Arthur C. Corcoran Memorial Lecture

Hormones and Vasoprotection

Suzanne Oparil

Abstract—There is a strong link between menopause and increased cardiovascular disease incidence in women, and observational studies suggest that postmenopausal hormone replacement therapy reduces cardiovascular disease risk by about half. Observational studies suffer from important limitations, however, and the only published prospective controlled trial of the effects of hormone replacement therapy on cardiovascular outcomes, the Heart Estrogen-Progestin Replacement Study (HERS), showed no net benefit of continuous estrogen plus synthetic progestin treatment in women with established coronary disease. Fundamental mechanistic studies of the cellular and molecular events by which hormones protect (or fail to protect) blood vessels from damage are needed to define the role of postmenopausal hormone replacement therapy in cardiovascular disease prevention. Most studies suggest that estrogen inhibits the neointimal response to acute injury in normal blood vessels, but this vasoprotective effect was not seen in vessels with preexisting atherosclerosis. Studies from our laboratory in the rat carotid injury model have shown that estrogen inhibits neointima formation via effects on all 3 layers of the vascular wall, including inhibition of medial smooth muscle cell migration and proliferation, stimulation of regrowth of endothelium, and inhibition of adventitial cell migration into neointima. Our laboratory is currently using transduced (lacZ) syngeneic fibroblasts as ‘reporter’ cells to delineate the factors that stimulate migration of adventitial cells into neointima after vascular injury and their modulation by estrogen and the other sex hormones. These fundamental studies will establish more rational strategies for therapeutic intervention in vascular diseases, including the basis for future gene therapy. (Hypertension. 1999;33[part II]:170-176.)

Key Words: hormones ■ gender ■ women ■ estrogen ■ progestin ■ fibroblast ■ muscle, smooth

Cardiovascular disease, including coronary artery disease and stroke, is the leading cause of death in women.1 There is a strong link between menopause and an increased incidence of cardiovascular disease,2 and observational studies suggest that postmenopausal hormone replacement therapy, including various estrogen preparations with or without progestin (most commonly synthetic progestin), reduces cardiovascular disease risk by about half.1,3–5 Studies also suggest that estrogen may slow the progression of existing coronary artery disease in postmenopausal women and limit the proliferation of vascular smooth muscle cells (VSMCs) after vascular injury [Table].6–10 For example, 6-month results of the Coronary Angioplasty Versus Excisional Atherectomy Trial (CAVEAT I) showed that women undergoing atherectomy who received hormone replacement therapy (estrogen±progestin) had significantly less late loss in minimal lumen diameter, larger lumen diameter, and lower restenosis rates (27% versus 57%, \( P=0.038 \) for >50% stenosis) than those not receiving estrogen.7 In contrast, estrogen had a minimal effect on restenosis after balloon angioplasty in this trial. Furthermore, 2 retrospective studies of women undergoing elective percutaneous transluminal coronary angioplasty (PTCA) showed improved survival and reduced cardiovascular event rates (death, nonfatal myocardial infarction, or nonfatal stroke) in women treated with hormone replacement; there was no difference between treatment groups in need for subsequent revascularization, suggesting no reduction in restenosis.8,9 These device-specific results are consistent with an inhibitory effect of estrogen on neointima formation, which is thought to play a greater role in restenosis after atherectomy than after balloon angioplasty, where later recoil is a major contributor.10

These studies, while provocative, suffer from the limitations of small patient numbers and noncomparability of the 2 treatment groups: women who take estrogen are on average better educated, have higher incomes and better access to health care, and are healthier even before starting therapy.11,12 This deficiency in available data concerning the cardiovascular benefits of hormone replacement therapy, combined with the known increased risk of breast and endometrial cancer and venous thromboembolism in long-term users of estrogen, has created controversy in the healthcare arena about the general advisability of hormone replacement.13

The recently published early results from the Heart Estrogen-Progestin Replacement Study (HERS) have added to the controversy.14–16 HERS was the first large-scale, randomized clinical trial to test the efficacy and safety of hormone replacement on clinical cardiovascular disease out-
Population Studies of Effects of Hormone Replacement Therapy on Cardiovascular Risk Factors, Atherosclerotic Disease, and Cardiovascular Outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Full Title</th>
<th>Intervention/End Point</th>
<th>Therapy</th>
<th>Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAVeAT</td>
<td>Coronary Angioplasty Versus Excisional Atherectomy Trial</td>
<td>Atherectomy; angioplasty</td>
<td>E2 + P</td>
<td>Lower restenosis rate</td>
</tr>
<tr>
<td>HERS</td>
<td>Heart and Estrogen/Progestin Replacement Study</td>
<td>CV disease outcomes</td>
<td>E2 + P</td>
<td>↑ early events, ↓ later events</td>
</tr>
<tr>
<td>WHI</td>
<td>Women’s Health Institute</td>
<td>Longitudinal outcomes</td>
<td>E2 + P</td>
<td>In progress</td>
</tr>
<tr>
<td>PEPI</td>
<td>Postmenopausal Estrogen/Progestin Intervention trial</td>
<td>HDL</td>
<td>E2 + MPA</td>
<td>Smaller ↑ in HDL with MPA</td>
</tr>
<tr>
<td>FOS</td>
<td>Framingham Offspring Study</td>
<td>CV risk factors</td>
<td>E2 + P</td>
<td>↑ PAI-1 with E2 ± MPA</td>
</tr>
<tr>
<td>NHS</td>
<td>Nurses’ Health Study</td>
<td>E2 and E2 + P</td>
<td>E2 + P</td>
<td>Decrease major CHD</td>
</tr>
</tbody>
</table>

E2 indicates estrogen; P, progestin; CV, cardiovascular; PAI-1, plasminogen activator inhibitor; and CHD, coronary heart disease.

comes in postmenopausal women. The study population included 2763 women with established coronary artery disease randomized to combined hormone replacement therapy or placebo who were followed up for an average of 4 years. Overall, there was no significant difference between groups for the primary outcome, nonfatal myocardial infarction or coronary heart disease death, or for several secondary cardiovascular end points. There was a statistically significant time trend, with more coronary heart disease events in the treatment group than in the placebo group in year 1 and fewer in years 3 and beyond (Figure 1). These results do not support instituting hormone replacement therapy in women with established coronary heart disease for the sole purpose of avoiding secondary events. HERS did not address the question of benefit (and risk) from estrogen alone or from combined hormone replacement in primary prevention, nor did it elucidate the mechanism of the apparently biphasic effect (early detriment, later benefit) of combined hormone replacement in women with atherosclerotic disease. Answers to the first two questions will come from the Women’s Health Initiative (WHI), a randomized trial of estrogen and combined hormone replacement therapy for primary prevention, which includes 10 times as many treated women as HERS and a longer (9 years) period of treatment, completing in the year 2005. The last question can be answered only by further research in both human subjects and animal models.

Effects of Progestin on Estrogen-Mediated Vasoprotection

Studies in humans and experimental animals have suggested that addition of a progestin, while needed to prevent the hyperplastic/neoplastic effects of unopposed estrogen on the endometrium, may reduce the beneficial effects of estrogen replacement therapy on the risk of cardiovascular disease. For example, in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, women randomized to conjugated equine estrogen and continuous or cyclical medroxyprogesterone acetate (MPA) had smaller increases from baseline in HDL cholesterol levels than women randomized to unopposed estrogen. Such an effect on HDL levels could compromise the cardioprotective effect of estrogen, given the inverse relationship between HDL cholesterol levels and cardiovascular disease in women. In the Framingham Offspring Study, postmenopausal women taking a combination of estrogen and progestin had higher levels of plasminogen activator inhibitor (PAI-1) than women taking unopposed estrogen when adjustments were made for age, risk factors, and other covariants, suggesting that these women might be less vulnerable to the development of intravascular thrombi and therefore cardiovascular events. Ovariectomized rhesus monkeys treated chronically with a combination of MPA plus 17β-estradiol manifested coronary vasospasm in response to infusion of serotonin plus a thromboxane A2 mimetic, while those treated with native progesterone plus 17β-estradiol did not. This study gives evidence that different progestins have different effects on vascular function, perhaps depending on their potency, androgenicity, and other yet to be evaluated properties.

Whether these putative adverse effects of progestins are clinically important is uncertain, particularly in light of the recent report from the Nurses’ Health Study, which found a
decrease in the risk of major coronary heart disease among women who took estrogen with progestin compared with women who took estrogen alone or did not use hormones. The recent demonstration of dose-dependent inhibition of DNA synthesis, expression of cyclin A and E mRNA levels, and proliferation in human and rat aortic smooth muscle cells in culture by native progesterone is consistent with the interpretation that some progestins can be vasoprotective. Whether native progesterone has effects similar to those of MPA on the response to vascular injury and whether interactions between progesterone and estrogen on the vasculature are mediated at the estrogen receptor level are unanswered questions of both scientific and clinical importance.

**Animal Models of Vascular Injury: Sex Hormone Effects**

Definitive answers to questions about the effects of sex hormones on injured blood vessels will come only from fundamental mechanistic studies of the cellular and molecular events by which estrogen and progestins protect (or fail to protect) blood vessels from damage. While much has been learned about mechanisms of estrogen-induced vasoprotection from studies carried out in women, insights derived from animal experiments have been indispensable in advancing our understanding of the vascular effects of the sex hormones, particularly estrogen. It has been clearly shown that endothelial cells and VSMCs possess estrogen receptors (ERs) and are thus targets for estrogen action. Cellular responses to estrogen may be elicited via both genomic and nongenomic mechanisms. These vascular effects have been recently reviewed in the context of graft atherosclerosis, experimental hypertension, and other models of vascular disease. Most human and animal studies suggest that estrogen inhibits the neointimal response in acutely injured blood vessels.

In contrast to findings in models in which mechanical injury is delivered to a presumably normal artery, estrogen treatment was recently shown not to inhibit neointima formation in a nonhuman primate model with preexisting atherosclerosis. In this study, estrogen effected only a transient decrease in arterial cell proliferation rate immediately after balloon injury but did not alter either neointimal area or arterial remodeling. Interestingly, estrogen treatment administered concurrently with a high fat diet slowed the progression of experimentally induced atherosclerosis in this model, but it did not modulate the acute injury response in the atherosclerotic artery. While the possible explanations may be numerous and complex, it is highly likely that this refractoriness of the injury response to estrogen in atherosclerotic vessels may reflect a greatly reduced density of ERs, as reported by Losordo et al in diseased human coronary arteries. This mechanism could also contribute to the lack of benefit of short-term hormone replacement therapy seen in the HERS trial.

Our laboratory has used balloon injury of the common carotid artery of the rat as an experimental model of localized and highly controllable vascular damage in which the response to injury can be studied in vivo. In this model, balloon inflation denudes endothelium and induces a highly reproducible intimal migration/proliferation of VSMCs over the entire length of the affected vessel. Our recent observations suggest that balloon injury also induces activation/migration of adventitial cells into the neointima in this model and that these cells contribute to the neointimal response to injury.

All experimental protocols for studies with the rat carotid injury model were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985). Our initial studies with the rat carotid injury model focused on the role of the early-response proto-oncogene c-myc and its transcriptional inhibitor mithramycin in regulating neointima formation after balloon denudation. Mithramycin is a guanine-cytosine (G-C)–specific DNA binding drug that selectively inhibits transcription of genes, such as the human c-myc gene, that have G-C–rich promoter sequences by preventing the binding of Sp1 and other proteins to the c-myc P1 and P2 promoters. We therefore tested the hypothesis that mithramycin can prevent neointima formation and expression of c-myc mRNA in the balloon-injured carotid artery. Administration of mithramycin in 2 systemic (intraperitoneal) injections 1 hour before and 1 hour after injury resulted in significant suppression (≈50%) of neointima formation compared with vehicle control rats. Expression of c-myc mRNA in undamaged carotid artery was barely detectable, but steady state c-myc mRNA levels increased promptly after injury, reaching maximal levels (10-fold increase from baseline) at 2 hours (Figure 2). Mithramycin inhibited expression of c-myc gene transcript levels by 66% at 2 hours. The inhibitory effects of mithramycin on the neointimal response to injury are consistent with previous demonstrations that downregulation of c-myc expression is associated with inhibition of cell growth and induction of differentiation. Accordingly, c-myc levels were used in subsequent experiments as an index of the robustness of the neointimal response to vascular injury.

We subsequently used the rat carotid injury model to test whether there is a sexual dimorphism in the neointimal response to balloon injury; if so, whether this dimorphism is estrogen or androgen dependent; and whether there is a sexual dimorphism in expression of the c-myc proto-oncogene in the injured carotid artery. We hypothesized...
that the sex hormones modulate neointima formation in response to balloon injury of the carotid artery, at least in part, by modulating expression of c-myc. Our initial study demonstrated that (1) neointima formation after balloon injury of the carotid artery was significantly greater in intact male Sprague-Dawley rats than in age-matched intact females; (2) gonadectomy of male rats did not alter the neointimal response; (3) gonadectomy of female rats was associated with a more robust neointimal proliferative response to injury, comparable to that seen in the male; and (4) estradiol replacement markedly attenuated neointima formation in gonadectomized rats of both sexes (Figure 3). Serum estradiol levels in estrogen-treated male and female rats were in the physiological (for females) range. Thus, the sexual dimorphism of neointima formation after balloon injury of the rat carotid artery is estrogen dependent. The most dramatic of these findings was the observation that 17β-estradiol inhibited the neointimal response to vascular injury by =60% in gonadectomized rat of both sexes, an effect greater than that previously observed in our laboratory with mithramycin and comparable to results observed with a variety of other agents in the rat carotid injury model.

Furthermore, steady state c-myc mRNA levels were significantly greater in uninjured carotid arteries of intact male rats than in intact females. Vascular injury was associated with significant increases in c-myc mRNA levels in male rats at 1 and 2 hours after the insult (+648% and +667%, respectively, compared with uninjured left carotid artery controls) (Figure 2). This response was comparable to that observed in male rats in our previous study. In contrast, the c-myc response in females was delayed and greatly attenuated. Thus, there is a sexual dimorphism in expression of this early-response gene in the setting of acute vascular injury, with the female being less responsive than the male. This suggests that estrogen may blunt the increased expression of c-myc in blood vessels in response to injury.

To test whether progestins, alone and in combination with estrogen, modulate the vascular response to injury, we administered 17β-estradiol, MPA, or a combination of the 2 to gonadectomized male and female Sprague-Dawley rats after balloon injury of the carotid arteries. At 2 weeks after injury, indices of neointima formation, including intimal areas and intima/media ratios, were reduced by estrogen but unaffected by MPA in gonadectomized rats of both sexes (Figure 3). Addition of MPA blunted but did not completely block the antiproliferative effects of 17β-estradiol.

A subsequent study examined the effects of estrogen and MPA, alone and in combination, against a background of endogenous sex hormones, on the vascular injury response in male and female rats with their gonads in place (Figure 3). Two weeks after injury, the neointimal area in male rats was significantly greater than in females and was unaffected by estrogen or MPA treatment. Neointima formation in estradiol-treated male rats was not significantly different from that in vehicle-treated males, while estradiol-treated females had significantly less (>70% less) neointima formation than vehicle-treated females. MPA treatment alone did not alter the neointimal response to balloon injury in male rats compared with that in vehicle controls, but it greatly increased the intima/media ratio in females compared with vehicle-treated controls. Neointima formation in MPA-treated females was significantly greater than in either vehicle- or estradiol-treated females, approaching male levels. This effect was associated with a significant reduction in serum estradiol levels compared with vehicle-treated females. Thus, MPA attenuated the vasoprotective effects of both endogenous and exogenous estrogen in females, in part by blocking the production of endogenous estrogen by the ovary. The most dramatic finding of this study was that administration of exogenous estrogen to male rats had no effect on the carotid injury response but reduced the injury response in female rats by >70%. This unresponsiveness to exogenous estrogen in the intact male contrasted with the female-like response previously elicited in the orchidectomized male. The mechanism of this sexual dimorphism of the vasoprotective effects of estrogen remains to be elucidated.

**Figure 3.** Effects of administration of estrogen (20 μg/kg per day) and MPA (10 mg/kg per day) on neointima formation in balloon-injured right common carotid artery of intact and gona-dectomized male and female Sprague-Dawley rats at 14 days after injury. Ratios of neointimal area to medial area are presented as mean±SEM. *P<0.05, compared with respective intact male groups; **P<0.05, compared with respective vehicle control groups.

**Cellular Mechanisms of the Vascular Injury Response: Modulation by Sex Hormones**

To define the cellular mechanisms by which estrogen inhibits neointima formation after vascular injury, it will be necessary to identify the critical estrogen-sensitive chemoattractants/growth factors/cytokines involved, along with their cellular sites of origin and of action. Numerous molecular responses and biological relationships have been identified during neointima formation in the rat carotid injury model. The relative contributions of these various mediators to the injury response are not understood, nor is it certain that all of the relevant mechanisms have been identified. There is, however, general agreement that in the rat carotid artery, the injury response begins with destruction of the endothelium and damage to medial smooth muscle cells and the internal elastic laminae (Figure 4). Importantly, recent in vitro studies in our own laboratory have shown that VSMCs activated by exposure to 10% FBS in culture release soluble factor(s) that stimulate the migration of adventitial fibroblasts, and furthermore that production, release, and/or posttranslational processing of this factor(s) are inhibited by estrogen via an
Adventitial Cell Translocation and Phenotypic Modulation in the Vascular Injury Response

Participation of the adventitia in the response to vascular injury has been suggested by pathologic findings of adventitial fibroblast migration and/or delivery of these cells to the adventitia via the circulation and the vasa vasorum is uncertain. The growth factors/cytokines involved in adventitial activation have not been identified. What is known from previous research is that inhibition of the early proliferative events in the injury response, which has been accomplished with antisense agents directed at cell cycle genes and early-response genes, as well as with mithramycin, a selective inhibitor of c-myc transcription, limits the extent of neointimal proliferation. This suggests that these interventions have long-lasting effects on later events in the injury response, including adventitial fibroblast migration and neointimal cell proliferation.

ER-dependent mechanism. Identification and characterization of this factor(s) are ongoing in our laboratory.

Our recent findings and data from other laboratories provide evidence that adventitial activation occurs within the first 24 hours after injury. Whether the signaling pathway responsible involves diffusion of fibroblast growth factor (FGF) and/or other chemoattractant/mitogenic factors from damaged smooth muscle cells through the media and external elastic lamina to the adventitia and/or delivery of these factors to the adventitia via the circulation and the vasa vasorum is uncertain. The growth factors/cytokines involved in adventitial activation have not been identified. What is known from previous research is that inhibition of the early proliferative events in the injury response, which has been accomplished with antisense agents directed at cell cycle genes and early-response genes, as well as with mithramycin, a selective inhibitor of c-myc transcription, limits the extent of neointimal proliferation. This suggests that these interventions have long-lasting effects on later events in the injury response, including adventitial fibroblast migration and neointimal cell proliferation.

ER-dependent mechanism. Identification and characterization of this factor(s) are ongoing in our laboratory.

Our recent findings and data from other laboratories provide evidence that adventitial activation occurs within the first 24 hours after injury. Whether the signaling pathway responsible involves diffusion of fibroblast growth factor (FGF) and/or other chemoattractant/mitogenic factors from damaged smooth muscle cells through the media and external elastic lamina to the adventitia and/or delivery of these factors to the adventitia via the circulation and the vasa vasorum is uncertain. The growth factors/cytokines involved in adventitial activation have not been identified. What is known from previous research is that inhibition of the early proliferative events in the injury response, which has been accomplished with antisense agents directed at cell cycle genes and early-response genes, as well as with mithramycin, a selective inhibitor of c-myc transcription, limits the extent of neointimal proliferation. This suggests that these interventions have long-lasting effects on later events in the injury response, including adventitial fibroblast migration and neointimal cell proliferation.

Adventitial Cell Translocation and Phenotypic Modulation in the Vascular Injury Response

Participation of the adventitia in the response to vascular injury has been suggested by pathologic findings of adventitial activation (inflammation and fibrosis) in coronary arteries of victims of fatal coronary artery disease. In some individuals who died suddenly at an early age, adventitial inflammation appeared to antedate intimal disease. Furthermore, neointima formation and/or atherosclerotic lesions have been observed in response to adventitial injury in various animal models, raising the possibility of alternative pathways of the vascular injury response and alternative routes of administering therapeutic agents. More recently, endoluminal injury of a porcine coronary artery was shown to result in significant remodeling of the adventitia, characterized by proliferation and differentiation of adventitial fibroblasts to myofibroblasts, which acquired α-smooth muscle actin. These adventitial responses were associated with increased neointima formation. The proliferating adventitial cells were subsequently shown to migrate into the neointima and transform into a myofibroblast phenotype. These findings suggest that adventitial fibroblasts may play an important role in the response to endothelial/medial vascular injury.

Current studies in our laboratory are testing whether this mechanism plays a role in neointima formation in the rat carotid injury model and, if so, whether estrogen and the other sex hormones modulate the adventitial activation/migration response to vascular injury. To test this hypothesis directly, our laboratory has harvested syngeneic fibroblasts from the carotid artery adventitia of ovariectomized Sprague-Dawley rats, expanded them in culture, and stably transduced them with retroviral particles (pLBg) coordinating expression of lacZ. Transduced fibroblasts were grown to 60% to 70% confluence, stained for β-galactosidase activity with fluorescein-di-galactoside (FDG), and sorted twice by FACS to reach >95% purity. The adhesive and migratory properties of the transduced fibroblasts were determined in vitro by counting the number of blue lacZ-labeled cells attached (4 hours) to Boyden-type chambers preconditioned (24 hours) under defined experimental conditions by VSMCs derived from the carotid artery of female Sprague-Dawley rats. Compared with media alone, chambers conditioned by VSMCs demonstrated a 2-fold increase in fibroblast migration, suggesting that activated VSMCs release a soluble chemoattractant factor(s) competent to bind the Boyden chamber membrane and promote fibroblast migration and adhesion. Treatment of VSMCs with varying doses (10^{-6} to 10^{-7} mol/L) of estrogen during preconditioning of the chamber induced a dose-dependent inhibition of fibroblast migration and attachment. Treatment of VSMCs with the estrogen receptor antagonist ICI182780 (10^{-6} mol/L) alone had no effect on fibroblast migration, but coadministration with estrogen blocked the inhibitory effect of estrogen. Progesterone did not antagonize the estrogen effect. These observations suggest a novel mechanism of hormonal vasoprotection, wherein estrogen directly modulates VSMC expression of factor(s) controlling activation and migration of adventitial fibroblasts via an estrogen receptor–dependent mechanism.

To test whether adventitial fibroblasts contribute to the vascular injury response in vivo, transduced fibroblasts were reintroduced into the adventitia of the right (injured) and left (uninjured) carotid artery of ovariectomized Sprague-Dawley rats at the time of injury. Our preliminary data showed that at defined kinetic time points after injury, blue lacZ-expressing fibroblasts migrated from the adventitia into the medial and neointimal compartments. No lacZ-labeled cells were seen in the left (uninjured) carotid artery. No lacZ staining was observed in injured carotid arteries of control rats that did not receive transduced fibroblasts. Results demonstrate that activation and migration of adventitial fibroblasts into neointima contribute to neointima formation in balloon-injured carotid arteries. This is the first definitive demonstration that adventitial fibroblasts migrate into the neointima of blood vessels after endoluminal injury. The observation that large populations of cells move through the vessel wall in an adventitia-to-lumen direction following endoluminal damage suggests novel therapeutic approaches to vascular injury. Ongoing studies will test the hypothesis...
that estrogen inhibits this adventitial contribution to the vascular injury response. These fundamental mechanistic studies will establish more rational strategies for therapeutic intervention in vascular diseases, including the basis for future gene therapy.

References


35. Shi Y, O’Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. Adven-


Hormones and Vasoprotection
Suzanne Oparil

*Hypertension*. 1999;33:170-176
doi: 10.1161/01.HYP.33.1.170

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 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/33/1/170

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/