Angiotensin-(1-7) Downregulates the Angiotensin II Type 1 Receptor in Vascular Smooth Muscle Cells

Michelle A. Clark, Debra I. Diz, E. Ann Tallant

Abstract—Angiotensin (Ang)-(1-7) is a biologically active peptide of the renin-angiotensin system that has both vasodilatory and antiproliferative activities that are opposite the constrictive and proliferative effects of angiotensin II (Ang II). We studied the actions of Ang-(1-7) on the Ang II type 1 (AT₁) receptor in cultured rat aortic vascular smooth muscle cells to determine whether the effects of Ang-(1-7) are due to its regulation of the AT₁ receptor. Ang-(1-7) competed poorly for [¹²⁵I]Ang II binding to the AT₁ receptor on vascular smooth muscle cells, with an IC₅₀ of 2.0 µmol/L compared with 1.9 nmol/L for Ang II. The pretreatment of vascular smooth muscle cells with Ang-(1-7) followed by treatment with acidic glycine to remove surface-bound peptide resulted in a significant decrease in [¹²⁵I]Ang II binding; however, reduced Ang II binding was observed only at micromolar concentrations of Ang-(1-7). Scatchard analysis of vascular smooth muscle cells pretreated with 1 µmol/L Ang-(1-7) showed that the reduction in Ang II binding resulted from a loss of the total number of binding sites [Bₘₐₓ 437.7±261.5 fmol/mg protein in Ang-(1-7)–pretreated cells compared with 607.5±301.2 fmol/mg protein in untreated cells, n=5, P<0.05] with no significant effect on the affinity of Ang II for the AT₁ receptor. Pretreatment with the AT₁ receptor antagonist L-158,809 blocked the reduction in [¹²⁵I]Ang II binding by Ang-(1-7) or Ang II. Pretreatment of vascular smooth muscle cells with increasing concentrations of Ang-(1-7) reduced Ang II–stimulated phospholipase C activity; however, the decrease was significant (81.2±6.4%, P<0.01, n=5) only at 1 µmol/L Ang-(1-7). These results demonstrate that pharmacological concentrations of Ang-(1-7) in the micromolar range cause a modest downregulation of the AT₁ receptor on vascular cells and a reduction in Ang II–stimulated phospholipase C activity. Because the antiproliferative and vasodilatory effects of Ang-(1-7) are observed at nanomolar concentrations of the heptapeptide, these responses to Ang-(1-7) cannot be explained by competition of Ang-(1-7) at the AT₁ receptor or Ang-(1-7)–mediated downregulation of the vascular AT₁ receptor. (Hypertension. 2001;37:1141-1146.)

Key Words: angiotensin II receptors, muscle, smooth, vascular

Angiotensin (Ang)-(1-7), the amino-terminal angiotensin heptapeptide, is an important biologically active peptide of the renin-angiotensin system (RAS).¹ Ang-(1-7) plays a role in vascular regulation that is distinct and often opposite that of angiotensin II (Ang II). Furthermore, these opposing effects of Ang II and Ang-(1-7) occur at similar concentrations of the 2 angiotensin peptides. For example, in contrast to the mitogenic effects of nanomolar Ang II, Ang-(1-7) inhibits vascular smooth muscle cell (VSMC) growth with an IC₅₀ value in the nanomolar range.² Ang-(1-7) reduces neointimal formation after vascular injury and increases urinary prostaglandin release at concentrations of the heptapeptide 2- to 3-fold higher than the endogenous circulating concentration of Ang-(1-7) or Ang II.³ ⁴ Nanomolar concentrations of Ang-(1-7) increased arachidonic acid release and prostacyclin production in rabbit VSMCs, with maximal effects observed at 100 nmol/L Ang-(1-7).⁵ Ang-(1-7) stimulated prostaglandin (PGE₂) and prostacyclin release from both porcine and rat VSMCs with EC₅₀ values in the nanomolar range.⁶ ⁷ Finally, Ang-(1-7) caused the vasodilation of rabbit renal afferent arterioles at nanomolar concentrations of the heptapeptide.⁸ In addition, the actions of endogenous Ang-(1-7) unmasked after converting enzyme inhibition⁹ or a low-salt diet¹⁰ appear to occur at submicromolar concentrations of the heptapeptide. These results indicate that Ang-(1-7) has both vasodilatory and antiproliferative effects that are observed at subpharmacological concentrations of the heptapeptide.

In contrast, various effects of Ang-(1-7) were reported at pharmacological doses of the heptapeptide. We observed a rapid transient increase in pressure preceding a prolonged depressor response when high doses of Ang-(1-7) were injected into pithed rats.¹¹ The pressor, but not the depressor, component of this response was blocked by the AT₁ receptor antagonist losartan, suggesting that high concentrations of Ang-(1-7) may stimulate the AT₁ receptor to increase blood pressure. Mahon et al¹² reported that micromolar concentrations of Ang-(1-7) caused a rightward shift in the dose-contractile response to Ang II in rabbit aortic rings.
and a concentration-dependent decrease in the maximal response, with a pA2 of 5.5, and suggested that Ang-(1-7) was an antagonist at the AT1 receptor at micromolar concentrations of the heptapeptide. Roks et al13 also demonstrated that Ang-(1-7) at a concentration of 10 \mu M reduced Ang I- and Ang II–induced contraction of human internal mammary arteries, suggesting that micromolar Ang-(1-7) was an antagonist at the AT1 receptor. Because Ang-(1-7) competes for binding to the vascular AT1 receptor at micromolar concentrations,7 pharmacological concentrations of Ang-(1-7) may oppose or counteract the actions of Ang II through competitive antagonism. Alternatively, Ang-(1-7) could oppose the vasoconstrictive and proliferative effects of Ang II through downregulation or desensitization of the AT1 receptor. This study was designed to determine whether Ang-(1-7) causes a downregulation of the AT1 receptor or desensitizes responses to Ang II by direct actions at the AT1 receptor.

Methods

Materials

[\textsuperscript{[125]I}]Sodium iodide was obtained from Amersham Pharmacia, and myo-[\textsuperscript{3}H]inositol was obtained from American Radiolabeled Chemicals, Inc. DMEM/Ham's F-12 (DMEM/F12), inositol-free DMEM, FBS, penicillin, streptomycin, and trypsin/EDTA were obtained from Gibco. Ang II and Ang-(1-7) were purchased from Bachem, L-158,809 were kindly provided by Merck and Co, Inc, and the AT2 receptor antagonist PD 123319 was a gift from Parke-Davis. All other chemicals were purchased from Sigma Chemical Co.

Cell Culture Procedures

Aortic VSMCs were isolated from 10- to 12-week-old male Hanover Sprague-Dawley rats bred and raised at the ALACC-accredited animal facility of Wake Forest University School of Medicine. Primary cultures of VSMCs were obtained from aortic explants as previously described. The cells were cultured in DMEM/F12 supplemented with 10% FBS, 100 \mu g/mL penicillin, 100 \mu g/mL streptomycin, and maintained at 37°C in a humified CO2 incubator (5% CO2 and 95% air). Confluent monolayers of VSMCs were prelabeled with [\textsuperscript{3}H]-labeled inositol phosphates in VSMCs prelabeled with myo-[\textsuperscript{3}H]-inositol, as previously described.\textsuperscript{16} Confluent monolayers of VSMCs in 6-well culture plates were labeled for 72 hours with myo-[\textsuperscript{3}H]-inositol (2 \muCi/well) in inositol-free DMEM that contained 0.5% FBS. The radiolabeled VSMCs were preincubated with increasing concentrations of [\textsuperscript{125}I]Ang II (0.1 to 10 \muM) in HEPS-buffered Krebs-Ringer solution (125 mmol/L NaCl, 5 mmol/L KCl, 1.2 mmol/L MgSO\textsubscript{4}, 6 mmol/L glucose, 1 mmol/L CaCl\textsubscript{2}, and 25 mmol/L HEPS, pH 7.4) and 10 mmol/L LiCl, for 30 minutes at 37°C. The cells were then treated with 10 mmol/L Ang II, and the reaction was continued for 30 minutes at 37°C. Inositol phosphates were extracted with ice-cold perchloric acid, separated through ion exchange chromatography, and quantified with liquid scintillation spectroscopy as previously described.\textsuperscript{16}

Statistics

All data are expressed as the mean±SEM of ≥3 experiments. Competition binding data were analyzed by nonlinear regression with the computer program Prism (GraphPad). Saturation isotherms were analyzed with the EBDA/LIGAND computer program (Elsevier-BIOSOFT). Repeated measures 1-way ANOVA or ANOVA with Dunnett’s post hoc test were used to compare treatment groups. The criterion for statistical significance was P<0.05.

Results

Competition for the AT\textsubscript{1} Receptor by Ang-(1-7)

VSMCs were incubated with increasing concentrations of Ang II and Ang-(1-7) to determine whether Ang-(1-7) competed for [\textsuperscript{125}I]Ang II binding to VSMCs. As shown in Figure 1, Ang-(1-7) competed poorly for binding to the AT\textsubscript{1} receptor on VSMCs isolated from thoracic aorta of Sprague-Dawley rats with an IC\textsubscript{50} value of 2.0 \muM. The [\textsuperscript{125}I]Ang II binding to VSMCs was characterized as AT\textsubscript{1} based on ≥95% competition of specific binding by either 10 \muM concentration of the AT\textsubscript{1} receptor antagonist losartan or EXP 3174 and no competition by 10 \muM PD 123319, an AT\textsubscript{2} receptor antagonist (Figure 1).

Regulation of the AT\textsubscript{1} Receptor by Ang-(1-7)

Ang II binds to AT\textsubscript{1} receptors on VSMCs and induces a rapid internalization of the Ang II–AT\textsubscript{1} receptor complex. Because we observed that Ang-(1-7) binds to the vascular AT\textsubscript{1} receptor, albeit at a 1000-fold higher concentration than Ang II, Ang-(1-7) binding to the AT\textsubscript{1} receptor could reduce subsequent responses to Ang II by causing internalization and

Second, the effect of pretreatment with Ang-(1-7) or Ang II on subsequent binding of [\textsuperscript{125}I]Ang II to VSMCs was determined through preincubation with increasing concentrations of peptide in Hanks’ balanced salt solution for 30 minutes at room temperature. After this preincubation, surface-bound Ang-(1-7) or Ang II was removed with a 5-minute treatment with ice-cold 50 mmol/L glycine/50 mmol/L HCl, pH 3.0, before the measurement of [\textsuperscript{125}I]Ang II binding.

Third, saturation isotherms were constructed after treatment (30 minutes at room temperature) of VSMCs with vehicle or 1 \muM/L Ang-(1-7). Surface-bound Ang-(1-7) was removed with acidic glycine, and acid-stripped cells were incubated with increasing concentrations of [\textsuperscript{125}I]Ang II (0.075 to 2.4 nmol/L) for 1 hour at room temperature.
subsequent loss of membrane AT1 receptors. To test this hypothesis, VSMCs were pretreated with increasing concentrations of Ang-(1-7) before the measurement of [125I]Ang II binding to determine whether treatment with Ang-(1-7) downregulates the AT1 receptor. As a control, cells were also pretreated with Ang II. After a 30-minute pretreatment with either Ang-(1-7) or Ang II, surface-bound peptide was removed through treatment with acidic glycine and [125I]Ang II binding was measured. Ang II caused a dose-dependent reduction in total binding, with a significant reduction of 51.4±10.5% (P<0.05) of total binding at 10 nmol/L (Figure 2). Ang-(1-7) also caused a similar reduction in Ang II binding, with significant inhibition of 36.1±7.8% of [125I]Ang II binding at 1 μmol/L Ang-(1-7). A higher concentration of Ang-(1-7), up to 10 μmol/L, caused no further reduction in [125I]Ang II binding.

To determine whether Ang-(1-7) reduced binding to the vascular AT1 receptor by increasing receptor affinity or by reducing the total number of AT1 receptors, VSMCs were pretreated with 1 μmol/L Ang-(1-7) for 30 minutes. The cells were subsequently treated with acidic glycine to remove surface-bound Ang-(1-7) and incubated with increasing concentrations of [125I]Ang II to saturate the receptor. Specific [125I]Ang II binding was measured and receptor density and affinity were determined through Scatchard analysis. Pretreatment with Ang-(1-7) had no significant effect on the affinity of Ang II for the AT1 receptor (Table). In contrast, pretreatment with 1 μmol/L Ang-(1-7) for 30 minutes caused a significant 66±8% decrease in the Bmax of Ang II receptors on VSMCs.

Effect of AT1 Receptor Antagonism on AT1 Receptor Downregulation

The AT1 receptor antagonist L-158,809 was used to further demonstrate that the reduction in [125I]Ang II binding by Ang-(1-7) was due to a direct interaction of the heptapeptide with the AT1 receptor. VSMCs were treated with 10 nmol/L L-158,809 before the addition of 1 μmol/L Ang-(1-7) to saturate the receptor. Specific [125I]Ang II binding was measured. Pretreatment with 1 μmol/L Ang-(1-7) reduced Ang II binding to the AT1 receptor to 84.3±2.2% of total binding. The inclusion of 10 nmol/L L-158,809 prevented the Ang-(1-7)–induced reduction in [125I]Ang II binding (Figure 3). Pretreatment with 100 nmol/L Ang II also reduced subsequent binding to the AT1 receptor to 74.2±7.0% of total binding, which was prevented by the inclusion of the AT1 receptor antagonist L-158,809. Pretreatment with the antagonist alone followed by treatment with acidic glycine had no significant effect on [125I]Ang II binding to the AT1 receptor, as would be expected with this antagonist.

Effect of Ang-(1-7) on Ang II–Induced PLC Activity

Ang II significantly stimulated inositol phosphate release from VSMCs, measured as the release of total inositol phosphates from cells prelabeled with myo-[3H]-inositol. A 30-minute incubation of VSMCs with Ang II (10 nmol/L) increased phosphoinositide-specific PLC activity by 102.6±19.6% above basal levels (P<0.05, n=5). A similar incubation with 1 μmol/L Ang-(1-7) had no effect on inositol

<table>
<thead>
<tr>
<th>Effect of Ang-(1-7) on the Binding Affinity and Density of the AT1 Receptors on VSMCs</th>
<th>Treatment</th>
<th>Kd, nmol/L</th>
<th>Bmax, fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5±0.35</td>
<td>607.5±301.2</td>
<td></td>
</tr>
<tr>
<td>Ang-(1-7)</td>
<td>1.18±0.29</td>
<td>437.7±261.5*</td>
<td></td>
</tr>
</tbody>
</table>

VSMCs were pretreated with vehicle or 1 μmol/L Ang-(1-7) for 30 minutes at room temperature. Surface-bound peptide was removed through treatment with acidic glycine. Acid-stripped cells were incubated with increasing concentrations of [125I]Ang II to saturate the receptor. Nonspecific binding was measured in the presence of 10 μmol/L Ang II. Scatchard analysis was used to calculate the Kd and Bmax values of the AT1 receptor in the presence and absence of Ang-(1-7). Each value is the mean±SEM of 4 or 5 experiments. *P<0.05 of Ang-(1-7)–treated cells compared with untreated cells.
After exposure to the peptides for 30 minutes at room temperature, surface-bound peptide and antagonist were removed by treatment with acidic glycine, and $[^{32}P] \text{Ang II}$ binding was measured. The data are presented as the percentage of specific binding. *$P<0.05$ compared with total specific binding in cells pretreated with vehicle. Each value represents the mean±SEM of 4 experiments with VSMCs isolated from different rat aortas.

Discussion

The pretreatment of VSMCs with Ang-(1-7) followed by treatment with acidic glycine to remove surface-bound peptide reduced subsequent binding of Ang II to the AT$_1$ receptor. However, the decrease in Ang II binding was detected only at micromolar concentrations of the heptapeptide, which is in agreement with the micromolar affinity of Ang-(1-7) for the AT$_1$ receptor. The reduction in Ang II binding by Ang-(1-7) resulted from a decrease in the number of receptors available for subsequent binding with no significant effect on receptor affinity. The downregulation of the Ang II receptor by Ang-(1-7) was blocked by pretreatment with the AT$_1$ receptor antagonist L-158,809, demonstrating that micromolar concentrations of Ang-(1-7) have direct effects at the AT$_1$ receptor to reduce the number of receptors available for subsequent binding. Finally, Ang-(1-7), at a concentration of 1 $\mu$mol/L, significantly attenuated the Ang II–mediated stimulation of PLC to reduce the production of inositol phosphates. These results clearly demonstrate that the short-term treatment of VSMCs with pharmacological concentrations of Ang-(1-7) downregulates the vascular AT$_1$ receptor.

Although 1 $\mu$mol/L Ang-(1-7) alone did not activate PLC in VSMCs, it did reduce subsequent binding of Ang II to the AT$_1$ receptor and attenuated activation of AT$_1$-coupled cellular responses by Ang II. The AT$_1$ receptor on VSMCs is rapidly internalized on stimulation by Ang II, a phenomenon accompanied by a reduction in subsequent responses. This suggests that Ang-(1-7), at micromolar concentrations, binds to the AT$_1$ receptor to induce receptor internalization without activating subsequent cellular responses. Previous work with substance P and serotonin receptors clearly indicates that receptor internalization can occur independent of changes in functional responsiveness. Studies by several investigators suggest that different motifs in the structure of the AT$_1$ receptor are required for receptor internalization compared with intracellular signal generation by Ang II.

The truncation of a section of the carboxyl terminus of the AT$_1A$ receptor prevented receptor internalization without affecting coupling to PLC. A point mutation in the AT$_1$ receptor that eliminates Ang II coupling to PLC (Asp$_{74}$→Asn) did not block receptor internalization. In addition, the potent antagonist [Sar$_1$,Ile$_8$]Ang II, a sarcosine analog of Ang II, was internalized but did not activate PLC in Chinese hamster ovary cells transfected with the AT$_1$ receptor, as well as in adrenal medullary cells. This suggests that Ang-(1-7), as well as sarcosine analogs of Ang II, bind to and activate the AT$_1$ receptor to produce receptor internalization without coupling to G proteins to activate PLC. In contrast, the nonpeptide antagonist L-158,809 did not cause internalization in our study. Thus, it is possible that high concentrations of Ang-(1-7) and the peptide antagonists produce receptor internalization through weak agonist properties. This would also be consistent with our observation of a weak pressor response to high doses of Ang-(1-7) in the pithed rat, an effect that was mediated by an AT$_1$ receptor.

Mahon et al. suggested that Ang-(1-7) is a noncompetitive antagonist at AT$_1$ receptors in rabbit aortic rings, causing a rightward shift in the dose-response contraction curve to Ang II administered 60 minutes after Ang-(1-7). However, the concentrations of Ang-(1-7) used in their study ranged from 1 to 30 $\mu$mol/L. Roks et al. also suggested that 10 $\mu$mol/L Ang-(1-7) was antagonistic at the AT$_1$ receptor in human VSMCs. This would be consistent with our observation that Ang-(1-7) had an IC$_{50}$ value of 2 $\mu$mol/L at the AT$_1$ receptor in VSMCs. Our evidence suggests that short-term exposure of cells to micromolar levels of Ang-(1-7) downregulates the AT$_1$ receptor over a time frame consistent with receptor interaction.
internalization. Thus, when Ang II is given after a prior exposure to Ang-(1-7) (as we did in our study) or in the presence of a large excess of Ang-(1-7) (as in studies described by Roks et al\textsuperscript{13} and Mahon et al\textsuperscript{12}), one might expect antagonist actions of Ang-(1-7). However, endogenous circulating levels of Ang-(1-7), as well as tissue concentrations of the peptide, are in the picomolar range.\textsuperscript{25–27} Although treatment with ACE inhibitors increased circulating levels of Ang-(1-7) from 9- to 25-fold,\textsuperscript{25,28} there is no evidence showing an increase in endogenous Ang-(1-7) levels to the micromolar range. In addition, the majority of the in vivo and in vitro actions of Ang-(1-7) occur at doses lower than 1 to 30 $\mu$mol/L. Because Ang-(1-7) downregulated Ang II binding and receptor activation only at micromolar concentrations, the vasodilatory and antiproliferative responses that occur at nanomolar concentrations of the heptapeptide cannot be explained by antagonist effects of Ang-(1-7) at the AT\textsubscript{1} receptor. Ueda et al\textsuperscript{29} recently reported that the infusion of Ang-(1-7) antagonized the Ang II-mediated vasoconstriction of human forearm resistance vessels, suggesting that Ang-(1-7) is an endogenous antagonist of the human AT\textsubscript{1} receptor. However, the authors reported that Ang-(1-7) significantly reduced the response to Ang II at a dose of 10 $\mu$mol/L, a concentration significantly lower than the IC\textsubscript{50} value for the AT\textsubscript{1} receptor ($>1$ $\mu$mol/L). In light of our observations, the responsiveness to Ang-(1-7) in the study by Ueda et al\textsuperscript{29} may be due to activation of a non-AT\textsubscript{1} receptor by Ang-(1-7) and the release of a compensatory vasodilator such as NO or prostaglandins.

In addition, evidence from several studies clearly shows that the actions of Ang-(1-7) are independent of AT\textsubscript{1} receptor activation. The vasodilation of canine or porcine coronary arteries induced by Ang-(1-7) was prevented by removal of the endothelium or by blockade of NO release,\textsuperscript{30,31} suggesting that Ang-(1-7) stimulated NO production to counter the vasoconstrictor effects of Ang II. These effects were not blocked by AT\textsubscript{1} or AT\textsubscript{2} receptor antagonists. Muthalif et al\textsuperscript{3} showed that Ang-(1-7) stimulated arachidonic acid release from rabbit VSMCs, an effect that was partially prevented by [D-Ala\textsuperscript{7}]Ang-(1-7), the selective Ang-(1-7) receptor antagonist, or by the AT\textsubscript{2} antagonist PD 123319 but not by an AT\textsubscript{1} receptor antagonist. Ang-(1-7) caused the vasodilation of renal afferent arterioles, an effect blocked by [D-Ala\textsuperscript{7}]Ang-(1-7).\textsuperscript{8} Further, Ang-(1-7) blocked mitogen-stimulated growth of rat VSMCs, responses that were not reversed by an AT\textsubscript{1} or AT\textsubscript{2} receptor antagonist\textsuperscript{2} but were prevented by the selective Ang-(1-7) receptor antagonist [D-Ala\textsuperscript{7}]Ang-(1-7),\textsuperscript{32} suggesting that Ang-(1-7) activates a unique receptor on VSMCs to counteract the mitogenic response to Ang II. These in vitro studies are further supported by observations in combined ACE inhibitor/losartan–treated rats where a monoclonal antibody to Ang-(1-7)\textsuperscript{9} or [D-Ala\textsuperscript{7}]Ang-(1-7)\textsuperscript{32} masked a role for endogenous Ang-(1-7), working in part through prostaglandins, in the blood pressure–lowering effects.\textsuperscript{34} Furthermore, there is evidence for a [D-Ala\textsuperscript{7}]Ang-(1-7)–sensitive, non-AT\textsubscript{1}, non-AT\textsubscript{2} receptor in the aorta\textsuperscript{32} and mesentery\textsuperscript{34} of the animals treated with ACE inhibitors and losartan. Thus, Ang-(1-7) has unique effects in the vasculature that are coupled to a non-AT\textsubscript{1} receptor and do not result from antagonism of the AT\textsubscript{1} receptor.

In the present study, we showed that prior treatment of VSMCs with Ang-(1-7) attenuated [$\textsuperscript{3}H$]Ang II binding to the AT\textsubscript{1} receptor and reduced Ang II–stimulated PLC activation. However, significant downregulation of Ang II binding and Ang II–mediated responsiveness occurred only at micromolar concentrations of Ang-(1-7), in agreement with its IC\textsubscript{50} value for competition at the AT\textsubscript{1} receptor. These results suggest that at micromolar concentrations, Ang-(1-7) acts as a weak agonist at the AT\textsubscript{1} receptor to downregulate the receptor and to reduce subsequent cellular responses to Ang II.

**Acknowledgments**

This work was supported in part by grants HL-51952 and NS-31664 from the National Institutes of Health and a Grant-in-Aid from the American Heart Association, North Carolina Affiliate.

**References**

Angiotensin-(1-7) Downregulates the Angiotensin II Type 1 Receptor in Vascular Smooth Muscle Cells
Michelle A. Clark, Debra I. Diz and E. Ann Tallant

_Hypertension._ 2001;37:1141-1146
doi: 10.1161/01.HYP.37.4.1141

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/37/4/1141

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/