Adrenomedullin Gene Delivery Attenuates Hypertension, Cardiac Remodeling, and Renal Injury in Deoxycorticosterone Acetate-Salt Hypertensive Rats

Eric Dobrzynski, Cindy Wang, Julie Chao, Lee Chao

Abstract—Adrenomedullin (AM) is a potent vasodilator and natriuretic peptide that plays an important role in cardiorenal function. In this study, we explored the potential protective role of AM in volume-dependent hypertension by somatic gene delivery. Adenovirus containing the human AM cDNA under the control of the cytomegalovirus promoter/enhancer was administered into deoxycorticosterone acetate (DOCA)-salt hypertensive rats via tail vein injection. A single injection of the human AM gene resulted in a prolonged reduction of blood pressure with a maximal reduction of 41 mm Hg 9 days after gene delivery. Human AM gene delivery enhanced renal function, as indicated by a 3-fold increase in renal blood flow and a 2-fold increase in glomerular filtration rate (n = 5, P < 0.05). Histological examination of the kidney revealed a significant reduction in glomerular sclerosis, tubular injury, luminol protein cast accumulation, and interstitial fibrosis as well as urinary protein. Human AM gene delivery caused significant decreases in left ventricular weight and cardiomyocyte diameter, which were accompanied by reduced interstitial fibrosis and extracellular matrix formation within the heart. Expression of human AM mRNA was detected in the kidney, adrenal gland, heart, aorta, lung, and liver; immunoreactive human AM levels were measured in urine and plasma. Significant increases in urinary and cardiac cAMP levels were observed in DOCA-salt rats receiving the human AM gene, indicating activation of the AM receptor. These findings showed that AM gene delivery attenuates hypertension, protects against cardiac remodeling and renal damage in volume-overload hypertension, and may have significance in therapeutic applications in cardiovascular and renal diseases. (Hypertension. 2000;36:995-1001.)

Key Words: deoxycorticosterone • adrenomedullin • genes • kidney • hypertrophy, cardiac
acutely in the DOCA-salt model.\textsuperscript{14,15} In the present study, unilateral nephrectomy, subcutaneous administrations of DOCA, and subsequent salt loading were used to create the volume-overload hypertensive animal model. We explored the effects of a continuous supply of AM via a single intravenous injection of a replication-deficient adenovirus harboring the human AM gene in DOCA-salt hypertensive rats. Our results showed that AM gene delivery resulted in a marked reduction in blood pressure, significant attenuation of cardiac hypertrophy, fibrosis, and renal injury, and enhanced renal function. These findings suggest that AM gene therapy could be a candidate for the treatment of volume-dependent hypertension as well as cardiovascular and renal diseases.

**Methods**

**Preparation of Replication-Deficient Adenoviral Vectors and Animal Preparation**

Adenoviral vectors harboring the human AM (Ad.CMV-AM) or luciferase cDNA (Ad.CMV-Luc) under the control of the cytomegalovirus enhancer/promoter (CMV) were constructed and prepared as previously described.\textsuperscript{16} Left unilateral nephrectomy was performed on 30 male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind) at 3.5 weeks of age. Experimental animals received weekly subcutaneous injections of DOCA (30 mg/kg body wt, Sigma Chemical Co) suspended in sesam oil and provided with 1% NaCl drinking water. Control animals were injected with sesam oil and provided with tap water. Each rat received 2×10\textsuperscript{8} plaque-forming units of either the adenovirus harboring the human AM gene or luciferase gene via the tail vein 2 weeks after the start of steroid/salt treatment.

**Systolic Blood Pressure Measurements**

Systolic blood pressures of the rats were measured with a manometer-tachometer (Natsume KN-210, Natsume Seisakusho Co Ltd) as previously described.\textsuperscript{17}

**Analysis of Human AM and Rat β-Actin mRNAs**

The reverse transcription–polymerase chain reaction Southern blot analysis specific for human AM and rat β-actin was performed as previously described.\textsuperscript{13} Total RNA was extracted from fresh rat tissues 5 days after gene delivery by using Trizol reagent, and cDNA synthesis was performed according to the manufacturer’s instructions (GIBCO-BRL). A 453-bp fragment of human AM was amplified by polymerase chain reaction (94°C, 1 minute; 58°C, 1 minute; and 72°C, 1 minute; 35 cycles) by use of human AM–specific oligonucleotides (5’-CGCTCGTGGATGTCG-3’ and 5’-CCGTGTGCTTGTGGCTTA-3’). A nested oligonucleotide (5’-CAACTTCCAAGGGCCTCC-3’) was end-labeled and used as an internal probe for hybridization at 42°C. The membrane was washed in 2× SSC at 55°C.

**Urine and Plasma Collection**

Twenty-four–hour urine samples were collected and used for measuring sodium, potassium, cAMP, cGMP, AM, and protein concentrations. EDTA-plasma samples were collected via the tail vein. Urine sodium and potassium levels were measured by flame photometry; protein concentrations were determined by micro-Lowry assay.

**RIA for Human AM, cAMP, and cGMP**

Immunoreactive human AM was determined in plasma and urine by a radioimmunoassay (RIA) for human AM with the use of rabbit anti-human AM 1-52 antiserum (Peninsula Laboratories Inc) as previously described.\textsuperscript{13} Urinary and cardiac cAMP and cGMP levels were determined by RIA.\textsuperscript{18,19}

**Measurement of GFR and RBF**

Renal function was evaluated at 16 days after AM gene transfer as previously described.\textsuperscript{20,21} Glomerular filtration rate (GFR) and renal plasma flow were determined from the clearance of polyfructosan and p-aminohippuric acid, respectively. Renal blood flow (RBF) was calculated from renal plasma flow and hematocrit. Clearance data were normalized to kidney weight.

**Morphological and Histological Analysis**

Rats were anesthetized with pentobarbital (50 mg/kg body wt), and hearts and kidneys were removed, washed in saline, blotted, and weighed. The intraventricular septum was included in the left ventricular weight. Sections of the kidney and heart were preserved in 4% buffered formaldehyde solution and embedded in paraffin. Four-micrometer-thick sections were cut and stained with hematoxylin-eosin, periodic acid–Schiff (PAS), Sirius red, and/or Gordon and Sweet silver staining. Cardiomyocyte diameter was determined as previously described.\textsuperscript{21} Final glomerular sclerosis was determined under PAS staining and was graded as previously described.\textsuperscript{22} ECM production was quantified with the use of Sirius red and Adobe Photoshop 5.0 (Adobe). All sections were evaluated by investigators under blinded conditions, without previous knowledge as to which section belonged to which rat.

**Statistical Analysis**

Results are expressed as mean±SEM. Comparisons among groups were made by ANOVA followed by the Fisher protected least significant difference or unpaired Student t test. Differences were considered significant at P<0.05.

**Results**

**Expression of Human AM in DOCA-Salt Rats**

Human AM mRNA in DOCA-salt hypertensive rats after gene delivery was analyzed by reverse transcription–polymerase chain reaction followed by Southern blot analysis. Human AM was detected in the adrenal gland, aorta, cortex, and medulla of the kidney, liver, heart, and lung of the DOCA-salt rat injected with Ad.CMV-AM. The expression of human AM mRNA was not detected in the control DOCA-salt rat, which received Ad.CMV-Luc. Similar levels of β-actin mRNA were detected in tissues of both experimental and control groups, indicating the integrity of RNA in these samples. The results show that recombinant human AM is expressed in tissues relevant to cardiovascular and renal function after gene transfer in DOCA-salt hypertensive rats.

Immunoreactive human AM levels in rats receiving AM gene delivery were measured by RIA for human AM. Linear displacement curves of serial dilutions of urine and plasma from rats injected with the human AM gene displayed parallelism with the human AM standard curve, which indicated their immunological identity (data not shown). Serial dilutions of control rat urine and plasma did not show parallelism with the human AM standard (data not shown). These results indicate that the rabbit anti-human AM antibody had some cross-reactivity with endogenous rat AM. However, rat AM differs from human AM, and they are not immunologically identical and are distinguished by the human RIA. After intravenous injection of Ad.CMV-AM to DOCA-salt hypertensive rats, immunoreactive human AM levels in rat urine 7 days after gene delivery reached a level of 71.2±27.6 ng/100 g body wt per day, whereas plasma AM levels 3 days after gene delivery were detected at a level of 8.6±4.1 ng/mL. The expression levels of human AM in this
assay are considered to be only semiquantitative because of the nonparallelism of rat AM with the human AM standard.

Effect of Adenovirus-Mediated Gene Delivery of Human AM on Systolic Blood Pressure and Physiological Parameters in DOCA-Salt Hypertensive Rats

Figure 1 shows the effect of human AM gene delivery on systolic blood pressure of DOCA-salt hypertensive rats. Before gene delivery, a difference of 31 mm Hg in blood pressure was observed between DOCA-salt and control unilaterally nephrectomized animals (183 ± 4 mm Hg for DOCA-salt rats versus 152 ± 2 mm Hg for control rats, n = 10, P < 0.01). In DOCA-salt rats, a significant reduction in blood pressure was observed 3 days after human AM gene delivery compared with luciferase gene delivery. A maximal difference of 41 mm Hg in blood pressure was observed 9 days after gene delivery between DOCA-salt hypertensive rats treated with AM versus control rats receiving the luciferase gene (178 ± 7 mm Hg versus 219.5 ± 10 mm Hg, respectively; n = 10; P < 0.01). DOCA-salt rats receiving Ad.CMV-AM maintained significantly lower blood pressures for nearly 20 days compared with rats receiving the control adenovirus (n = 10, P < 0.05).

The Table shows the results of physiological analysis of DOCA-salt hypertensive rats after gene delivery. No apparent changes occurred in body weight or heart rate in rats injected with Ad.CMV-AM compared with DOCA-salt rats receiving Ad.CMV-Luc. Also, water intake and urine volumes were of similar levels 15 days after gene delivery between the DOCA-salt rats injected with Ad.CMV-AM and those injected with Ad.CMV-Luc. Urinary sodium and potassium levels were also measured 15 days after gene delivery and were not significantly altered between the 2 groups.

Effects of Human AM Gene Delivery on Renal Morphology and Renal Function in DOCA-Salt Hypertensive Rats

Morphological evaluation of the renal cortex (Figure 2, top panels) and medulla (Figure 2, bottom panels) revealed a beneficial effect of AM gene delivery on DOCA-salt hypertensive rats. PAS stained glycoprotein moieties bright red, clearly delineating the brush boarders and tubule epithelium. The cortex and medulla of unilaterally nephrectomized control rats fed a normal salt diet appeared normal (Figure 2A and 2D), whereas rats treated with DOCA salt and injected with Ad.CMV-Luc developed significant renal injury, occurring in both the cortex and medulla (Figure 2B and 2E). The damage in the cortex of DOCA-salt rats injected with Ad.CMV-Luc included tubular dilatation, loss of brush bor-

Physiological Analysis of DOCA-Salt Hypertensive Rats After Human AM Gene Delivery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>DOCA/Luc</th>
<th>DOCA/AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>280.5 ± 11.4</td>
<td>281.1 ± 9.1</td>
<td>275.3 ± 8.4</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>389 ± 7</td>
<td>390 ± 19</td>
<td>386 ± 9</td>
</tr>
<tr>
<td>Heart wt, g/100 g body wt</td>
<td>3.85 ± 0.11</td>
<td>5.20 ± 0.39</td>
<td>4.40 ± 0.14*</td>
</tr>
<tr>
<td>Cardiac cAMP pmol/mg protein</td>
<td>4.18 ± 0.96</td>
<td>4.90 ± 0.37</td>
<td>6.40 ± 0.30*</td>
</tr>
<tr>
<td>Cardiac cGMP pmol/mg protein</td>
<td>22.4 ± 1.8</td>
<td>18.9 ± 3.2</td>
<td>25.0 ± 3.2</td>
</tr>
<tr>
<td>Kidney wt, g/100 g body wt</td>
<td>0.62 ± 0.61</td>
<td>0.92 ± 0.41</td>
<td>0.76 ± 0.15*</td>
</tr>
<tr>
<td>Glomerular sclerosis score</td>
<td>0.27 ± 0.06</td>
<td>2.92 ± 0.20</td>
<td>1.93 ± 0.20†</td>
</tr>
</tbody>
</table>

Values for each group are mean ± SEM (n = 4 to 8).
Control animals were uninephrectomized, drank tap water, and were injected with Ad.CMV-Luc. Statistical significance between the groups was determined by ANOVA.

* P < 0.05 vs DOCA/Luc; † P < 0.01 vs DOCA/AM.
ders in proximal tubules, luminal protein cast formation, areas of inflammation, and glomerular sclerosis. Renal damage in the cortex was attenuated by human AM gene delivery (Figure 2C). In the medulla, DOCA-salt rats injected with the luciferase gene developed large colloidal casts within renal tubules (Figure 2E). Human AM gene transfer greatly reduced the size and number of protein casts present in the tubules compared with the sections from the rat treated with the luciferase gene (C vs B and F vs E).

The Table compares an overall quantification of glomerular sclerosis in DOCA-salt hypertensive rats 16 days after gene delivery. DOCA-salt rats injected with the AM gene had a significant reduction in glomerular sclerosis compared with those injected with the luciferase gene (Table). Figure 2F shows significantly attenuated left ventricular weight in DOCA-salt rats injected with the AM gene compared with DOCA-salt rats injected with the luciferase gene (0.71 ± 0.02 versus 0.76 ± 0.03 g/total heart wt, respectively; n = 5; P < 0.05). Further morphological evaluation by Gordon and Sweet silver staining showed that AM gene delivery significantly reduced cardiomyocyte diameter in DOCA-salt rats compared with the control rats injected with the luciferase gene (21.15 ± 1.37 versus 27.15 ± 0.30 μm, respectively; n = 300; P < 0.01) (Figure 4B).

Fibrosis of the left ventricle was examined under Sirius red staining as shown in Figure 5. Cardiomyocytes stained yellow, and ECM, such as collagen, stained red. To quantify ECM, red staining was expressed as a percentage of the total tissue area and was used as an index of fibrosis.

Human AM Gene Delivery Attenuates Cardiac Hypertrophy and Fibrosis

Global cardiac damage was attenuated in DOCA-salt hypertensive rats receiving AM gene delivery. The DOCA-salt rats that were injected with the AM gene had a significant reduction in total heart weight compared with those injected with the luciferase gene (Table). Figure 4A shows significantly attenuated left ventricular weight in DOCA-salt rats injected with the AM gene compared with DOCA-salt rats injected with the luciferase gene (0.71 ± 0.02 versus 0.76 ± 0.03 g/total heart wt, respectively; n = 5; P < 0.05).

Figure 3 confirms that AM gene delivery prevents renal injury by enhancing renal function of DOCA-salt hypertensive rats. Sixteen days after gene delivery, renal function was evaluated in all rats. The DOCA-salt rats receiving the AM gene, compared with the DOCA-salt rats receiving the luciferase gene, developed a significant increase in RBF (6.73 ± 1.26 versus 2.37 ± 0.63 mL/min·g kidney wt⁻¹, respectively; n = 5; P < 0.05) and GFR (0.41 ± 0.05 versus 0.23 ± 0.01 mL/min·g kidney wt⁻¹, respectively; n = 5; P < 0.05). Significant reduction in kidney weight was observed in rats receiving Ad.CMV-AM versus Ad.CMV-Luc (Table).

![Figure 2. Histological sections of kidney cortex (A through C) and medulla (D through F) stained by PAS. A and D, Control animals, which were injected with sesame oil. B and E, DOCA-salt hypertensive rats receiving control adenovirus, Ad.CMV-Luc. Note the tubular dilation and protein casts in the cortex (B) and medulla (E) and the glomerular sclerosis (B). C and F, DOCA-salt hypertensive rats receiving the human AM viral vector, Ad.CMV-AM. Note the decreased proximal tubular dilation and fewer protein casts compared with the sections from the rat treated with the luciferase gene (C vs B and F vs E).](http://hyper.ahajournals.org/)

![Figure 3. GFR (A) and RBF (B) of DOCA-salt hypertensive rats 16 days after adenovirus-mediated human AM gene delivery. Unilaterally nephrectomized rats and DOCA-salt hypertensive rats receiving Ad.CMV-Luc were used as controls. Data are expressed as mean ± SEM (n = 4). *P < 0.05 vs DOCA/Luc rats.](http://hyper.ahajournals.org/)

![Figure 4. Quantification of left ventricular weight/heart weight ratio (A) and cardiomyocyte diameter (B) after human AM gene delivery. Data are expressed as mean ± SEM (n = 4 for left ventricular weight/heart weight ratio and n = 300 for cardiomyocyte diameter). †P < 0.01 vs DOCA/Luc animals.](http://hyper.ahajournals.org/)
Adrenomedullin in DOCA-Salt Hypertension

Dobrzynski et al

Figure 6. Histological sections of rat hearts stained with Sirius red. A, Control unilaterally nephrectomized rats. B, DOCA-salt hypertensive rats receiving Ad.CMV-Luc. C, DOCA-salt hypertensive rats receiving Ad.CMV-AM. Tissues were harvested 16 days after gene delivery. Heart tissue sections stained by Sirius red protocol depict ECM accumulation in DOCA-salt hypertensive rats. D, Quantification of cardiac ECM accumulation after human AM gene delivery. ECM accumulation is expressed as percent collagen per total cardiac tissue (n=6). †P<0.05 vs DOCA/Luc animals.

Effects of Human AM Gene Delivery on cAMP and cGMP Levels

Urinary and cardiac cAMP and cGMP levels of DOCA-salt hypertensive rats receiving AM or luciferase gene delivery are shown in the Table. Human AM gene delivery significantly increased both urinary (7 days after gene delivery) and cardiac (16 days after gene delivery) cAMP levels in DOCA-salt rats compared with DOCA-salt rats receiving Ad.CMV-Luc. However, urinary and cardiac cGMP levels were not altered in DOCA-salt rats receiving human AM gene delivery versus rats receiving control virus.

Discussion

In the present study, we showed that adenovirus-mediated gene delivery of human AM not only produced a sustained reduction of blood pressure but also attenuated cardiac and renal dysfunction in DOCA-salt hypertensive rats. Human AM mRNA was present in key tissues involved in cardiovascular and renal function, such as the heart, aorta, and kidney. Immunoreactive human AM was detected in the plasma and urine of DOCA-salt hypertensive rats receiving AM gene delivery, indicating that human AM was secreted from the liver and kidney. Morphological evaluation demonstrated that somatic human AM gene delivery is capable of enhancing renal function and preventing cardiac remodeling in DOCA-salt hypertensive rats, leading to a reduction in both cardiovascular and renal damage. This protective ability provides significant insight regarding the ability of AM to regulate blood pressure via a cAMP-dependent pathway, which aids in the prevention of cardiovascular and renal damage in volume-dependent hypertension.

In the present study, we observed a blood pressure-lowering effect from adenovirus-mediated AM gene delivery, which is in agreement with previous studies reporting that infused AM or naked plasmid DNA containing the AM gene reduces blood pressure in rats.5,13 AM has also been reported to be a potent systemic and renal vasodilator.1,4,5 Work by Uehara et al22 suggests that a reduction of glomerular pressure can mediate local renal protection. Even though a slight reduction in blood pressure is widely accepted to provide a therapeutic value, a regional protective effect from a locally activated AM system may play an important role in the attenuation of renal and cardiovascular damage.

The biological effects of AM have been reported to be mediated by both cAMP and cGMP signaling pathways.6,7 It is well documented that cAMP is a potent vasodilator.23,24 Our results indicate that the mechanism of AM gene delivery on blood pressure reduction and organ protection in DOCA-salt hypertension appears to be mediated via a cAMP second-messenger cascade. We showed increased cAMP but not cGMP levels in the urine and hearts of DOCA-salt hypertensive rats after AM gene delivery. The elevation of cAMP is most likely due to AM binding to specific receptors on cell surfaces, such as vascular smooth muscle cells, endothelial cells, and glomerular mesangial cells.5,25–27 Further investigation is required to distinguish actual roles of renal protection that are due to reduced systemic blood pressure versus locally mediated renal affects of AM. Long-term infusion of AM at subdepressor levels and the use of an AM receptor–specific antagonist would aid in elucidating local renal protective effects of AM.

Discussion

In the present study, we failed to observe a natriuresis or diuresis effect in the DOCA-salt hypertensive rats receiving the human AM gene. This may indicate that human AM peptide does not affect natriuresis or diuresis in the DOCA-salt hypertensive animal model or that expression of recombinant human AM at the levels achieved in this study was not high enough to produce an effect. DOCA-salt rats treated with the luciferase gene exhibited renal damage in the form of elevated urinary protein levels (Table). Increased urinary protein levels were most likely due to renal injury, secondary to increased glomerular pressure. This notion was confirmed by morphological and renal function studies. Hematoxylin-eosin staining showed increased general interstitial inflammation, and Sirius red staining detected increased ECM...
protein production (data not shown), whereas PAS staining showed marked glomerular sclerosis, glomerular basement membrane thickening, renal tubular dilation, disruption of the proximal tubular brush border, and luminal protein cast accumulation in DOCA-salt rats receiving control virus (Figure 2). AM gene delivery significantly reduced renal damage compared with luciferase gene delivery in DOCA-salt rats. The renal protective effects of AM gene delivery are consistent with previous studies demonstrating that manipulation of the kallikrein-kinin system or the AM system can produce a marked reduction in blood pressure, leading to a reduction in renal injury.\(^{21,22}\) Within the kidney, increased cAMP levels due to AM interaction can inhibit mesangial cell growth, suppress the generation of reactive oxygen metabolites, and increase renovascular dilation, thus preventing renal damage.\(^{5,12,27,28}\) Therefore, the observed renal protection could be attributed to blood pressure reduction and to a direct interaction of AM with mesangial cells.\(^{28}\)

AM has also been suggested to play a major role in cardiac function in addition to blood pressure regulation and renal function.\(^ {9,10}\) Our results showed that DOCA-salt rats injected with Ad.CMV-AM had significant reductions in left ventricular weight, heart weight, and cardiomyocyte diameter as well as increased cardiac cAMP levels (Table). These results are consistent with previous observations by Tsuruda et al.,\(^ {11}\) who reported that AM inhibited protein synthesis in cultured neonatal rat cardiomyocytes and may thus attenuate hypertrophy in vivo. The AM-treated DOCA-salt rats had a marked reduction in both cardiac fibroblast activation and ECM accumulation compared with DOCA-salt rats treated with the luciferase gene, as evidenced by staining with both hematoxylin-eosin and Sirius red. Consistent with our results, in vitro cell culture study showed that AM peptide directly acts to inhibit collagen synthesis in cardiac fibroblasts mediated via a cAMP pathway in an autocrine/paracrine fashion.\(^ {29}\) The reduced damage observed in both the heart and kidney is most likely due to a mixture of direct and indirect mechanisms. Indirect organ protection may be provided by significant reduction in blood pressure.

Adenoviral gene delivery is capable of producing high levels of the transgene, but because of the lack of viral genome integration, transgene expression is only temporary. Adenoviral vectors can stimulate host immune responses, thus leading to inflammation, loss of infected host cells, immune response to foreign proteins, and the inability to readminister the viral vector.\(^ {29}\) To minimize the host immune and inflammatory responses, it is essential to develop improved viral vectors for prolonged transgene expression.

In the present study, we have shown that adenovirus-mediated gene delivery of human AM not only led to a sustained blood pressure reduction but also protected against cardiovascular and renal injuries in volume-overload hypertensive rats. The ability of AM gene delivery to produce these beneficial effects may compensate for the dysfunction of blood pressure regulatory systems and raise the potential for AM as a candidate for treatment of hormonal, neural, or salt-related hypertension as well as cardiorenal diseases.

**Acknowledgment**

This work was supported by National Institutes of Health grant HL-29397.

**References**

19. Gettys TW, Okonogi K, Tarry WC, Johnston J, Horton C, Taylor JL. Examination of relative rates of cAMP synthesis and degradation in crude


Adrenomedullin Gene Delivery Attenuates Hypertension, Cardiac Remodeling, and Renal Injury in Deoxycorticosterone Acetate-Salt Hypertensive Rats
Eric Dobrzynski, Cindy Wang, Julie Chao and Lee Chao

Hypertension. 2000;36:995-1001
doi: 10.1161/01.HYP.36.6.995

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/36/6/995

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/