein expression and enzymatic activity, effects of the overexpression were only
evident during Ang II infusion. This latter finding would suggest that an effect of catalase expression on vascular phenotype can only be seen under conditions in which the amount of H$_2$O$_2$ generated is increased.

ROS have been mechanistically implicated in the development of genetic and pharmacological forms of hypertension. However, it is not clear which ROS are the causative agents in hypertension. A number of studies using animals with genetic forms of hypertension (spontaneously hypertensive rats, salt-dependent Dahl hypertensive rats) demonstrated a strong association between the production of ROS and blood pressure. Hypertensive patients have been reported to have significantly higher levels of plasma peroxides when compared with normotensive subjects. In addition, it appears that plasma H$_2$O$_2$ production correlates directly with plasma renin activity, suggesting a role for H$_2$O$_2$ in Ang II–related regulation of blood pressure. Systemic treatment with SOD attenuates Ang II–induced hypertension, suggesting that superoxide may be the predominant ROS that cause hypertension. This may occur via direct effects on the VSMCs, via degradation of NO, or both. It has been suggested to induce vasoconstriction and vasorelaxation. A recent study by Yang et al concluded that the overexpression of catalase in a nontissue-specific fashion did significantly reduce the hypertensive effect of Ang II. It is important to note that these authors showed an effect of catalase overexpression on norepinephrine-induced hypertension as well. This finding is somewhat surprising given that other studies have shown that norepinephrine-induced hypertension is not mediated by ROS. We observed no differences in blood pressure between the Tgcat-SMC and wild-type mice under either baseline conditions or during Ang II infusion using the noninvasive tail-cuff technique to measure blood pressure. This technique is useful for measuring significant changes in systolic blood pressure but may not be as useful as the more invasive telemetry techniques. Thus, we may have not been able to detect more subtle changes in blood pressure. However, the similarity in the systolic blood pressures in the Ang II–treated Tgcat-SMC mice and the Ang II–treated wild-type mice suggest that H$_2$O$_2$ in VSMCs does not significantly contribute to the regulation of blood pressure in this model of hypertension. Together with other published studies, it appears that either superoxide or H$_2$O$_2$ in a different cell type is likely to be more important in terms of arterial hypertension.

H$_2$O$_2$ has also been implicated as a critical second messenger for Ang II–induced vascular hypertrophy. We found no difference in the aortic wall thickness between Tgcat-SMC and wild-type mice under baseline conditions. However, in the setting of Ang II–induced hypertension, vascular wall hypertrophy was significantly and dramatically reduced in the Tgcat-SMC mice. The reduction of vascular hypertrophy was concurrent with the inhibition of Ang II–induced H$_2$O$_2$ production in the Tgcat-SMC mice. Importantly, this inhibition of vascular wall hypertrophy occurred in the face of no obvious change in arterial blood pressure. These findings indicate that at least part of the hypertrophic effect of Ang II occurs independently of the hypertensive effect of Ang II infusion. This finding is consistent with the results from studies performed using cultured VSMCs, showing that Ang II–mediated protein synthesis and cellular hypertrophy in vitro are mediated by H$_2$O$_2$ resulting from activation of the NAD(P)H oxidase.

There are several aspects of this model that warrant discussion. First, because H$_2$O$_2$ is generally considered to be freely diffusible across the cell membrane, overexpression of catalase in smooth muscle does not necessarily mean that the source of H$_2$O$_2$ is the smooth muscle. However, the results obtained in our experimental model demonstrate that intracellular H$_2$O$_2$ in the VSMCs is a critical mediator of vascular hypertrophy. In addition, it is also important to realize that we did not study resistance vessels in this model. Our findings in aortic tissues may not be directly applicable to resistance vessels. Finally, it has been proposed recently that catalase may have peroxidase-like activity that could potentially yield a biological response independent of reductions in hydrogen peroxide. Although the potential conflicting effects of a peroxidase-like function is a possibility, peroxidase-like activity has only been demonstrated in the setting of the addition of significant concentrations of exogenous catalase to a nonvascular cell culture system.

In summary, we used a novel transgenic mouse model with SMC-specific overexpression of the human catalase gene to examine the physiological importance of H$_2$O$_2$ production by SMCs. We have shown that VSMC-derived H$_2$O$_2$ is an important mediator of Ang II–induced vascular hypertrophy in vivo. However, we also demonstrated that VSMC-derived H$_2$O$_2$ does not appear to have a significant effect on blood pressure. We conclude from this that different ROS can have differing physiological consequences and that not all pathophysiologic responses to Ang II can be tied to a single molecular species of ROS.

Acknowledgments

This work was supported by grants from the National Institutes of Health (PO1 HL58000 and HL38206) and the southeast affiliate of the American Heart Association.

References


Vascular Hypertrophy in Angiotensin II–Induced Hypertension Is Mediated by Vascular Smooth Muscle Cell–Derived H$_2$O$_2$

Yong Zhang, Kathy K. Griendling, Anna Dikalova, Gary K. Owens and W. Robert Taylor

_Hypertension_. 2005;46:732-737; originally published online September 19, 2005; doi: 10.1161/01.HYP.0000182660.74266.6d

_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/46/4/732

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2005/11/01/01.HYP.0000182660.74266.6d.DC1

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/