Abstract—Defining the genetic basis of common forms of human essential hypertension is most informative when correlated with physiological mechanisms that underlie blood pressure regulation. A polymorphism of the alpha-adducin gene as been associated with elevated blood pressure in the rat, but previous studies of the 460Trp polymorphism of the human alpha-adducin gene have not clearly identified an association with hypertension. In this study, the frequency of the 460Trp allele was 19% and 9 of 279 subjects (3.2%) were homozygous for the 460Trp allele. The systolic blood pressure response to changes in dietary sodium was significantly greater in subjects homozygous for the 460Trp allele (25±4 mm Hg) compared with subjects heterozygous for 460Trp (12±2 mm Hg) or homozygous for the 460Gly allele (14±1 mm Hg). Intracellular erythrocyte sodium content, sodium-lithium countertransport, and renal fractional excretion of sodium were significantly decreased in subjects homozygous for the 460Trp polymorphism (P<0.05). There was a significant association between homozygosity for the 460Trp allele and low-renin hypertension. Subjects heterozygous for the 460Trp allele did not have increased salt-sensitivity or an increased frequency of low-renin hypertension. Therefore, this study demonstrates a common genetic basis for altered cellular sodium homeostasis, impaired renal sodium handling, and salt-sensitivity of systolic blood pressure in individuals homozygous for the 460Trp polymorphism of the alpha-adducin gene. Homozygosity for this alpha-adducin allele may be an important determinant for approximately 10% of individuals with low-renin hypertension. (Hypertension. 2002;39:191-196.)

Key Words: hypertension, essential ■ genetics ■ sodium, dietary ■ water-electrolyte balance ■ ion transport

Essential hypertension is recognized as a polygenic syndrome and most classifications of hypertension have been based on characterization of intermediate phenotypes. For example, hypertensive individuals have been characterized by salt-sensitivity, a low- or normal-renin state, or by renal and adrenal responses to angiotensin II infusion (non-modulation). With few exceptions, the relationship of a specific gene defect with the development of hypertension has not been well-characterized. The genetic basis of common phenotypic forms of hypertension has not been identified. In addition, the mechanisms by which salt intake can mediate changes in blood pressure in individuals with different salt-sensitive intermediate phenotypes have not been characterized.

The alpha-adducin gene was first characterized in the Milan Hypertension rat (MHR), an animal model of salt-sensitive hypertension in which the 316Tyr single nucleotide polymorphism (SNP) of alpha-adducin was associated with the hypertensive phenotype. In vitro studies have shown that expression of the 316Tyr form of alpha-adducin is associated with altered cellular sodium homeostasis. In human alpha-adducin, a SNP should encode Trp in place of Gly at amino acid residue 460 (Gly460Trp). There have been many previous clinical studies of hypertensive individuals with this alpha-adducin SNP, but these studies have been performed in heterogeneous populations and have yielded conflicting results. Thus, it has been difficult to confirm a role for alpha-adducin in the development of human hypertension.

This study was conducted on a large group of hypertensive subjects that were subgrouped into several intermediate phenotypes and in which detailed studies of sodium homeostasis were performed. The results of this study show that homozygosity for the 460Trp alpha-adducin SNP is associated with altered cellular sodium homeostasis, impaired sodium handling, and salt-sensitivity of systolic blood pressure. Hypertensive humans homozygous for this polymorphism are more likely to have one specific form of salt-sensitive hypertension, low-renin hypertension.
Methods

Subjects
All subjects were between 18 and 68 years of age. Hypertension was defined as a seated diastolic blood pressure of at least 100 mm Hg without treatment, at least 90 mm Hg on one antihypertensive medication, or the need for two or more medications. Secondary causes of hypertension and other significant medical problems, including diabetes mellitus, renal insufficiency, and heart disease, were excluded by history, physical, and laboratory evaluation. Patients with a body mass index (BMI) >31 kg/m², for women, or >33 kg/m², for men, were not studied. Race was self-identified by each subject. The study protocol was approved by the Institutional Review Board at each site and subjects gave written informed consent before study.

Study Design
Subjects (n=279) were studied as part of the HYPERpath consortium in the clinical research centers (CRC) of the Brigham and Women’s Hospital, Boston; the University of Utah Medical Center, Salt Lake City; and the Hospital Broussais, Paris. ACE inhibitors were discontinued 3 months before study. Subjects with a persistent untreated diastolic blood pressure >110 mm Hg were removed from the study. Subjects were instructed to maintain a high dietary sodium intake (>200 mmol sodium/d) for at least 3 days before the first admission to a clinical research center. One week before the second admission, subjects received one dose of hydrochlorothiazide (20 mg) and started an isocaloric diet providing 10 mmol sodium, 100 mmol potassium, and 800 mg calcium per day. High and low sodium balance were confirmed with 24 hours urinary sodium excretion of ≥160 mmol/d and ≤32 mmol/d, respectively. After achieving low salt balance, subjects underwent an upright posture study for 1 hour between 9 to 11 AM and a blood sample was drawn for determination of plasma renin activity. On each diet, subjects were admitted to a CRC and remained fasting and recumbent overnight. In the morning, supine morning blood pressure was calculated as the mean of three measurements obtained at 5 minute intervals by indirect recording sphygmomanometer (Dinamap; Critikon).

Techniques and Procedures
Genomic DNA was extracted from peripheral leukocytes using standard procedures. SNPs at codon 460 of alpha-adducin were identified by PCR and allele-specific oligonucleotide hybridization as previously described. Subjects homozygous for the 460Gly or 460Trp alleles were identified as G/G or T/T, respectively, while 460Trp alleles were identified as G/G or T/T, respectively, while

Results
Salt-Sensitivity and Alpha-Adducin Genotype
Salt-sensitivity of blood pressure was assessed by comparing recumbent morning blood pressures obtained after reaching sodium balance on high and low sodium diets. The systolic blood pressure change in response to a change in dietary sodium intake was significantly greater in T/T subjects (25±4 mm Hg) compared with subjects with the G/T (12±2 mm Hg) or G/G (14±1 mm Hg) genotypes (Figure 1). There was no significant difference in the blood pressure response between G/G and G/T subjects, suggesting that, in these two groups, the role of adducin in regulation of blood pressure was similar. Among subjects with the three alpha-adducin genotypes, there were no significant differences in the response of diastolic blood pressure to changes in dietary sodium content (G/G: 7±1 mm Hg, G/T: 7±1 mm Hg, T/T: 8±2 mm Hg).

Sodium Handling and Alpha-Adducin Genotype
To study possible mechanisms of salt sensitivity, cellular sodium homeostasis was assessed by the in vitro assays of intracellular sodium and potassium content and of sodium-lithium countertransport (Figure 2). The intracellular sodium content was significantly lower in the T/T subjects.
were also demonstrated by the lower fractional excretion of sodium (FENa). FENa was measured over 24 hours in subjects with high dietary sodium intake. FENa was significantly \( (P<0.05) \) lower in subjects with the T/T genotype compared with other subjects. 

\( (24.5 \pm 1.0 \text{ mmol/kg hemoglobin}) \), than in non-T/T subjects \( (33.2 \pm 0.1 \text{ mmol/kg Hb}) \). There was no significant difference in intracellular potassium content between the two groups of subjects \( (T/T: 340 \pm 15 \text{ mmol/kg Hb; non-T/T: 359} \pm 3 \text{ mmol/kg Hb}) \).

Sodium-lithium ion countertransport (SLC) was significantly lower in T/T subjects \( (0.16 \pm 0.04 \text{ mmol/L/h}) \) than in non-T/T subjects \( (0.28 \pm 0.01 \text{ mmol/L/h}) \). However, the mean SLC of all low-renin hypertensive subjects \( (0.25 \pm 0.02 \text{ mmol/L/h}) \) was not significantly different than the mean SLC of normal-renin hypertensive subjects \( (0.26 \pm 0.01 \text{ mmol/L/h}) \).

The physiological consequences of altered sodium handling were also demonstrated by the lower fractional excretion of sodium (FENa) observed in T/T subjects \( (0.84 \pm 0.12\%) \) compared with non-T/T subjects \( (1.33 \pm 0.05 \%) \) (Figure 3). Among non-T/T subjects, there was no significant difference in FENa between G/T \( (1.28 \pm 0.08\%) \) and G/G \( (1.36 \pm 0.05\%) \) subjects.

**Plasma Renin Activity and Alpha-Adducin Genotype**

Because altered sodium homeostasis may affect the renin response to changes in salt balance, plasma renin activity was measured in all subjects after they had reached low salt balance (Table 1). Although there was a trend to the T/T genotype being associated with a lower PRA recumbent level, a significant difference could not be shown between T/T and non-T/T subjects. There was also a trend to lower PRA levels after upright posture in subjects with the T/T genotype compared with other subjects, but a significant association could not be demonstrated between genotype and upright PRA level as a quantitative trait.

**TABLE 1. Plasma Renin Activity and Alpha-Adducin Genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
</tr>
<tr>
<td>All subjects</td>
</tr>
<tr>
<td>Normal renin</td>
</tr>
<tr>
<td>Low renin</td>
</tr>
</tbody>
</table>

\( G \) indicates 460Gly and \( T \) indicates 460Trp allele of the alpha-adducin gene. Plasma renin activity \( (\mu g/L/h \pm \text{SEM}) \) is measured as described in the Methods after subjects have reached sodium balance on a low salt diet. \*\( P<0.05 \) compared with normal renin subjects of same condition and genotype.

**Low-Renin Hypertension and Alpha-Adducin Genotype**

Hypertensive subjects were subclassified with the discrete traits of low-renin or normal-renin hypertension by using the rigorous stimuli of confirmed low salt balance and upright posture at a standard time of day. Subjects were classified with low-renin hypertension if the upright PRA was equal to or less than 2.4 \( \mu g/L/h \), whereas subjects with an upright PRA greater than 2.4 \( \mu g/L/h \) were classified with normal-renin hypertension. In this population, the mean PRA after upright posture was 1.4±0.1 \( \mu g/L/h \) in low-renin hypertensive subjects compared with 9.6±0.5 \( \mu g/L/h \) in normal-renin hypertensive subjects (Table 1). Similarly, basal PRA obtained after the subject remained recumbent overnight was significantly lower in subjects with low renin hypertension compared with the PRA in subjects with normal renin hypertension. Plasma renin activity in subjects with the T/T genotype remained significantly different between low-renin and normal-renin subjects, but did not differ from levels in non-T/T subjects. There were no significant differences in age, BMI, or gender among low-renin and normal-renin hypertensive patients (Table 2). Subjects with low-renin hypertension had a significantly higher systolic blood pressure than subjects with normal renin hypertension, but did not have a significantly different systolic blood pressure response to changes in dietary sodium intake.

The frequency of the 460Trp allele and the frequency of the homozygous T/T genotype were similar to that seen in studies performed in European populations. Nine of 279 subjects were homozygous for the 460Trp allele (T/T genotype) with a significant \( (P<0.005) \) association with low-renin hypertension. Six of 66 (9.0\%) low-renin hypertensive subjects had the T/T genotype, but only 3 of 214 (1.4\%) of normal-renin subjects were homozygous for 460Trp (Table 3). Compared with subjects with normal-renin hypertension, subjects with low-renin hypertension did not have an increased incidence of the heterozygous G/T genotype. In hypertensive subjects, there was a significant association between T/T genotype and

**TABLE 2. Characteristics of Subjects with Low-Renin and Normal-Renin Hypertension in the Study Population**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low-Renin Hypertension</th>
<th>Normal-Renin Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.4±6.8</td>
<td>46.6±7.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2±3.3</td>
<td>27.9±3.9</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>32/35</td>
<td>127/85</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>159±2*</td>
<td>149±1</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>92±1</td>
<td>89±1</td>
</tr>
<tr>
<td>Systolic BP sodium response, mm Hg</td>
<td>17±2</td>
<td>13±1</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Systolic and diastolic BP were obtained during screening on an ad lib dietary sodium intake. Systolic BP sodium response is the difference in supine systolic BP on confirmed high and low dietary sodium intakes. BP values are expressed as mean±SD; age and BMI are expressed as mean±SEM. *\( P<0.05 \) vs normal-renin hypertensive subjects.

**Low-Renin Hypertension, Sodium, α-Adducin**

Figure 3. Effect of alpha-adducin genotype on fractional urinary excretion of sodium (FENa). FENa was measured over 24 hours in subjects with high dietary sodium intake. FENa was significantly \( (P<0.05) \) lower in subjects with the T/T genotype compared with other subjects.
TABLE 3. Distribution of Alpha-Adducin Genotype in Subjects with Low-Renin and Normal-Renin Hypertension

<table>
<thead>
<tr>
<th>Adducin Genotype</th>
<th>Low-Renin Hypertension</th>
<th>Normal-Renin Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G or G/T</td>
<td>61 (91.0%)</td>
<td>209 (98.6%)</td>
</tr>
<tr>
<td>T/T</td>
<td>6 (9.0%)</td>
<td>3 (1.4%)</td>
</tr>
</tbody>
</table>

G indicates 460Gly and T indicates 460Trp allele of the alpha-adducin gene. Percentage of column totals are in parentheses. $\chi^2 = 9.27, P = 0.0023$.

being classified with low-renin hypertension, with 6 of 9 (66.7%) T/T subjects classified as low-renin compared with a 23.8% incidence of low-renin classification in other subjects. There was no increased risk for low-renin hypertension among subjects with the heterozygous G/T genotype.

Other Characteristics Not Affected by Adducin Genotype

There were no significant differences in basal serum sodium or potassium levels, fasting plasma glucose, or insulin levels among subjects with different alpha-adducin genotypes (Table 4). Although there was a trend toward decreased urinary free cortisol excretion in T/T individuals, no significant association could be demonstrated between alpha-adducin genotype and cortisol excretion. There was no significant association between self-identified race and adducin genotype. Among 9 subjects with the T/T genotype, 8 (88.9%) were of European origin and 1 (11.1%) was of African origin. Among the 270 subjects with non-T/T genotypes, 227 (84.1%) were of European origin, 41 (15.1%) were of African origin, and 2 (0.7%) were of Asian origin.

Discussion

The syndrome of essential hypertension is heterogeneous and has been classified into subgroups based on easily measured intermediate phenotypes. Low-renin hypertension is defined by an impaired PRA response to upright posture. It is associated with salt sensitivity, characterized by an inability to excrete a salt load without experiencing a concurrent increase in arterial blood pressure. Many criteria have been used to define salt-sensitivity as a discrete trait and approximately one-half of hypertensive individuals are salt-sensitive. Salt-sensitivity is frequently associated with a family history of high blood pressure and, in nonhypertensive individuals, may be a genetic risk factor for the later development of essential hypertension. Previous studies have tried to determine if salt-sensitivity is due to an intrinsic renal mechanism or is mediated by the effect of circulatory or neural factors on renal physiology. Characteristics such as insulin resistance have been associated with salt-sensitivity, and increased production of mineralocorticoids in glucocorticoid remedial hyperaldosteronism (GRA) leads to increased tubular resorption of sodium. However, other studies have demonstrated that monogenic primary renal disorders, such as Liddle’s syndrome and the syndrome of apparent mineralocorticoid excess, may alter sodium handling and lead to salt-sensitivity and low-renin hypertension.

In this study of 279 hypertensive subjects, we have characterized the association of the intermediate phenotypes of salt-sensitivity of systolic blood pressure and low-renin hypertension with a SNP in the alpha subunit of the cytoskeleton protein adducin. In the MHR, SNPs have been identified in the genes encoding each adducin isotype, and cross breeding studies have confirmed that the 316Tyr SNP of the alpha-adducin gene contributes to a hypertensive phenotype. It has been more difficult to confirm a role for alpha-adducin in the development of human hypertension. Before the cloning of the human alpha-adducin gene, linkage analysis using markers mapping near the adducin locus suggested an association between alpha-adducin and the phenotype of hypertension. After the human alpha-adducin gene was cloned, a SNP was identified that predicts a substitution of Trp for Gly at residue 460 of the alpha-adducin peptide. Many research groups have attempted to identify an association between the 460Trp allele and essential hypertension.

Some studies have reported an association between 460Trp and an elevation in blood pressure, whereas others have found no significant association between this allele and elevated blood pressure. Kamitani found no significant alteration in measures of sodium handling, such as basal PRA or isotopically determined body fluid volume associated with the SNP. However, other investigators have found decreased PRA levels in subjects carrying the 460Trp allele. In one detailed study of salt-sensitivity, subjects carrying either one or two 460Trp alleles were grouped together into one study group. These subjects had a greater decrease in mean arterial blood pressure after acute thiazide-induced sodium depletion and had a lower basal PRA and a diminished fractional urinary excretion of FENa. Other recent studies have given conflicting findings in identifying an association between the Gly460Trp polymorphism and hypertension in specific ethnic or racial groups. Therefore, the results of previous studies have been inconsistent in identifying a role for the Gly460Trp SNP in the development of essential hypertension, but did suggest a possible association of the 460Trp alpha-adducin allele with increased salt-sensitivity of blood pressure or suppression of PRA levels. These previous studies did not examine an association of the alpha-adducin SNP with particular subgroups of essential hypertension.

In the present study, hypertensive subjects were subclassified with either low-renin or normal-renin hypertension by using the rigorous stimuli of very low salt diet and upright posture at a standard time of day. In hypertensive subjects, there was a significant association between low-renin hypertension and homozygosity for the 460Trp allele, with 6 of 9 (66.7%) homozygous (T/T) subjects classified as low-renin

TABLE 4. Effect of Alpha-Adducin Genotype on Electrolyte and Hormone Levels in Hypertensive Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-T/T</th>
<th>T/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma sodium, meq/L</td>
<td>140±2</td>
<td>141±3</td>
</tr>
<tr>
<td>Plasma potassium, meq/L</td>
<td>4.2±0.06</td>
<td>4.1±0.04</td>
</tr>
<tr>
<td>Urinary free cortisol excretion, nmol/d</td>
<td>212±11</td>
<td>149±22</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2±0.1</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td>Fasting plasma insulin, IU/L</td>
<td>8.5±0.6</td>
<td>7.6±1.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM.
compared with a 23.8% incidence of low-renin classification in non-T/T subjects. In the present study, there was no increased risk for low-renin hypertension among subjects with the heterozygous G/T genotype, and unlike some previous reports, these heterozygous individuals did not demonstrate increased salt sensitivity as assessed by a difference in blood pressures measured while in low-salt and high-salt balance.

Studies of sodium homeostasis provide insight into possible mechanisms by which the T/T genotype may contribute to the development of some forms of essential hypertension. SLC and erythrocyte intracellular sodium content were decreased in individuals with the T/T genotype. Although not previously studied in humans, this finding is consistent with studies of intracellular sodium in the MHR. In the MHR, expression of the 316Tyr form of alpha-adducin results in decreased levels of both membrane-bound and cytoplasmic adducin in rat kidney tubule cells. In vitro studies demonstrated that mutations in the alpha and beta adducin genes are associated with increased cell-surface expression of the sodium-potassium transporter with an increased effective Vmax of the pump. These results were consistent with previous findings in the MHR that showed an increase in ion transport in renal tubular cells and erythrocytes that would predict decreased intracellular sodium content.

The decrease in SLC is not present in all subjects with low-renin hypertension. Therefore, a lower SLC is not a hallmark of low-renin hypertension, but may be a phenotypic consequence of homozygous expression of the 460Trp allele of alpha-adducin. The nonmodulation phenotype is characterized by an increased SLC. Although nonmodulation is a more common salt-sensitive intermediate phenotype, the effect of the less common homozygous T/T genotype is powerful enough to produce a decreased SLC in association with low-renin hypertension. This suggests that the effect of altered alpha-adducin function on blood pressure is different than the underlying mechanisms of nonmodulation. Alteration in the function of a sodium transporter, such as Na/K-ATPase, would result in perturbation of intracellular volume regulation with compensatory down-regulation of SLC. Thus, homozygous expression of the T/T genotype leads to altered cellular sodium homeostasis and impaired sodium handling.

Impaired sodium handling was demonstrated by the lower FENa observed in T/T subjects. FENa did not differ between G/T and G/G individuals. Thus, a single copy of the Gly460 allele is sufficient for normal alpha-adducin function in its role to regulate renal sodium handling. In individuals with impaired alpha-adducin function, increased tubular resorption of sodium should produce sodium retention and intravascular volume expansion with suppression of plasma renin activity. Consequently, T/T individuals with this alteration in renal sodium handling will have increased sensitivity of blood pressure to sodium intake and are at increased risk for developing low-renin hypertension. The strong association of the T/T genotype with increased salt sensitivity of systolic, but not diastolic, blood pressure suggests that regulation of these two measures of blood pressure may be physiologically and genetically separable. The Gly460 SNP had no significant effect on fasting glucose or insulin levels or on urinary cortisol excretion. Therefore, the findings of this study are consistent with the hypothesis that low-renin hypertension can be produced by intrinsic renal mechanisms such as altered sodium homeostasis. However, individuals with the ability to compensate for the alteration in sodium homeostasis could be protected from the development of hypertension. Finally, the lack of the homozygous T/T genotype in most subjects with low-renin hypertension demonstrates that alterations in alpha-adducin are not the only way in which to develop low-renin hypertension.

The goal of a study such as this one is to identify the genetic basis of essential hypertension. However, blood pressure is controlled by the expression of many genes and the syndrome of essential hypertension includes a number of distinct phenotypic subtypes with different likely genetic mechanisms. Although the products of many genes are involved in renal sodium handling, a mutation in only one or a few of these genes may be sufficient to alter salt sensitivity in any one individual. This study demonstrates that homozygous expression of one SNP of the alpha-adducin gene modifies sodium homeostasis and is associated with the intermediate phenotypes of salt-sensitivity and low-renin hypertension. The alterations in intracellular sodium homeostasis and renal sodium excretion in subjects with the T/T mutation are consistent with the hypothesis that altered renal sodium handling may contribute to the development of low-renin hypertension. Therefore, this study has demonstrated a consistent genetic basis for altered cellular sodium homeostasis, impaired renal sodium handling, and salt-sensitivity of blood pressure in individuals with the 460Trp SNP of alpha-adducin. In addition, the 460Trp SNP may be an important contributor to the elevated blood pressure in approximately 10% of individuals with low-renin hypertension.

Acknowledgments

This work was supported in part by a Specialized Center for Research (SCOR) (NIH grant HL55000), by NIH grants HL47651 and DK53538, and by INSERM. The authors thank Valeria Roccio and Alicia Rivera, PhD, for technical assistance, Lynn Braley, MS, Shelley Horwitz, PhD, and Natapong Koshachuhanan, MD, for assistance with data management and analysis, and the nursing, dietary, and laboratory staffs of the 3 General Clinical Research Centers (GCRCs) for their expert assistance in facilitating these studies.

References

196  Hypertension  February 2002


Low-Renin Hypertension, Altered Sodium Homeostasis, and an α-Adducin Polymorphism

Frederick D. Grant, Jose R. Romero, Xavier Jeunemaitre, Steven C. Hunt, Paul N. Hopkins, Norman H. Hollenberg and Gordon H. Williams

doi: 10.1161/hy0202.104273

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 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/39/2/191

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/