Afferent and Efferent Arteriolar Vasoconstriction to Angiotensin II and Norepinephrine Involves Release of Ca$^{2+}$ From Intracellular Stores

Edward W. Inscho, John D. Imig, Anthony K. Cook

Abstract Renal vascular responses to angiotensin II (Ang II) and norepinephrine (NE) are reported to involve both mobilization of calcium from intracellular stores and activation of calcium influx pathways. The present study was conducted to determine the contribution of calcium release from intracellular stores to afferent and efferent arteriolar responses to Ang II and NE. Experiments were performed in vitro using the blood-perfused, juxtamedullary nephron technique combined with videomicroscopy. The responses of afferent and efferent arterioles to Ang II and NE were determined before and after depletion of intracellular calcium pools with 1 mmol/L thapsigargin. Positive control responses were obtained with 55 mmol/L KCl. Ang II concentrations of 0.1, 1.0, and 10 nmol/L decreased afferent arteriolar diameter by 10±4%, 17±4%, and 29±6%, respectively (P<0.05, n=8). NE also decreased afferent diameter by 5±1%, 7±2%, and 14±4%, respectively (P<0.05, n=6). Thapsigargin treatment shifted the afferent arteriolar concentration-response curves for both Ang II and NE significantly to the right. Nevertheless, KCl evoked a pronounced vasoconstriction and decreased afferent diameter by 56±7% (P<0.05, n=6). Postglomerular responses to Ang II and NE were abolished by thapsigargin. During the control period, efferent diameter decreased by 3±1%, 7±2%, and 14±4% for the three Ang II concentrations and 3±1%, 5±1%, and 15±4% in response to the three NE concentrations, respectively. These responses were completely eliminated in the presence of thapsigargin, whereas KCl evoked an efferent arteriolar vasoconstriction of 57±9% (P<0.05). These data demonstrate that agonist-induced calcium release from intracellular stores represents an essential component in the afferent and efferent arteriolar response to Ang II and NE. Furthermore, they suggest that efferent arteriolar responses to these agents may rely more heavily on calcium release from this store, whereas afferent responses may include activation of other pathways. (Hypertension. 1997;29[part 2]:222-227.)

Key Words • afferent arteriole • efferent arteriole • calcium mobilization • thapsigargin • microcirculation • kidney • renal

Afferent and efferent arterioles respond in concert with hormonal and paracrine influences to efficiently regulate renal blood flow and glomerular filtration rate. Elevation of cytosolic calcium concentration represents an important component in the renal microvascular response to vasoconstrictor agonists. Increasing cytosolic calcium concentration in renal microvascular smooth muscle can occur through activation of calcium influx pathways involving ion channel activation and/or mobilization of calcium release from intracellular pools. Studies have shown that Ang II stimulates segmentally distinct signal transduction pathways to elicit vasoconstriction of afferent and efferent arterioles. Afferent arteriolar vasoconstriction relies largely on calcium influx through voltage-gated calcium channels, and this response can be blocked by dihydropyridine calcium channel antagonists. In contrast, Ang II vasoconstricts efferent arterioles through mechanisms independent of L-type calcium channel activation. With the recent development of agents that selectively perturb the filling state of intracellular calcium pools, it is now possible to manipulate these pools and determine the influence of calcium store integrity on microvascular responsiveness to vasoconstrictors. Indeed, in a recent report by Ruan and Arendshorst, infusion of agents capable of inhibiting the release of calcium from intracellular pools significantly attenuated the maximum decrease in renal blood flow produced by Ang II. These data suggest that calcium release from intracellular stores participates in the renal vascular response to Ang II, however, the specific vascular segments affected by these release inhibitors were not determined.

The present studies were performed to directly determine the effect of calcium store depletion on the afferent and efferent arteriolar response to Ang II and NE. Direct assessment of afferent and efferent arteriolar responsiveness to Ang II or NE was performed before and during depletion of intracellular calcium pools with the Ca$^{2+}$-ATPase inhibitor thapsigargin. Thapsigargin has been shown to deplete IP$_3$-sensitive intracellular calcium stores, thereby interrupting IP$_3$-dependent, agonist-mediated responses.

Methods

Materials

Thapsigargin was purchased from Calbiochem Inc. NE (Levophed) was obtained from Winthrop Pharmaceuticals. Enalaprilat was a gift from Merck Sharp and Dohme. All other reagents were purchased from Sigma Chemical Co.

Kidney Preparation

Studies were approved by the Tulane University Advisory Committee for Animal Resources. Experiments were conducted from the Department of Physiology, Tulane University School of Medicine, New Orleans, La. Correspondence to Edward W. Inscho, Department of Physiology, Tulane University School of Medicine, 1430 Tulane Ave, New Orleans, LA 70112. E-mail enscho@mailhost.tcs.tulane.edu © 1997 American Heart Association, Inc.
in vitro with the blood-perfused juxtamedullary nephron technique as previously described.

For each experiment, two male Sprague-Dawley rats (350 to 400 g) were anesthetized with sodium pentobarbital (40 mg/kg IP) and pretreated for 30 minutes with the converting enzyme inhibitor enalaprilat (2 mg IV). Perfusion blood was collected from the kidney donor and a blood donor and was prepared as previously described. The anesthetized blood donor rat was nephrectomized and, 30 minutes later, exsanguinated into a syringe containing heparin (500 U). The plasma and erythrocyte fractions were separated from the centrifuged blood, and the leukocyte fraction was discarded. The plasma was filtered (0.2 μm) before being mixed with erythrocytes to yield a hematocrit of ≈33. The reconstituted blood was filtered through a 5-μm nylon mesh.

The right renal artery of the kidney donor was cannulated, and the kidney was immediately perfused with a Tyrode’s buffer solution containing 52.0 g/L BSA (Sigma Chemical Co) and a complement of L-amino acids. The perfused kidney was removed and sectioned along the longitudinal axis, leaving the intact papilla on the dorsal two thirds of the kidney. The papilla was reflected back, and the pelvic mucosa was removed to expose the tip and was set at 100 mm Hg. The inner cortical surface of the kidney was continuously superfused with warmed (37°C) Tyrode’s buffer containing 10.0 g/L BSA, and the kidney was allowed to equilibrate for at least 15 minutes.

The perfusion chamber containing the prepared kidney was affixed to the stage of a Nikon Xenophot microscope (Nikon Corp) equipped with long-working-distance objectives. Video images were generated with a high-resolution Newvicon camera (NC-67 m, Dage-MTI) were electronically enhanced (image processor MFJ-1425, MFJ Enterprises Inc) and displayed on a video monitor while being simultaneously recorded on videotape for analysis. Afferent and efferent arteriolar IDs were measured at a single site with an image shearing monitor (model 901, Instrumentation for Physiology and Medicine) calibrated with a stage micrometer. The steady-state diameter of each arteriole was calculated for each experimental period by averaging the 10 determinations taken during the last 2 minutes of that period. Afferent arterioles were selected for study on the basis of the clarity of the vascular walls and the adequacy of blood flow through the vessel lumen.

Protocols

These studies were conducted to determine the role of calcium release from the thapsigargin-sensitive intracellular calcium pool on the Ang II- and NE-mediated vasoconstriction of juxtamedullary afferent and efferent arterioles. After a 15-minute equilibration period, a protocol was initiated consisting of consecutive 5-minute treatment periods. Treatments were administered by bathing the tissue with a superfusate solution whose composition was varied accordingly. After an initial control period, the tissue was exposed to increasing concentrations of Ang II over the range of 0.1, 1.0, and 10 nmol/L and followed by a recovery period. After the recovery period, the superfusion solution was changed to one containing a Ca2 + -ATPase–inhibiting sesquiterpene lactone, thapsigargin (1.0 μmol/L). The concentration of thapsigargin was selected from previous experiments in this preparation, which demonstrated that 1.0 μmol/L thapsigargin attenuated the afferent response to 200 nmol/L NE. The kidneys were exposed to thapsigargin for 10 minutes before arteriolar responses to Ang II at the concentrations used in the control conditions were reassessed.

A second series of experiments was performed to determine the effect of calcium store depletion with thapsigargin on the afferent and efferent arteriolar responses to NE. These experiments were performed according to a protocol identical to that described above except that NE was used as the agonist at concentrations of 10, 100, and 1000 nmol/L.

Positive control responses were obtained at the conclusion of each experiment. Microvascular responses to 55 nmol/L KCl were determined to confirm that arterioles remain responsive to calcium store–independent vasoconstrictor stimuli and to confirm that 1.0 μmol/L thapsigargin was not impairing voltage-dependent Ca2 + influx pathways.

Statistical Analysis

Statistical comparisons within each series were made by one-way ANOVA for repeated measures combined with the Newman-Keuls multiple-range test. Comparisons across treatment groups were made by paired t test. Values of P < 0.05 were considered significant. All data are reported as mean±SEM.

Results

Ang II Studies

The effect of thapsigargin on the afferent arteriolar response to Ang II is shown in Fig 1. Eight afferent arterioles from seven kidneys averaged 22.1±1.4 μm during the control period, and this diameter decreased in a concentration-dependent manner in response to sequential exposure to 0.1, 1.0, and 10 nmol/L Ang II. Afferent calibers decreased significantly to 19.7±1.2, 18.2±1.0, and 15.3±1.2 μm, respectively. Thapsigargin treatment initially caused afferent diameter to decline during the first 1 to 3 minutes before reaching a steady-state diameter similar to control (22.5±1.3 μm). Subsequent addition of Ang II in the continuing presence of thapsigargin resulted in a significant shift to the right in the concentration-response profile. Whereas angiotensin concentrations of 0.1 and 1.0 nmol/L evoked significant vasoconstriction during the control conditions, thapsigargin (1.0 μmol/L) attenuated the afferent response to 200 nmol/L NE. The kidneys were exposed to thapsigargin for 10 minutes before arteriolar responses to Ang II at the concentrations used in the control conditions were reassessed.

![Graph](http://hyper.ahajournals.org/)

**Fig 1.** Effect of thapsigargin on the afferent arteriolar response to Ang II. Changes in afferent diameter are expressed as a percentage of the control (CON) diameter. Afferent arteriolar responses to increasing Ang II concentrations of 0.1, 1.0, and 10 nmol/L are shown during superfusion with the control buffer (solid circles) and then again during exposure to 1 μmol/L thapsigargin (shaded circles). Afferent diameter averaged 22.3±1.6 and 22.4±1.5 μm during the control and thapsigargin periods, respectively. n=8 afferent arterioles from seven kidneys. *P<0.05 vs control, tP<0.05 vs Ang II alone.
Thapsigargm treatment almost completely abolished the efferent arteriolar response to NE, with only a slight tendency for vasoconstriction evident with 1000 nmol/L NE. Thapsigargm treatment completely abolished the NE-mediated efferent arteriolar vasoconstrictor response. Efferent diameter averaged 23.5 ± 1.4 μm during thapsigargm treatment alone and remained at that diameter during exposure to each NE concentration.

To control for the possibility that thapsigargm treatment might interfere with microvascular contractile response through nonspecific mechanisms, we confirmed the ability of these arterioles to respond to receptor-independent stimulation with 55 mmol/L KCl. KCl was administered in the presence of thapsigargm to ensure that the efferent arterioles remained unchanged in response to these Ang II concentrations in the presence of thapsigargm. Only the 10 nmol/L Ang II concentration evoked a significant efferent arteriolar vasoconstriction in the presence of thapsigargm, and the magnitude of that response was markedly attenuated compared with the control response.

Efferent arteriolar responses to NE were also assessed; the results of those studies are presented in Fig 2. Six efferent arterioles from six kidneys averaged 22.6 ± 1.2 μm during the control period. Only 10 nmol/L Ang II caused efferent arteriolar diameter to decrease significantly to 19.6 ± 1.8 μm (P < 0.05 versus control), however, as shown in Fig 2, efferent diameter tended to decrease progressively with each subsequent Ang II concentration. The magnitude of the efferent arteriolar vasoconstriction evoked by 10 nmol/L Ang II was not significantly different from the efferent arteriolar response to the same Ang II concentration. The response of efferent arterioles to thapsigargm treatment was similar to the response of afferent arterioles. Efferent diameter slowly declined during the initial exposure to thapsigargm before stabilizing at a diameter not significantly different from control. In the continuous presence of thapsigargm, the efferent response to Ang II was completely abolished. Vessel diameter averaged 23.2 ± 1.1, 23.2 ± 1.2, and 23.1 ± 1.1 μm for each concentration, respectively.

**Norepinephrine Studies**

The effect of thapsigargm on the efferent arteriolar response to NE was also examined; the results of those studies are illustrated in Fig 3. Six efferent arterioles examined from six kidneys averaged 20.3 ± 1.3 μm during the control period, and this diameter decreased in a concentration-dependent manner in response to sequential exposure to 10, 100, and 1000 nmol/L NE. Afferent caliber decreased significantly to 19.2 ± 1.3, 17.8 ± 1.3, and 8.6 ± 1.8 μm, respectively. Thapsigargm treatment almost completely abolished the efferent arteriolar response to NE, with only a slight tendency for vasoconstriction evident with 1000 nmol/L NE.

Efferent arteriolar responses to NE were also assessed, the results of those studies are presented in Fig 4. Six efferent arterioles from six kidneys averaged 24.6 ± 1.3 μm during the control period. NE concentrations of 100 and 1000 nmol/L caused efferent arteriolar diameter to decrease significantly, to 22.9 ± 1.3 and 20.6 ± 1.8 μm, respectively (P < 0.05 versus control). The magnitude of the efferent arteriolar vasoconstriction evoked by 1000 nmol/L NE was significantly smaller than the efferent arteriolar response to the same NE concentration. Similar to the efferent response to Ang II, thapsigargm treatment completely abolished the NE-mediated efferent arteriolar vasoconstrictor response. Efferent diameter averaged 23.5 ± 1.4 μm during thapsigargm treatment alone and remained at that diameter during exposure to each NE concentration.
influence of thapsigargin was not interrupted. Afferent arteriolar diameter declined by 36±7% (P<0.05 versus thapsigargin alone), from an average of 23.5±2.1 to 10.3±1.9 μm, in response to KCl exposure. Similarly, efferent arteriolar diameter decreased by 57.1±8% (P<0.05 versus thapsigargin alone), from 24.8±1.2 to 10.6±3.3 μm during KCl superfusion.

Discussion

In the present study, we directly examined the effect of intracellular calcium store depletion on renal microvascular responses to vasoconstrictors. The results of these studies demonstrate that juxtamedullary microvascular responses to Ang II and NE involve release of Ca²⁺ from thapsigargin-sensitive, intracellular calcium stores. These studies further demonstrate that preglomerular and postglomerular arterioles of juxtamedullary nephrons rely on differing degrees on Ca²⁺ released from intracellular calcium pools to effect the receptor-mediated vasoconstrictor response to Ang II or NE.

The importance of extracellular calcium in the regulation of renal microvascular responses to vasoactive agents has been recognized for some time, however, the potential role for calcium release from intracellular pools in agonist-mediated vasoconstrictor responses has not been directly evaluated. Earlier studies have suggested that calcium mobilization from intracellular stores is not a major contributor to autoregulatory responses in the dog kidney. Subsequent work revealed that infusion of a purported calcium release inhibitor, TMB-8, into the renal artery markedly attenuated the renal vasoconstriction induced by bolus intrarenal injection of Ang II or vasopressin. More recently, Ruan and Arendshorst reported that ≈50% of the Ang II-mediated decrease in rat renal blood flow was inhibited by blockade of L-type calcium channels. Approximately 50% of the renal blood flow response was also inhibited by TMB-8 or heparin. Both TMB-8 and heparin are reported to stabilize Ca²⁺ binding to the calcium stores and thereby inhibit Ca²⁺ release from IP³-sensitive intracellular calcium pools.

This observation suggests that Ang II stimulates calcium mobilization from intracellular pools to evoke renal vasoconstriction and thus reduce renal blood flow. These studies provide provocative in vivo evidence for the involvement of a phospholipase C-IP³-sensitive signal transduction step in the renal vascular response to Ang II, but they do not indicate which intrarenal vascular segments are involved in the response.

Previous studies have focused on the role of L-type calcium channels and calcium influx from the extracellular medium to evoke afferent and efferent arteriolar vasoconstriction. Administration of L-type calcium channel antagonists preferentially inhibits afferent arteriolar vasoconstrictor responses to Ang II, whereas it does not markedly alter Ang II-mediated efferent arteriolar vasoconstriction. Ang II increases cytosolic calcium in both afferent and efferent arterioles with EGTA-containing solutions with a reduced extracellular Ca²⁺ concentration attenuate the rise in cytosolic Ca²⁺ concentration and blunt the vasoconstriction of afferent and efferent arterioles in response to Ang II. Also in low-Ca²⁺ conditions, administration of the putative sarcoplasmic reticulum calcium release blocker dantrolene markedly attenuated the Ang II-mediated vasoconstriction of isolated perfused afferent and efferent arterioles. These data implicate a role for calcium release from intracellular stores in the preglomerular vasoconstriction elicited by Ang II. Unfortunately, the specificity of dantrolene has not been clearly demonstrated, and the simultaneous exposure of isolated vascular segments to dantrolene plus 110 mmol/L Ca²⁺ in EGTA-containing solutions may have nonspecifically compromised the reactivity of these arterioles by reducing intracellular Ca²⁺ concentration to unacceptable levels.

Thapsigargin is a Ca²⁺-ATPase–inhibiting sesquiterpene lactone that irreversibly inhibits the sarcoplasmic and endoplasmic reticulum Ca²⁺-ATPases (SERCA a, b₁, b₂, and c) expressed from cDNA. Thapsigargin inhibits each of these SERCA ATPases with equal potency. Calcium ATPase inhibition results in depletion of intracellular calcium stores as they are emptied via endogenous leak pathways without being replenished through the activity of the sarcoplasmic reticulum Ca²⁺-ATPase. This eliminates the availability of a readily mobilizable calcium reservoir to be accessed by store-dependent vasoconstrictor agonists even in the presence of normal extracellular Ca²⁺ concentrations. In the present report, thapsigargin treatment did not significantly alter resting afferent or efferent arteriolar diameter, however, thapsigargin did alter the microvascular response to Ang II and NE. Thapsigargin administration attenuated the afferent arteriolar response to these vasoconstrictor agents and abolished the efferent arteriolar response. Unlike a previous study, microvascular responses by intracellular store depletion were altered with the extracellular Ca²⁺ concentration in the normal physiological range.

Some studies have suggested that, in addition to its Ca²⁺-ATPase-inhibitory properties, thapsigargin may also inhibit Ca²⁺ influx through voltage-dependent, L-type calcium channels. Other studies have suggested that thapsigargin may stimulate calcium influx through a nocardipine-insensitive Ca²⁺ channel. Despite such reports, these nonspecific effects do not appear to be confounding the results of the present study, for the following reasons: Positive control experiments were performed to confirm that arterioles, which were unresponsive to Ang II during thapsigargin treatment, retained responsiveness to store-independent vasoconstrictor stimuli. Membrane depolarization with KCl vasoconstricts afferent and efferent arterioles by activation of L-type calcium channels. In the present study, 55 mmol/L KCl consistently decreased afferent and efferent arteriolar diameter by ≈50%, even with the continuous presence of thapsigargin in the bathing medium. This observation confirms that thapsigargin treatment did not significantly impair the activity of L-type calcium channels in these arterioles under these conditions and that these arterioles retained the ability to contract to vasoconstrictor stimuli that are not dependent on an intact intracellular calcium pool. Nonspecific effects due to stimulation of a nocardipine-insensitive calcium current are also an unlikely explanation, since this would serve to increase cytosolic calcium and thereby facilitate renal microvascular vasoconstriction. As noted, thapsigargin treatment did not significantly alter resting afferent or efferent arteriolar diameter, nor did it enhance responsiveness to Ang II or NE. Therefore, the data presented here support the hypothesis that thapsigargin treatment depletes afferent and efferent arteriolar calcium stores and thus eliminates a pivotal component of the signal transduction cascade induced by Ang II and NE.
Although Ang II stimulates vasoconstriction of afferent and efferent arterioles, divergent mechanisms are used by the preglomerular and postglomerular arterioles to activate the response. Numerous studies have shown that Ang II stimulates sustained vasoconstriction of afferent arterioles through activation of voltage-gated calcium channels. In contrast, Ang II-mediated vasoconstriction of efferent arterioles does not appear to involve L-type calcium channel activation. It is thus tempting to speculate that the efferent arteriolar response to Ang II is largely dependent on the mobilization of intracellular calcium, whereas afferent arteriolar responses are more dependent on the influx of extracellular calcium. The results of the present studies using juxtamedullary microvascular segments are consistent with this hypothesis.

Concentration-response curves for the afferent arteriolar response to Ang II or NE were not totally abolished by thapsigargin but rather were shifted to the right. This suggests that although the response is markedly blunted, thapsigargin-treated afferent arterioles still retain the ability to respond to these agents in the concentration ranges we examined. The mechanism(s) responsible for the remaining vasoconstrictor reserve remains to be identified. Several different pathways can be considered as viable explanations. Investigators have suggested that multiple calcium storage pools exist within cells and that not all of these pools are sensitive to thapsigargin. Therefore, it is possible that Ang II or NE stimulates calcium release from both thapsigargin-sensitive and thapsigargin-insensitive intracellular calcium pools to effect afferent arteriolar vasoconstriction. Intervention into the function of one of these calcium sources may not interfere with the ability of other sources to respond to Ang II or NE stimulation. Alternatively, it has been reported that isolated afferent arterioles use chloride channel activation in their response to Ang II. Cultured rat mesangial cells respond to Ang II with activation of phospholipase C-γ1, with subsequent activation of a low-conductance chloride channel. However, the link between agonist-induced calcium mobilization and chloride channel activation in microvascular smooth muscle has not been shown. Nevertheless, it is possible that the depolarizing influence of chloride channel activation can persist in thapsigargin-treated arterioles and thus lead to vasoconstriction through activation of voltage-dependent pathways. In contrast to the afferent arteriole, thapsigargin treatment totally abolished the efferent response to Ang II and NE at the concentrations used. The remarkable difference in the efficacy of thapsigargin treatment in altering afferent and efferent arteriolar responsiveness to Ang II and NE strongly suggests that calcium release from thapsigargin-sensitive intracellular stores plays a pivotal role in the ability of efferent arterioles to respond to these agents. Clearly, these data are consistent with the work of others demonstrating disparate afferent and efferent sensitivity to L-channel blockade in altering preglomerular and postglomerular responsiveness to Ang II.

In summary, thapsigargin treatment markedly attenuates juxtamedullary afferent arteriolar vasoconstrictor responses to Ang II or NE. In addition, depletion of intracellular calcium pools with thapsigargin completely abolishes the response of juxtamedullary efferent arterioles to these vasoconstrictor agents. The results of this study using juxtamedullary microvascular segments are in good agreement with the observations of other investigators using arterioles isolated from different regions of the kidney as well as in vivo studies involving whole-kidney hemodynamics. These data support the hypothesis that calcium release from a thapsigargin-sensitive intracellular calcium pool represents a critical link in the signal transduction pathway used by Ang II and NE to evoke afferent vasoconstriction. Furthermore, efferent arteriolar responses to these agents may rely more heavily on calcium release from this store, whereas afferent responses may include activation of other pathways.

Acknowledgments

These studies were supported by grants from the American Heart Association and the National Institutes of Health (DK-44628). Dr. John D. Imig is the recipient of a Grant-in-Aid from the American Heart Association. Dr. Edward W. Inceho is an Established Investigator of the American Heart Association. The authors wish to thank Vy Mui for her excellent technical assistance.

References

7. Carmenes PK, Navar LG. Depolarizing effects of Ca channel blockade on afferent and efferent arteriolar responses to Ang II. *Am J Physiol* 1989;256:F1015-F1020
18. Ogawa N, Ono H. Effect of 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8), an inhibitor of intracellular Ca2+ release, on
autoregulation of renal blood flow in the dog. *Naunyn Schmiedebergs Arch Pharmacol* 1988, 338:293-296
Afferent and Efferent Arteriolar Vasoconstriction to Angiotensin II and Norepinephrine Involves Release of Ca^{2+} From Intracellular Stores
Edward W. Inscho, John D. Imig and Anthony K. Cook

_Hypertension_. 1997;29:222-227
doi: 10.1161/01.HYP.29.1.222

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1997 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/29/1/222

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/