Effect of Acute Volume Alterations on Norepinephrine and Dopamine-β-Hydroxylase in Normotensive and Hypertensive Subjects

C. Raymond Lake, M.D., Ph.D., and Michael G. Ziegler, M.D.

SUMMARY  Fifty-six patients with essential hypertension and 29 normotensive controls were hospitalized and volume expanded with saline or volume depleted with furosemide. We measured plasma norepinephrine (NE) and dopamine-β-hydroxylase (DBH) to evaluate their sympathetic nervous activity. NE and DBH levels and their response to volume changes were the same in normal and hypertensive subjects. Volume depletion increased plasma NE in recumbent

SYMPATHETIC NERVOUS ACTIVITY maintains blood pressure during changes in posture or after acute blood loss and elevates blood pressure during acute stress. The role of the sympathetic nervous system in the maintenance of blood pressure over long periods of time is uncertain. Several authors have implicated noradrenergic-mediated maintenance of high blood pressure in patients with hypertension of long duration, since they found higher plasma norepinephrine (NE) concentrations in hypertensive than in control subjects. Recent studies indicate that plasma NE increases with age. When we age matched outpatient controls and hypertensives we found no difference between NE levels of hypertensives and controls. Several groups have proposed that the response of hypertensives to mental stress or postural stress is exaggerated, and that this may lead to essential hypertension. To investigate if hypertensives who are under stress to their sympathetic nervous system have abnormal sympathetic nervous activity we hospitalized hypertensive and control subjects under identical conditions and evaluated their plasma NE and DBH responses after volume depletion and volume expansion.

NE is the neurotransmitter of the sympathetic nerves and plasma levels of NE reflect the level of nerve activity. Dopamine-β-hydroxylase (DBH) is the enzyme which converts dopamine to NE and is present in sympathetic nerve endings where it is released along with NE upon nerve stimulation. DBH activity has been proposed as an index of sympathetic tone but this postulate is controversial. Although DBH activity in normal subjects varies widely, the same adult individual has a consistent range of DBH activity which is genetically controlled. Plasma NE levels reflect sympathetic nervous activity over short time periods because the half-life of NE is less than three minutes. DBH levels may reflect sympathetic nerve activity over a longer time period.

Volume depletion by salt restriction or diuretic therapy lowers blood pressure, and sympathetic nerves respond to increase peripheral vascular resistance and cardiac output to maintain blood pressure. Hypertensive patients are reported to have a diminished response of DBH levels to the stress of diuresis and standing and some hypertensives are reported to have an exaggerated output of NE in response to postural changes. The sympathetic nervous response to volume expansion in normotensive and hypertensive subjects has not been documented.

Methods

Twenty-nine healthy normotensive volunteers and 56 patients with essential hypertension participated in the study after giving their written informed consent. Blood pressure was measured by auscultation both in the clinic and by the patients at home on a regular schedule. Patients included in the study had more than 80% of their diastolic blood pressures above 90 mm Hg in over 25 separate measurements. Patients were excluded who had symptomatic cardiac disease, malignant hypertension, diabetes mellitus, primary aldosteronism, renovascular disease or other known causes of their hypertension. All subjects were medication free for at least two weeks before beginning the study. Hypertensive patients underwent a thorough physical exam and laboratory investigation including minute-sequence intravenous pyelogram. Subjects were tested on three occasions all within a three day period: first, while medication free and on a 109 meq sodium diet; second, after a 2 L infusion of normal saline given over four hours while on the 109 meq sodium diet; and third, 24 hours after beginning a 9 meq sodium diet and receiving 120 mg furosemide in three divided doses. All subjects were weighed on the same scale before and after saline and after furosemide. The entire procedure was performed on normotensive and hypertensive subjects identically while hospitalized at the Clinical Center of the National Institutes of Health.

For each test procedure the subject was supine and had an indwelling catheter inserted in an arm vein. At least 20 minutes after insertion of the indwelling catheter but not before the subject was subjectively relaxed and had a stable pulse rate, blood pressure was measured by auscultation and a 12 ml sample of blood was withdrawn (basal sample). Each subject stood for five minutes and pulse, blood pressure and a second blood sample were obtained. This procedure was repeated with each subject after volume expansion and volume depletion and DBH, NE and plasma protein were measured from the same blood samples.

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TABLE 1. Normotensive (NT) and Essential Hypertensive (HT) Subjects

<table>
<thead>
<tr>
<th>Untreated subjects</th>
<th>Norepinephrine (pg/ml)</th>
<th>DBH (units)</th>
<th>Blood pressure (torr)</th>
<th>Pulse rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT (N = 29)</td>
<td>Age</td>
<td>Basal</td>
<td>Stand</td>
<td>Basal</td>
</tr>
<tr>
<td>40</td>
<td>+3</td>
<td>253</td>
<td>458</td>
<td>673</td>
</tr>
<tr>
<td>HT (N = 56)</td>
<td>+2</td>
<td>249</td>
<td>437</td>
<td>588</td>
</tr>
</tbody>
</table>

P (NT vs HT) NS NS NS NS NS <0.01 <0.01 <0.01 <0.01

Blood samples for assay of NE, DBH and plasma protein were cooled in iced 20 ml vacutainer tubes containing 2 ml acid-citrate-dextrose (ACD) anticoagulant. Samples were centrifuged at 4°C and the plasma transferred to polystyrene tubes and frozen at −70°C until assayed within two weeks. We measure NE by the radioenzymatic method of Henry et al., as modified by Lake et al., which converts NE to 3H-methyl-ephedrine by the phenylethanolamine N-methyltransferase catalyzed transfer of a 3H-methyl group from 3H-methyl-S-adenosylmethionine to the primary amine of NE. The assay has a sensitivity (two blank) of about 20 pg/ml of plasma.

We used aliquots of the same sample to measure DBH activity by the radioenzymatic method of Weinsilboum and Axelrod using phenylethylamine as substrate in the presence of 3.3 × 10^-4 M CuSO4 at pH 5.5 with 4 ml of plasma in an incubation volume of 300 ml. The ACD anticoagulant did not interfere with the assay. DBH activity is expressed as units which equal one nM of phenylethanolamine generated from phenylethylamine per ml of plasma per hour of incubation. Fifty µl of the plasma dilution used to measure DBH was assayed for protein content by the method of Lowry et al. Statistical tests used included Student’s two-tailed t-test and the paired two-tailed t-test when appropriate. To evaluate the relationship of NE with age we used the natural logarithm of the NE level (ln NE) to normalize the distribution of NE levels.

Results

Pretreatment Comparison of Hospitalized Normotensive and Essential Hypertensive Subjects (table 1)

Normotensive and hypertensive subjects had the same NE and DBH while supine and standing; hypertensive subjects had a higher pulse rate while supine (P < 0.001) and standing (P < 0.01). Although an effort was made to test normotensive subjects the same age as the patients, the hypertensives were slightly but not significantly older.

Effects of Volume Expansion (table 2)

The administration of 2 L of normal saline over four hours failed to significantly alter basal plasma NE and caused a significant, but small, decrease in plasma NE only in hypertensives after standing (P < 0.02). Volume expansion also diluted plasma proteins, including DBH. Blood pressure was increased in both groups but this effect was more striking after standing. Normotensive subjects increased their basal pulse rate more than hypertensive subjects (P < 0.02).

Effects of Volume Depletion (table 3)

Dietary salt restriction and 120 mg furosemide led to large, significant increases in plasma NE while subjects were recumbent or standing (P < 0.001). DBH levels did not change and plasma protein concentration increased slightly. Volume depletion increased the pulse rate (P < 0.001) and decreased systolic blood pressure of normotensive and hypertensive subjects (P < 0.05), but significantly decreased the diastolic blood pressure of only the hypertensive group (P < 0.001). Volume depletion had the same effects on both groups except that hypertensive patients lowered their blood pressure more.

As seen in table 4 weight changes after each procedure of volume alteration were significant and as expected. Weight change does not significantly correlate with change in plasma levels of NE. The hypertensive subjects weighed more than the controls (P < 0.02), and tended to retain less

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TABLE 2. Effects of Normal Saline Infusion in Normotensive and Essential Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Norepinephrine (pg/ml)</th>
<th>DBH (units)</th>
<th>Plasma protein (g/l)</th>
<th>Pulse rate (beats/min)</th>
<th>Blood pressure (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Stand</td>
<td>Basal</td>
<td>Stand</td>
<td>Basal</td>
</tr>
<tr>
<td>NT (N = 29)</td>
<td>0</td>
<td>127</td>
<td>197*</td>
<td>187*</td>
<td>10.51</td>
</tr>
<tr>
<td>HT (N = 52)</td>
<td>1.19</td>
<td>126</td>
<td>134</td>
<td>30</td>
<td>0.3</td>
</tr>
</tbody>
</table>

P (NT vs HT) NS NS NS NS NS <0.02 NS NS NS

*Post saline different from pre-saline by paired Student’s t-test; P <0.05.
**Post saline different from pre-saline by paired Student’s t-test; P <0.01.

The change in NE, DBH, plasma protein, pulse rate and blood pressure after infusion of 2 L of normal saline over a 4 hour period in normotensive and hypertensive subjects. This is the change from values given in table 1.
fluid after saline and lose more with furosemide than the normotensives (table 4).

In order to evaluate our data by previously published methods we calculated percent change in DBH induced by volume depletion and standing and evaluated young and old hypertensive subjects. The percent increase in supine DBH levels resulting from standing and furosemide is 17 ± 7% in 28 control subjects and 21 ± 7% in 54 hypertensive subjects. The 21 hypertensive patients younger than 44 years increased their DBH after furosemide 22 ± 7% and the 33 hypertensive patients older than 43 increased their DBH by 20 ± 10%. None of these differences in DBH increments between groups were significant. There were no significant positive correlations between increases in NE and increments of DBH after standing or after volume depletion and standing in either hypertensive or normotensive groups. In the normotensives but not hypertensives the increases in NE and pulse rate after volume depletion and standing correlated weakly (r = 0.61).

**Discussion**

We found no difference in plasma NE levels of hypertensive and normotensive subjects. This supports some studies and contradicts others. We found no difference in plasma DBH levels in essential hypertensive and normotensive subjects, supporting some studies and contradicting others. Nor did hypertensive subjects have a different response of their plasma NE or DBH levels to the stresses of postural changes, volume depletion, or volume expansion. Thus we found no evidence that patients with essential hypertension have an abnormal level of peripheral sympathetic nervous discharge during stresses to the sympathetic nervous system. Some authors suspect that hypertensive patients have an exaggerated response of NE output to stress, so both groups were treated identically under strictly controlled conditions. The subjects were not 'blind' to their diagnosis, because they knew some of their blood pressure measurements, so the hypertensives might be more anxious than control subjects. Regardless, blood levels of NE and DBH did not differ between the two groups.

Administration of 2 L of normal saline over four hours led to a significant volume expansion as evidenced by the resultant rise in blood pressure and body weight. Supine subjects did not decrease their level of noradrenergic activity even when volume expansion elevated blood pressure. Since heart rate increased at the same time there does not appear to be an increase in parasympathetic vagal tone so the autonomic nervous system appears unresponsive to volume expansion sufficient to increase blood pressure in both normal and hypertensive subjects. The significant increase in pulse rate in the normotensive subjects after saline is difficult to explain.

When a person stands, sympathetic nerve activity acts to maintain blood pressure. Intravascular volume expansion should decrease the amount of vasoconstriction needed to maintain blood pressure, but changes in the NE levels are minimal before and after volume expansion in standing subjects. Diminished sympathetic activity upon standing can effectively lower the blood pressure, but these subjects maintained normal NE levels and increased their blood pressure. Since 2 L was infused in all patients regardless of height and weight, volume expansion may have been inadequate to suppress sympathetic activity in some subjects.

Volume depletion with dietary salt restriction and furosemide increased NE levels, 50% in recumbent subjects and 70% in standing subjects. Compensatory mechanisms do not maintain blood pressure at prior levels, however, particularly in the hypertensive patients. Salt restriction and diuretic therapy is customary initial treatment for hypertension and antihypertensive drug treatment might be particularly effective after a diuretic if it decreases sympathetic outflow, such as reserpine, clonidine or fensulamide.

The hypertensive subjects retained less fluid after the saline infusion (P < 0.1) and had a greater diuresis after

**Table 3. Effects of Volume Depletion in Normotensive and Essential Hypertensive Subjects on NE and DBH**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive vs Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE (pg/ml)</td>
<td>70.44 ± 2.48</td>
<td>78.66 ± 2.21</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>DBH (units)</td>
<td>1.31 = 0.10</td>
<td>1.02 = 0.13</td>
<td>0.1 &gt; P &gt; 0.05</td>
</tr>
<tr>
<td>Plasma protein (g%)</td>
<td>1.51 = 0.15</td>
<td>1.86 = 0.12</td>
<td>0.1 &gt; P &gt; 0.05</td>
</tr>
</tbody>
</table>

**Table 4. Weight Changes with Volume Alterations**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Change with saline</th>
<th>Change with furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>70.44 ± 2.48</td>
<td>1.31 = 0.10</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>78.66 ± 2.21</td>
<td>1.02 = 0.13</td>
</tr>
<tr>
<td>Normotensives vs Hypertensives</td>
<td>P &lt; 0.02</td>
<td>0.1 &gt; P &gt; 0.05</td>
</tr>
</tbody>
</table>

*Weights expressed in kg, mean ± srm.
Subjects were medication free for 2 wks and weighed before the saline infusion or furosemide and 9 mg salt diet.
Different from ad lib weight, P < 0.001 (paired t-test).
furosemide \( (P < 0.1) \) than did the normotensive subjects (table 4). Although these comparisons are only at borderline significance these data may represent exaggerated natriuresis possibly on the basis of the higher perfusion pressure at the hypertensive kidneys both with volume loading and depletion.

There is a very weak association between the logarithm of plasma NE and age in these control subjects \( (r = 0.14) \) and no correlation between NE and age in these hypertensives. Other studies have noted a relationship between NE and age in normotensive\(^5\), \(^6\) and hypertensive\(^7\) subjects. Since this relationship has not been found to be quite weak, \(^6\), \(^8\) it is not surprising that we did not find a relationship.

There are reports of elevated\(^27\), \(^44\) and normal\(^40\)-\(^43\) levels of DBH in essential hypertension. The hypertensive patients in the present study have normal DBH levels and their DBH responds normally to stress. We found no differences in DBH alterations between groups in response to volume depletion and standing or between young and old hypertensive patients in contrast to another report.\(^27\) DBH levels should, in theory, provide an index of noradrenergic activity.\(^14\), \(^15\) Levels of plasma DBH generally change in the same direction as those of plasma NE, but the changes are relatively small and partially accounted for by changes in total plasma proteins. There were no significant correlations between changes in NE and DBH with volume alterations. Compared with NE levels, DBH activity is an insensitive indicator of sympathetic activity and basal DBH levels are more than twice as variable as basal levels of NE (table 1). Even though plasma DBH activity can be measured easily, it is difficult to estimate levels of sympathetic activity from DBH measurements.

These hypertensive patients have hemodynamic abnormalities. They have a faster heart rate at rest than controls, as others have found.\(^83\), \(^84\) When volume expanded, they have a more rapid diuresis than control subjects which Uryeh et al.\(^85\) have shown to be associated with an increase in cardiac output in hypertensives. These hemodynamic abnormalities may be the result of increased sensitivity of \( \beta \)-adrenergic receptors in hypertensives,\(^86\) we find no evidence that they are due to an abnormal level of sympathetic nerve discharge.

Acknowledgment

We thank Drs. A. A. Taylor, J. L. Pool, D. Rollins and J. R. Mitchell for providing us with plasma samples for NE, DBH and protein determinations and for allowing us to publish blood pressure, pulse rate and weight changes from their investigations of the effect of acute volume changes in normo- and hypertensive patients. The authors also wish to cite Messrs. Frank Guglested, Bill Allen, John George, Gary Birkmaier, and Ms. Carol Basin and Toni Cridlin for their technical assistance.

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Coronary Sinus Reflux

A Source of Error in the Measurement of Thermodilution Coronary Sinus Flow

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JOHN V. TYBERG, M.D., PH.D., JON LEKVEN, M.D.,
BRUCE BRUNDAGE, M.D., AND WILLIAM W. PARMLEY, M.D.

SUMMARY In seven patients thermodilution coronary sinus flow (TD-CSF) was higher (164 ± 21 ml/min) during ventricular pacing than during atrial pacing (119 ± 21 ml/min, P < 0.005) at identical heart rate, without an increase in the determinants of myocardial oxygen consumption. To assess the possibility of right atrial admixture in coronary sinus blood during ventricular pacing we compared electromagnetic coronary arterial blood flow (CBF) with TD-CSF in nine dogs during interventions that increased right atrial pressure. During ventricular pacing, rapid atrial pacing, pulmonary artery constriction and increased intrathoracic pressure, right atrial pressure increased and electromagnetic CBF was significantly less (41–166%) than TD-CSF. Marked reflux from the right atrium to the coronary sinus was also demonstrated by bolus injection of cold saline into the right atrium and continuous infusion of contrast material into coronary sinus. Caution needs to be exercised in interpreting TD-CSF in the presence of changing right atrial pressure.

THE MEASUREMENT OF CORONARY SINUS BLOOD FLOW by the thermodilution technique has been used by many investigators, because it is a safe, simple and inexpensive method which can be applied easily in man. Its main advantage is the rapidity with which changes in flow can be detected.

We applied this technique in patients to evaluate the effect of atrial and ventricular pacing at identical heart rate on myocardial blood flow, assuming that changes in thermodilution coronary sinus flow (TD-CSF) reliably reflect changes in myocardial blood flow. It was the surprising finding of this study that ventricular pacing produced an increase in TD-CSF, although there was a decrease in the determinants of myocardial oxygen consumption. To verify this observation we performed animal experiments, where in addition to TD-CSF we also measured coronary arterial blood flow (CBF) with electromagnetic flow transducers. The measurements were done during various interventions that are expected to cause a change in coronary blood flow, so that CBF and TD-CSF could be compared.
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