Altered Dopaminergic Responses in Hypertension

Barbara A. Clark, Robert M. Rosa, Franklin H. Epstein, James B. Young, and Lewis Landsberg

Biogenic amine metabolism may be altered in hypertension and thus contribute to its pathophysiology. This report describes an abnormality in dopamine excretion in hypertensive subjects in the postabsorptive state that persists despite an increase in dietary precursors for dopamine supplied by a protein meal. We studied seven normotensive and six nonmedicated hypertensive men after two different meals: 60 g protein and a noncaloric electrolyte-equivalent broth. Overall mean sodium excretion was 56% higher in the hypertensive group throughout both meal studies (p<0.01), implying higher chronic dietary sodium intake. Despite this, overall urinary excretion of dopamine tended to be lower in hypertensive than in normotensive subjects (p=0.06). Hypertensive also differed from normotensive subjects in their response to protein feeding. In the normotensive subjects there was a 23% increase in urinary dopamine excretion (p<0.05), which was not seen after the noncaloric meal. In the hypertensive subjects, there was no change in urinary dopamine after the protein meal. In the normotensive subjects there was a 74% increase in sodium excretion (p<0.01) after the protein meal, but no significant change was seen in the hypertensive subjects. There were no differences in baseline renal plasma flow or glomerular filtration rate between the groups and no statistically significant differences between the groups in their renal hemodynamic responses to the meals. In summary, hypertensive subjects have less renal dopamine production for the amount of sodium ingested and a decreased renal dopamine production in response to a protein load as compared with normotensive subjects, consistent with a renal defect in conversion of DOPA to dopamine. (Hypertension 1992;19:589–594)

KEY WORDS • dopamine • kidney • protein • sodium excretion • essential hypertension

Among the physiological actions of dopamine are a decrease in systemic vascular resistance, natriuresis, and suppression of renin and aldosterone secretion.1 The renal actions of dopamine include natriuresis, through direct inhibition of sodium potassium ATPase activity and sodium-hydrogen ion exchange in renal tubules2–4 and enhancement of renal blood flow and glomerular filtration rate.5,6 Furthermore, endogenous dopamine appears physiologically important in the natriuretic response to salt loading.7–9 Through these actions, endogenous dopamine production may play a vital role in the homeostasis of sodium, renal perfusion, and blood pressure. Indeed, Kuchel and colleagues10,11 have demonstrated a number of alterations in the renal dopaminergic system in patients with essential hypertension. Hypertensive subjects, particularly those with "salt sensitive" hypertension, fail to increase urinary dopamine in response to dietary salt loading.12,13 Previous studies from our laboratory have shown that dopamine may be important in the renal response to acute dietary protein loading. Urine dopamine rises in normotensive subjects after a protein meal, presumably due to the increased availability of the precursor amino acid tyrosine.14 Thus, dietary protein loading is a useful method to investigate the pathways of biogenic amine metabolism.

The effect of protein loading in hypertensive subjects has not been previously reported. We hypothesized that abnormalities might exist in dopamine formation when precursor availability is increased after a protein meal in subjects with essential hypertension and that these defects might be associated with alterations in renal sodium handling.

Methods

Six men with mild, untreated essential hypertension (aged 48–67 years) and seven healthy normotensive male volunteers of similar age (44–68 years) were studied. All subjects were Caucasian, had normal renal function, no history of diabetes, were receiving no medications, and were otherwise healthy. Hypertension had been known to be present for 2–5 years duration. None of the subjects had ever been on antihypertensive medication. Mild hypertension was defined by a blood pressure of greater than 140/90 but less than 180/100 mm Hg, measured at least eight times throughout the day and on three separate occasions. The hypertensive subjects were not taking any antihypertensive medications either because the hypertension was relatively mild or they had never been advised by a physician to take medication. All studies were conducted in the
Clinical Research Center of the Beth Israel Hospital. All protocols were approved by the Committee on Clinical Investigations at the Beth Israel Hospital, and informed consent was obtained from all subjects before study.

All subjects continued their usual ad libitum diet before study. Each subject underwent two different meal studies, in random order, separated by an interval of at least 2 weeks. Subjects were admitted to the Clinical Research Center the evening before testing and were studied after an overnight fast. Subjects remained supine throughout the study but were permitted to stand to void to aid in complete bladder emptying. Urine samples were obtained hourly for 9 hours by spontaneous voiding, beginning at 7 AM. The average of three 1-hour urine collections was used to represent a basal period (−3 to 0 hours), the first 3 hours after the meal (0 to 3 hours), and the second 3 hours after the meal (3 to 6 hours). To ensure frequent voiding, all subjects received an oral water load of 300 ml at 6:30 AM. Subsequent urine output was replaced milliliter for milliliter throughout the study. Blood samples were taken at hourly intervals for 9 hours via a retrograde catheter placed in a dorsal hand vein. This hand was kept in a warming box maintained at 70°C to obtain "arterialized" venous blood samples. In addition to the water load, subjects received a priming dose of 50 mg/kg 10% inulin (Iso-tex Diagnostics, Friendswood, Tex.) and 10 mg/kg para-aminohippurate (PAH) (Merck Sharp & Dohme, West Point, Pa.) at 7 AM. Thereafter, inulin and PAH were infused continuously using a constant-speed infusion pump (Flo-gard 6100, Travenol Laboratories, Deerfield, Ill.) at a rate of 1 ml/min in 0.9% saline at a concentration calculated to achieve plasma concentrations of approximately 25 mg/dl and 2.5 mg/dl, respectively. After a 60-minute equilibration period, clearances were measured hourly. At 10 AM the subjects were given the test meal to ingest over 15-25 minutes. The test meal consisted of either 60 g protein (by our own previous analysis).14

Blood samples for norepinephrine and DOPA were drawn into chilled heparinized syringes and placed into prechilled test tubes containing sodium heparin (Vacutainer, Becton Dickinson, Rutherford, N.J.) to which 8 mg glutathione was added. Blood samples were immediately centrifuged at 4°C, and plasma was frozen at −70°C until assayed.

Aliquots of each hourly urine sample were placed into two separate containers for electrolytes, creatinine, serotonin and dopamine, norepinephrine, and epinephrine determinations. The aliquot for dopamine, norepinephrine, and epinephrine was placed into a chilled container containing 6N HCl, and pH was then adjusted to between 2 and 4. Sodium, potassium, and creatinine were assayed immediately after the completion of each study. All other urine samples were frozen immediately at −20°C until assayed.

Inulin was determined by the method of Fjeldbo and Stamey15 and PAH was measured as described by Harvey and Brothers.16 To minimize errors due to variation in bladder emptying, clearances were calculated using the constant infusion method described by Cole et al17:

\[
\text{IR/P} = I / (R + P)
\]

where \(I\) is the concentration of either inulin or PAH in the sustaining solution, \(R\) is the rate of the sustaining infusion in milliliters per minute, and \(P\) is the concentration of inulin or PAH in the plasma.

Serum and urine sodium and potassium were assayed using an ion electrode analyzer (Labyte, Beckman Instruments, Inc., Brea, Calif.). Serum and urine creatinine were determined using a rate-dependent modification of the Jaffe reaction that minimizes the influence of slow reacting, noncreatinine chromogens (SMAC II, Technicon Instruments, Inc., Tarrytown, N.Y.). Serum glucose was determined using glucose oxidase methodology (glucose analyzer II, Beckman). Serum insulin levels were determined using radioimmunoassay kits purchased from ICN Biomedicals, Costa Mesa, Calif.

Urine and plasma catecholamines and serotonin were assayed using high-performance liquid chromatography as previously described.14 Intra-assay coefficients of variation for urine samples are 4–6% for epinephrine, norepinephrine, and dopamine; interassay coefficients of variation are 6–7% for all three catecholamines. Intra-assay coefficients of variation are 4% for plasma DOPA and 2–4% for norepinephrine.

Blood pressure and heart rate were obtained hourly during the study via an automated sphygmomanometer (Dinamap, Critikon Inc., Tampa, Fla.). Mean arterial blood pressure was calculated using the formula:

\[
\text{Mean arterial blood pressure} = (\text{systolic} - \text{diastolic pressure}) / 3 + \text{diastolic pressure}
\]

**Data Analysis**

Statistical analysis was done with repeated-measures analysis of variance with covariance using the absolute values of each of the parameters measured. All results are expressed as mean±SEM. Results were considered statistically significant at a value of \(p<0.05\). Probability values between 0.05 and 0.07 were considered to be of borderline significance.

**Results**

**Baseline Measurements**

The baseline values (Table 1) are derived from the mean of the premeal values in an individual for both meal studies. By definition, mean arterial blood pressure was significantly greater in the hypertensive than the normotensive subjects. The two groups were otherwise well matched for age and body mass index (weight in kg/height in meters squared). Baseline premeal sodium excretion was presumed to reflect chronic dietary sodium intake before the study and was approximately twofold greater in the hypertensive subjects. Despite this, baseline urinary excretion of dopamine tended to be lower in the hypertensive subjects, although this did not reach statistical significance (\(p=0.09\)). Premeal fasting plasma DOPA and norepinephrine were not different between the two groups, nor was urinary excretion of epinephrine or norepinephrine. Urinary excretion of serotonin tended to be higher in the normotensive subjects, although this did not reach...
TABLE 1. Average Baseline Parameters in the Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.0±3.5</td>
<td>61.2±2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2±1.1</td>
<td>26.0±1.1</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>84.1±1.4</td>
<td>107.8±2.2 *</td>
</tr>
<tr>
<td>C_G (ml/min)</td>
<td>120±5</td>
<td>129±6</td>
</tr>
<tr>
<td>C_IN (ml/min)</td>
<td>98±4</td>
<td>99±5</td>
</tr>
<tr>
<td>C_PAH (ml/min)</td>
<td>599±48</td>
<td>617±53</td>
</tr>
<tr>
<td>U_IN (µg/ml)</td>
<td>0.24±0.04</td>
<td>0.28±0.04</td>
</tr>
<tr>
<td>U_NA (µg/ml)</td>
<td>122±16</td>
<td>239±35 *</td>
</tr>
</tbody>
</table>

Plasma

| NE (µg/ml)                         | 291±26       | 298±41       |
| DOPA (pg/ml)                       | 2,393±132    | 2,293±181    |

Urine

| Dopamine (µg/hr)                   | 10.7±0.8     | 8.8±0.4      |
| NE (µg/hr)                         | 1.46±0.58    | 1.34±0.26    |
| Epi (µg/hr)                        | 0.24±0.04    | 0.28±0.04    |
| Serotonin (µg/hr)                  | 4.56±0.39    | 3.75±0.22    |

Groups were similar in age and body mass index (BMI). Premeal sodium excretion was higher in the hypertensive subjects. Baseline dopamine tended to be lower in the hypertensive subjects (p=0.09). All other premeal parameters except for mean arterial blood pressure (MABP) were similar. C_G, creatinine clearance; C_IN, inulin clearance; C_PAH, para-aminohippurate clearance; U_IN, sodium excretion; NE, norepinephrine; Epi, epinephrine. *p<0.05.

There were no differences in the average premeal clearance of creatinine, inulin, or PAH between the normotensive and hypertensive subjects or between these measurements before a protein meal or a noncaloric meal. Basal serum glucose and insulin were also similar.

Response to Meals

Biogenic amines. Urinary dopamine excretion increased by 23±5% in the normotensive subjects after the protein meal but did not change in the hypertensive subjects (p<0.05, Figure 1). There were no significant changes in urinary dopamine in either group after the control meal. However, urinary dopamine remained somewhat higher in the normotensive than the hypertensive subjects throughout the control study (p=0.06). There was also a small but consistent increase in urine serotonin (27±9%) after the protein meal in the normotensive but no significant change in the hypertensive subjects (p<0.05, Figure 2). Plasma DOPA was slightly higher after the protein meal as compared with the control meal only in the normotensive subjects, although this did not reach statistical significance (p=0.09, Figure 3). There were no significant changes in plasma norepinephrine, urine norepinephrine, or epinephrine after any of the meals nor were there group differences in these responses.

Sodium excretion. In normotensive subjects, natriuresis increased significantly above baseline for the entire 6 hours after the protein meal (p<0.05) but did not significantly increase after the noncaloric meal. In contrast, the hypertensive subjects had no consistent increase in sodium excretion (Figure 4) above basal levels after either meal, although the sodium excretion remained higher than in the normotensive subjects throughout the studies (p<0.01).

Glomerular filtration rate and renal plasma flow. Creatinine clearance and PAH clearance were higher after the protein meal as compared with the noncaloric meal in both the hypertensive and control subjects (p<0.05) (Table 2). Inulin clearance tended to increase in the control group after the protein meal, but this increase did not reach the level of statistical significance (p=0.10). Although the changes in all parameters after a protein meal seemed more apparent in normotensive than in hypertensive subjects, these differences were not statistically significant.

Glucose and insulin. Serum glucose did not change after the noncaloric or protein meal in either group. Serum insulin rose to a similar degree in response to the protein meal (99% versus 73%, normotensive versus hypertensive) in both groups. There were no significant differences in insulin or glucose responses between groups.

Blood pressure. Mean arterial blood pressure remained elevated throughout the studies in the hypertensive subjects. There was no significant meal effect on blood pressure or heart rate in either group.

Discussion

Dopamine may be an important regulator of renal function as well as systemic blood pressure. This study...
FIGURE 2. Bar graphs show urinary serotonin responses. Urinary serotonin increased after the protein meal in the normotensive subjects (p<0.05). No significant changes were seen in either group after the noncaloric meal. *Significant changes compared with baseline (p<0.05).

provides evidence in subjects with essential hypertension of a defect in urinary dopamine excretion despite increased precursor availability provided by a protein load.

In these experiments basal and overall sodium excretion were nearly twofold greater in hypertensive than in normotensive subjects, implying higher chronic dietary sodium intake in subjects with hypertension. Nevertheless, overall renal dopamine excretion throughout both meal studies tended to be lower in the hypertensive subjects, indicating a dissociation between dopamine and sodium. This observation is consistent with the findings of others in hypertensive subjects of a failure to increase urine dopamine in response to dietary sodium loading.12,13

Since renal dopamine production is believed to be limited by the availability of dopamine precursors,1 the aim of the present study was to determine if defects in dopamine excretion are still apparent in subjects with essential hypertension when precursor availability is increased. Dopamine is formed from tyrosine via the intermediate compound, DOPA. A protein meal provides an acute amino acid load, and protein feeding increases urine dopamine in normotensive subjects.14 After a protein meal, tyrosine is converted to DOPA via tyrosine hydroxylase predominantly in peripheral tissues that contain catecholamine-synthesizing cells such as sympathetic nerve terminals and chromaffin-containing tissues.18 Urinary dopamine is then produced by the action of L-amino acid decarboxylase on DOPA. In prior studies a rise in plasma DOPA after protein ingestion was only detectable when metabolism of DOPA was blocked by carbidopa (a competitive inhibitor of DOPA decarboxylase). It was concluded that the DOPA formed in response to protein ingestion is rapidly removed from plasma and converted to dopamine. Because of this short half-life of DOPA in the circulation, the rise in venous plasma DOPA concentration was only demonstrable in the presence of decarboxylase inhibition. In the present study, plasma DOPA rose slightly but not significantly after the protein meal in normotensive subjects, but did not rise at all in hypertensive subjects. Because of the short circulatory half-life of DOPA, we cannot rule out the possibility of a defect in the formation of DOPA from tyrosine in the hypertensive subjects. Further study of hypertensive and normotensive subjects after protein ingestion in the presence of carbidopa might help clarify this issue.

An additional possible explanation for the lower urinary dopamine response in hypertensive subjects concerns the DOPA decarboxylase step, responsible for the transformation of DOPA to dopamine. The most obvious defect in the hypertensive subjects was a deficiency in renal dopamine formation. Circulating DOPA is rapidly taken up by tissues and converted to dopamine by DOPA decarboxylase.19,20 In the kidney this conversion occurs predominantly in the renal proximal tubule cells, and urinary dopamine excretion reflects renal production.21-23 Since DOPA decarboxylase is widely distributed throughout the body,18 defects in renal production of dopamine may reflect biogenic amine metabolism in other tissues as well.

In the present studies protein ingestion was associated with a natriuresis in the normotensive subjects,
associated with the increase in urinary dopamine. The natriuretic response to salt or protein loading can be prevented by blockade of dopamine receptors or synthesis (with carbidopa), suggesting that in normotensive subjects, dopaminergic responses may indeed be important in the natriuresis that occurs after a protein meal. The hypertensive subjects in our study showed minimal change in dopamine or sodium excretion after the protein meal. Although differences in sodium excretory responses to protein loading between normotensive and hypertensive subjects may be difficult to interpret because of the different basal sodium excretion, it does appear that urinary dopamine and sodium excretion are dissociated in the hypertensive subjects. Endogenous dopamine regulates sodium excretion in the normotensive rat but does not appear to play an important role in regulating sodium excretion in the hypertensive rat. The present studies suggest that this may also be true in humans with essential hypertension.

The normotensive subjects also demonstrated an increase in urinary serotonin excretion in response to the protein meal. Serotonin, like dopamine, is formed in proximal renal tubular cells by the action of DOPA decarboxylase on the precursor substrate, in this case the amino acid tryptophan. Therefore, formation of serotonin in response to an acute protein load is likely to parallel that of dopamine. Although serotonin has been reported to have antinatriuretic actions, a protein meal generally produces an increase in sodium excretion, presumably due to overriding effects of natriuretic substances such as dopamine. The hypertensive subjects failed to increase urine serotonin as well as dopamine after the protein meal. This provides further evidence for defective precursor uptake or defective DOPA decarboxylase activity in these hypertensive subjects.

In normotensive humans, protein meals have been reported to increase renal plasma flow and glomerular filtration rate. The magnitude of the change in renal plasma flow and glomerular filtration rate in response to protein loading in normotensive subjects may vary considerably and is dependent on the amount of protein ingested. A prior study by Hostetter with a similar protein load produced an increase in inulin clearance varying from 0% to 48%, with an average increase of 28%. The present studies were associated with an increase of approximately 10% in renal plasma flow as assessed by PAH clearance. Inulin clearance rose slightly after the protein meal (predominantly in the normotensive subjects), although this did not reach the level of statistical significance (p=0.10). In contrast, creatinine clearance increased substantially by approximately 20%. This dis-

### Table 2. Renal Hemodynamic Responses in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 0 to 3 hours 3 to 6 hours</td>
<td>Baseline 0 to 3 hours 3 to 6 hours</td>
</tr>
<tr>
<td>C_{P} (ml/min)</td>
<td>NTN 130±7 127±3 118±5 117±4 141±8* 137±6*</td>
<td>HTN 134±10 123±6 122±5 128±5 136±6 140±7*</td>
</tr>
<tr>
<td></td>
<td>HTN 101±5 102±5 98±5 97±6 99±10 97±11</td>
<td>HTN 101±5 102±5 98±5 97±6 99±10 97±11</td>
</tr>
<tr>
<td>C_{PAH} (ml/min)</td>
<td>NTN 583±37 633±64 656±83 615±50 682±59* 691±62*</td>
<td>HTN 609±63 591±46 569±37 624±50 684±59* 657±54*</td>
</tr>
</tbody>
</table>

There were no significant changes in creatinine clearance (C_{C}), inulin clearance (C_{P}), or p-aminohippurate clearance (C_{PAH}) after the control meal. C_{P} and C_{PAH} rose slightly after the protein meal, but there were no significant group differences. Changes in C_{P} after the protein meal did not reach statistical significance. NTN, normotensive subjects; HTN, hypertensive subjects.

*p<0.05.
crepancy can probably be attributed to the exogenous creatinine load of the ingested animal protein with a subsequent increase in tubular secretion of creatinine. These studies confirm the observation that creatinine clearance may overestimate the change in glomerular filtration rate after a protein meal. They also suggest that dopamine is not solely responsible for the changes in renal plasma flow that occur after a protein meal, since both normotensive and hypertensive subjects demonstrated an increase in renal plasma flow despite differences in the dopamine responses.

In summary, the present studies provide further evidence for an alteration in the renal dopaminergic system in subjects with essential hypertension. Dopamine excretion is lower than in normotensive subjects despite higher chronic sodium intake, and urinary dopamine fails to increase in response to an acute protein load. Possible reasons for these differences that remain to be elucidated include abnormalities in the processing of tyrosine to DOPA, in the uptake of DOPA by the Na-K-ATPase, and might conceivably reflect an abnormality in catecholamine metabolism in other tissues.

Acknowledgments

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