Cerebrospinal Fluid and Plasma Dopamine-Beta-Hydroxylase Activity in Human Hypertension

LUIGI X. CUBEDDU, M.D., PH.D., JUAN DAVILA, M.D., DEITRICH ZSCHAECK, M.D., YARISMA R. BARBELLA, M.D., PABLO ORDAZ, M.D., AND JORGE DOMINGUEZ, M.D.

SUMMARY Cerebrospinal fluid (CSF) and plasma dopamine-beta-hydroxylase (DBH) activity was measured in 22 normotensive (NT), 31 essential hypertensive (EH), and 11 renal hypertensive (RH) patients. Although no differences were observed in their plasma DBH, the mean CSF-DBH activity and specific activity of EH were significantly lower than those of NT and RH patients. Very low CSF-DBH (< 0.15 units/ml of CSF or < 0.5 units/mg of CSF protein) was found only in EH (26% of EH). Of the 31 EH patients, 19 (60%) had CSF-DBH activities lower than 0.5 units/ml, whereas only 5 of 22 NT (23%) and no RH fell within this range. Nevertheless, 20% of EH, 55% of NT, and 40% of RH patients had CSF-DBH activities above the mean value for NT (> 0.9 units/ml). NT subjects with very low plasma DBH (< 50 units/ml) had CSF-DBH activities that fell within normal range. With the exception of these subjects, the specific activity of CSF-DBH was always lower than that of the plasma enzyme.

The concentration of albumin, alpha-, beta, and gamma globulins was measured in plasma and CSF obtained from the last five NT, four EH, and two RH patients. A positive linear relationship was obtained when the log of the plasma/CSF concentration ratio for these proteins was plotted against their molecular weight. Similar slopes and intercepts were obtained for these patients, suggesting that no major differences seem to exist in their blood-brain-barrier permeability to proteins. The results suggest that measurements of CSF-DBH could be of help in the differential diagnosis of human hypertension and in the neurochemical characterization of EH. If CSF-DBH reflects central noradrenergic activity, its reduction might indicate the existence of a central catecholaminergic defect in a subgroup of EH patients. (Hypertension 3: 448-455, 1981)

KEY WORDS • dopamine-beta-hydroxylase • cerebrospinal fluid • plasma • hypertension

norepinephrine (NE)- and epinephrine (E)-containing neurons are located in brain areas involved in cardiovascular control. It is known that intraventricular and direct micro-injection of these amines into the nucleus of the solitari tract in the medulla oblongata reduces systemic blood pressure (BP). Experimental evidence also indicates that antihypertensive drugs like clonidine and alpha-methyldopa appear to exert their central BP-lowering action via activation of catecholaminergic receptors in the brain. Changes in catecholamine (CA) content and turnover, and in the levels of their synthesizing enzymes have been reported in the brain of hypertensive rats. In addition, changes in BP can modify the release of CA from the posterior hypothalamus of the cat. These and other studies provide evidence in favor of a participation of central catecholaminergic neuronal systems in the regulation of BP and in the development of certain forms of animal hypertension. However, little is known about the activity of central noradrenergic and adrenergic neurons in human hypertensive disease.

Dopamine-beta-hydroxylase (DBH), the enzyme that converts dopamine to NE, has been shown to be present in peripheral and central noradrenergic neurons and in the plasma and CSF of humans and animals. It has been proposed that CSF-DBH could be used as a tool to evaluate the activity of central noradrenergic neuronal systems. Based on measurements of CSF-DBH, we had previously suggested a deficit in central noradrenergic neurons in patients with established essential hypertension (EH). It therefore appeared of interest to evaluate whether a reduced CSF-DBH activity in hypertensives is pathognomonic of EH or if it is found associated with other forms of human hypertension. In addition, we investigated whether a diminished CSF-DBH activity...
The enzyme activity proved to be directly proportional to the amount of CSF (10-50 μg/ml) and catalase were essential for optimal enzyme activity. The addition of CuSO₄, use of a final concentration, and dilutions were used to obtain optimal enzyme activity (1-10 μM, final concentration). The DBH step was incubated for 20 minutes and the PNMT step for 45 minutes. Each sample was assayed in triplicate. The variability between replicate assay was 4.2% ± 0.8%.

The CSF-DBH was measured as described by Goldstein and Cubeddu. After the collection, the CSF was kept on ice and the assay, run within 2 hours. Plasma DBH was measured as described by Cubeddu et al. Heparinized blood samples were centrifuged at 12,000 × g for 10 minutes at 2°C, and the plasma was stored at −40°C. Before the assay, the plasma was diluted (1:200 to 1:500) with ice-cold distilled water. Appropriate copper sulfate concentrations were used to obtain optimal enzyme activity (1-10 μM, final concentration). The DBH step was incubated for 20 minutes and the PNMT step for 45 minutes. Each sample was assayed in triplicate. The variability between replicate assay was 4.2% ± 0.8%.

The CSF-DBH was measured as described by Goldstein and Cubeddu. After the collection, the CSF was kept on ice and the assay, run within 2 hours. The DBH activity was expressed in units. One unit represented the formation of 1 nanomole of octopamine per hour of incubation. The results were expressed as units per milliliter of plasma or CSF, or as units per milligram of protein (specific activity).

Appropriate dilutions of plasma and CSF were used for protein determinations. The CSF and plasma were diluted 1:2 and 1:200 respectively in ice-cold distilled water. Linearity was obtained for up to 150 μg of bovine serum albumin. Four ml of CSF were concentrated in Amicon cones to a final concentration of 60-80 mg/ml. Five μl of concentrated CSF and plasma were used for the electrophoretic separation of proteins on cellulose acetate.

**Patients**

We studied 22 normotensive subjects (11 women and 11 men), 31 patients with primary hypertension (21 women and 10 men), and 11 patients with hypertension secondary to kidney disease (9 women and 2 men). Their ages ranged from 15 to 62 years (table 1).

**Renal Hypertensives (RH)**

The renal section of the Department of Medicine of the Perez Carreño Hospital provided patients with hypertension secondary to renal failure or renal vascular disease; their ages ranged from 25 to 44 years. Eight patients had chronic parenchymal disease, and three had renovascular hypertension. Six of the patients with parenchymal damage had chronic glomerulonephritis (1 woman, 5 men), one woman had chronic pyelonephritis and one man had polycystic kidney disease. Seven of the uremic patients were receiving weekly standard hemodialysis with a

<table>
<thead>
<tr>
<th>TABLE 1. Patient Characteristics</th>
<th>Sex</th>
<th>Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Normotensives (NT)</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Essential hypertensives (EH)</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Renal hypertensives (RH)</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

*Significantly different from NT at p < 0.01.
Travenol coil artificial kidney, and their volume-dependent hypertension was controlled by ultrafiltration. The diagnosis of chronic parenchymal disease was based on clinical, biochemical, and x-ray findings, and in one case confirmed by the histopathological picture. Three of 11 patients with renal hypertension had renal vascular disease documented by rapid sequence intravenous pyelography, renal arteriography, and selective renal vein renin measurements. Their renal function was within normal range.

Uremic patients received multivitamins, folic acid, oral iron, and aluminum hydroxyde, and were on a protein-, sodium-, and potassium-restricted diet. Prior to dialysis the patients received heparin. In the uremic patients, the lumbar puncture was always performed 1 day before the hemodialysis.

Essential Hypertensives (EH)

The EH patients (ages, 32 to 62 years) were provided by the Cardiology Department of the Central University Hospital. Essential hypertension was diagnosed by exclusion of other causes. The routine screening included: measurements of serum urea and creatinine levels, urinalysis, creatinine clearance, intravenous pyelography, repeated serum electrolyte levels, urinary excretion of 17-OH, 17-keto steroids, and vanillylmandelic acid (VMA). In 20% of the subjects renal arteriography was performed. We chose for the study only mild or moderate fixed hypertensives, without important end-organ damage; we eliminated those with a history of angina pectoris, myocardial infarction, heart failure, cardiac arrhythmia, renal failure, or cerebrovascular insufficiency. Six of the 31 EH patients had mild-to-moderate left ventricular hypertrophy. Creatinine values ranged from 0.6 to 1.67 mg%. In no case were hemorrhages or exudates found upon fundoscopy. None of the patients had received digitalis, anticoagulants, antiarrhythmic drugs, or diuretics other than thiazides. When admitted to the study, all EH had BPs greater than 140/95 mm Hg because of poor compliance or inadequate treatment; their BPs at admission were: 177 ± 4 mm Hg systolic and 112 ± 3 mm Hg diastolic.

Experimental Protocol

Any antihypertensive medication was discontinued 2 weeks before the lumbar puncture. During this "washout" period, the patients received one capsule of 250 mg lactose (placebo) twice a day, and were examined every 2 days. In no case was it necessary to reinstate the antihypertensive medication, since no important BP increases were observed (table 1).

On the day of the study, the patients underwent physical examinations and then rested for 60 minutes in the supine position. The BP was taken with a sphygmomanometer upon arrival and every 30 minutes thereafter. Lumbar puncture was performed under standard conditions (between 8:30 to 9:30 a.m.), and the CSF pressure measured before and after withdrawal of the spinal fluid. The first 2 ml sample of CSF was used for biochemical and cytological examinations. Subsequently, 4 ml samples of 1.5 to 2 ml each were collected in ice-cold polypropylene tubes, at 2-minute intervals. In some patients, an additional 4.5 ml sample was taken for protein determination and electrophoresis. The CSF samples were clear and colorless and had normal biochemistry and cell counts. No red blood cells were detected. Immediately after termination, a 5 ml blood sample was obtained by antecubital vein puncture. Afterward the patients remained recumbent for 3 hours under supervision. Patients were discharged after medical examination and reinstitution of antihypertensive therapy. The protocol was approved by the National Council for Scientific and Technological Research, and patients and their families gave informed consent.

Unless otherwise stated, the data are presented as mean ± SEM. Statistical probability of differences was calculated by Student's t test, and regression lines and correlation coefficients by the method of least squares.

Results

Cerebrospinal Fluid and Dopamine-Beta-Hydroxylase Activity (CSF-DBH)

Storage of CSF at -4°C in plastic tubes produced a complete loss of DBH activity in 48 hours. The addition of bovine serum albumin (1 mg/ml CSF) and of catalase (2000 U/ml CSF) improved the stability of the CSF-DBH. With this treatment, 7 days of storage at -4°C reduced the CSF-DBH activity by 46.5% ± 8.8%. In all cases, however, the CSF-DBH activity was measured within 2 hours of CSF collection. No significant differences were observed in the enzyme activity present in samples of CSF taken serially, or between the first and second 5 ml aliquot of CSF obtained from the same lumbar puncture, in NT, EH, and RH subjects (fig. 1).

![Figure 1. Dopamine-beta-hydroxylase (DBH) activity in serially collected samples of human cerebrospinal fluid (CSF) withdrawn by lumbar puncture from normotensive and hypertensive subjects. The first 2 ml of CSF were used for routine cytochemical determinations. The enzymatic activity was measured in five consecutive 1 ml of CSF taken at 2-minute intervals, and in a final 5-ml aliquot. Results are expressed as percentage of the DBH activity present in the first sample. Shown are mean values ± SEM of 11 determinations.](image-url)
The mean CSF-DBH activity in the absence and presence of 3 and 10 μM CuSO₄ was: 0.79 ± 0.16, 0.82 ± 0.13 and 0.75 ± 0.17 units/ml respectively. The addition to human CSF of 0.9 units of DBH partially purified from bovine adrenals gave a 103% ± 4% recovery of enzymatic activity. Dilution of CSF (1:2) with distilled water gave 48% ± 3% of the DBH activity present in the undiluted CSF. No differences were observed in the response of the CSF to CuSO₄ on the dilution and addition of adrenal DBH, in NT, EH, and RH subjects.

**Blood Pressure, Age, Sex, and DBH Activities**

The NT subjects had significantly lower systolic, diastolic, and mean BPs than EH and RH patients (table 1). The age of EH patients was higher than that of NT and RH (table 1). No significant correlation was found between the BP, age, and sex of any of the groups of patients or for all patients combined as a single group.

**CSF and Plasma DBH Activity**

In the three groups of patients, the concentration of plasma DBH was at least 1000 times greater than that of CSF-DBH (fig. 2). Large variations in enzyme activity were found in the plasma and CSF. The wider range of plasma DBH activities found in NT (22 to 2280) and EH (17 to 2129) was due to subjects with "low" plasma DBH (<50 units/ml), who accounted for 6.5% and 12.5% of the EH and NT subjects respectively. If these patients were omitted from the calculations, the ranges for plasma DBH activity of NT (212 to 2288) and EH (206 to 2129) were much closer to those of RH (274 to 1594).

No differences in plasma DBH activity were observed between NT, EH, and RH patients (fig. 2). However, the mean CSF-DBH activity of the EH was significantly lower than that of NT and RH, and there was no difference in the mean CSF-DBH activity of NT and RH.

**TABLE 2. Classification of Essential Hypertensive Patients According to their Cerebrospinal Fluid-Plasma Dopamine-Beta-Hydroxylase (CSF-DBH) Activity**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>CSF-DBH activity (n mole/hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.5 (n = 18)</td>
</tr>
<tr>
<td>Age</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Blood pressure:</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>182 ± 7</td>
</tr>
<tr>
<td>Diastolic</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>Mean</td>
<td>137 ± 6</td>
</tr>
<tr>
<td>CSF-DBH activity</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Plasma-DBH activity</td>
<td>796 ± 150</td>
</tr>
<tr>
<td>CSF-DBH SA</td>
<td>0.39 ± 0.1</td>
</tr>
<tr>
<td>CSF protein concentration</td>
<td>0.29 ± 0.03</td>
</tr>
</tbody>
</table>

*Significantly different at p < 0.001. SA = specific activity; CSF = cerebrospinal fluid; DBH = dopamine-beta-hydroxylase.

Nearly 60% of the EH had CSF-DBH activities lower than 0.5 units/ml (mean value for EH), whereas only 5 of 22 NT and no RH fell within this range of activities. Very low CSF-DBH activities (<0.15 units/ml) were found only in EH (26% of patients). Interestingly, no differences were observed between the mean age, BP, and plasma DBH activity of the EH patients with CSF higher and lower than 0.5 units/ml (table 2). Nearly 20% of the EH, 55% of the NT, and 40% of the RH had CSF-DBH activities greater than the mean value obtained for the group of NT patients (0.9 units/ml).

Similar values for plasma and CSF-DBH activities were obtained in RH patients, whether they were uremic or not (plasma DBH activity: 861 ± 270...
uremics; 1103 ± 280 nonuremics; CSF-DBH activity: 1.2 ± 0.1 uremics; 1.1 ± 0.1 nonuremics).

The mean plasma/CSF-DBH activity ratios are shown in fig. 2; in RH they were significantly lower than in EH patients.

No significant correlations were found between CSF-DBH, plasma DBH, age and BP in NT, EH, and RH patients separately or combined as a single group; with the exception of a low but significant correlation observed between plasma and CSF-DBH activity in EH (fig. 3).

The EH subjects with "low" plasma DBH (17, 43, and 99 units/ml) had CSF-DBH activities of 0.24, 0.59 and 0.33 units/ml respectively. In the same group of EH patients, a plasma value of 1276 units/ml was accompanied by a very low CSF-DBH activity (0.065 units/ml). Two of the NT subjects had "low" plasma DBH activity (22 and 23 units/ml) and a CSF-DBH activity of 0.46 and 0.89 units/ml.

CSF and Plasma Protein Concentrations and DBH Specific Activities

A similar specific activity of plasma DBH was found in NT, EH, and RH patients (16.5 ± 2.5, 15.1 ± 1.8, 13.1 ± 1.8 units/mg protein); whereas in the CSF of ET patients the specific activity of DBH (1.5 ± 0.2) was significantly lower (p < 0.05) than that of NT (2.3 ± 0.2) and RH (1.9 ± 0.2) patients. The RH patients had a higher CSF-protein concentration than NT and EH (RH = 0.6 ± 0.1, NT = 0.4 ± 0.04, EH = 0.43 ± 0.03 mg/ml CSF), whereas their plasmas had a similar protein concentration. With the exception of the subjects with "low" plasma DBH activity, the specific activity of the plasma DBH (15.6 ± 1.8 units/mg protein) was always higher than that of CSF-DBH (1.9 ± 0.2 units/mg protein). Interestingly, a very low specific activity of CSF-DBH was found only in EH patients (table 2).
No correlation was found between the CSF-DBH specific activity, plasma DBH specific activity, age, and blood pressure of the patients. Only in RH was a significant correlation ($r = 0.7, p < 0.05$) encountered between the CSF-protein concentration and the CSF-DBH activity.

The concentration of albumin, alpha, beta, and gamma globulins was measured in plasma and CSF obtained from the last five NT, four EH, and two RH patients. A positive linear relationship was observed when the log of the plasma/CSF concentration ratio for these proteins was plotted against their molecular weight (fig. 4). No differences in the slopes and intercepts of the regression lines were observed between the NT, EH, and RH patients studied.

Discussion

In the present study we measured the activity of DBH in the CSF of NT, EH, and RH patients. Although no differences in plasma DBH were detected among the three groups, there was a reduction in CSF-DBH activity in the EH when compared to the NT and RH groups. In smaller groups of patients we previously reported a diminished CSF-DBH activity in EH when compared to NT subjects. Interestingly, in the larger group of patients of our present study only EH patients showed very low CSF-DBH activities (< 0.15 units/ml of CSF). Nevertheless, nearly 20% of the EH patients had CSF-DBH activities greater than the mean value obtained for NT and RH subjects. These findings open the possibility of using CSF-DBH levels in the differential diagnosis of human hypertension and in the biochemical characterization of EH.

The significance of CSF-DBH activity largely depends on the origin of the enzyme. The DBH in CSF could originate from central adrenergic or noradrenergic neurons. In addition, and as shown for other proteins, the CSF-enzyme could derive from plasma by a process of molecular sifting. This latter view is supported by the following observations made in humans: 1) the existence of a large plasma-CSF gradient for DBH activity (present study); 2) the lower specific activity of CSF-DBH compared to plasma DBH (present study); 3) the weak, although significant correlation observed between plasma and CSF-DBH activities (present study); 4) The very high preoperative plasma and CSF-DBH activities and the reduction in both enzyme activities observed in a NT patient with large bilateral pheochromocytomas. On the other hand, the following observations suggest that CSF-DBH might well derive from central adrenergic or noradrenergic neurons: 1) the large molecular size of DBH (290,000 daltons); 2) the very low or lack of correlation between plasma and CSF-DBH activities observed in other studies (present study); 3) the finding that subjects with "low" plasma DBH, in whom the gradient plasma/CSF for DBH is reduced 25- to 100-fold, have CSF-DBH activities within normal range (present study); 4) the selective reduction in CSF-DBH activity found in 60% of EH patients, in spite of the fact that their plasma DBH activities and blood-brain-barrier permeability to proteins were normal (present study); 5) the selective reduction in CSF-DBH activity reported in depressed patients treated with monoamine oxidase inhibitors, without concomitant changes in their plasma DBH activities, a treatment is known to decrease central noradrenergic firing; 6) in rabbits, pentamethylenetetrazol increases both plasma and CSF-DBH activity; however, destruction of central noradrenergic neurons by 6-OH-dopamine reduces the basal levels and the drug-induced increase in CSF-DBH, without affecting the drug-evoked increase in plasma DBH.

In summary, these results indicate that CSF-DBH activity cannot be explained solely by the molecular

![Figure 4. Blood-brain-barrier permeability to proteins in normotensive (NT), essential hypertensive (EH) and renal hypertensive (RH) subjects. Proteins were measured in plasma and CSF in the last five NT (open circles), four EH (black circles), and two RH (open triangles) patients examined. The log of the protein concentration ratio of plasma/CSF was plotted against the molecular weight of the most abundant protein for each fraction. A regression line was calculated for each patient and the following mean values ± SEM for the slopes, intercepts, and correlation coefficients ($r$), were obtained:

NT: $r = 0.81 ± 0.06$; intercept = $2.08 ± 0.06$; slope = $7.0 ± 2.0 × 10^4$

EH: $r = 0.86 ± 0.06$; intercept = $2.11 ± 0.09$; slope = $6.7 ± 1.7 × 10^4$

RH: $r = 0.67 - 0.78$; intercept = $2.07 - 2.33$; slope = $2.3 - 3.2 × 10^4$

No significant differences at $p < 0.05$ were obtained among the groups of patients.](http://hyper.ahajournals.org/)
sifting of this large enzyme molecule from the plasma to the CSF. Therefore, central CA-containing neurons might also contribute to the enzyme levels in the CSF. Accordingly, the lower enzyme activity found in 60% of our EH patients, together with an apparently normal blood-brain-barrier permeability to proteins, suggests the existence of a defect in central adrenergic and/or noradrenergic neurons in some forms of EH. A lower neuronal turnover or a reduced number of NE- or E-containing neurons, a defect in the quality or amount of enzyme synthesized, and a lower proportion of releasable DBH could determine the reduced CSF-DBH activity observed in these patients. An augmented clearance of proteins from CSF in EH could also account for the diminished CSF-DBH activity found; however, the normal CSF-protein concentration found in EH patients speaks against this possibility.

The firing of central catecholaminergic neurons could be modified by antihypertensive medications and by BP changes.14 Our EH patients were withdrawn from any antihypertensive medication 2 weeks prior to lumbar puncture and did not present a significant increase in BP during the "washout" period; consequently, it is rather unlikely that the reduced CSF-DBH activity observed in 60% of our EH patients was related to the drug therapy. The greater medical care given to the patients during the "washout" period, the use of a placebo regimen, and the selection of patients who had elevated BPs because of poor compliance or inadequate treatment possibly account for the lack of a significant increase in BP after medication withdrawal.

Changes in mood stages have been reported to modify CSF-DBH activity.4 Therefore, it might be possible that the anxiety involved in the spinal tap procedure would induce changes in central catecholaminergic neuronal firing, and thus in CSF-DBH. If so, our results would indicate a different reactivity to stress of the central catecholaminergic neurons of some EH patients.

Recently, increased NE concentrations were found in the CSF of younger, labile hypertensives with primary hypertension.15 Based on their findings, these investigators suggest that central noradrenergic tone is enhanced in some hypertensive patients, especially younger subjects with increased plasma NE and renin activity. Further studies are required to establish the relationships between NE and DBH in CSF, to determine whether they represent the activity of spinal or brain noradrenergic neurons, to understand their dynamics in the lumbar CSF, and to establish their relationships with the central and peripheral noradrenergic tone and with changes in the permeability of the blood-brain barrier in physiological and pathological states.

References


5. de Jong W: Catecholamines of the nucleus tractus solitarii and blood pressure regulation. International Symposium 32: Central Adrenaline Neurons: Basic Aspects and Their role in Cardiovascular Functions, Stockholm, August 27, 1979

6. de Jong W, Nijkamp FP, Bohus B: Role of noradrenaline and serotonin in the central control of blood pressure in normoten- sive and spontaneously hypertensive rats. Arch Intern Pharmacodyn Ther 213: 272, 1975


22. Okada T, Ohta T, Kato T, Ikuta K, Nagatsu T: Dopamine-
beta-hydroxylase activity in serum and cerebrospinal fluid in neuropsychiatric diseases. Neuropsychobiology 2: 139, 1976
Cerebrospinal fluid and plasma dopamine-beta-hydroxylase activity in human hypertension.
L X Cubeddu, J Davila, D Zschaecck, Y R Barbella, P Ordaz and J Dominguez

Hypertension. 1981;3:448-455
doi: 10.1161/01.HYP.3.4.448

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 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/3/4/448

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/