Imbalance of Renal Production Between 5-Hydroxytryptamine and Dopamine in Patients With Essential Hypertension Complicated by Microalbuminuria

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BACKGROUND
In the kidney, 5-hydroxytryptamine (5-HT) and dopamine (DA) are formed by the same enzyme, l-aromatic amino acid decarboxylase, but act on renal function and glomerular structure in an opposite direction. The present study was designed to explore whether rates of renal production of 5-HT relative to that of DA are altered in patients with essential hypertension and microalbuminuria.

METHoDS
We measured urinary levels of 5-HT and DA, reflecting renal production of 5-HT and DA as well as 24-hour ambulatory blood pressure and urinary albumin excretion in 82 consecutive untreated, essential hypertensives without overt proteinuria.

RESULTS
Urinary 5-HT excretion and the ratio of urinary 5-HT to DA were significantly higher in 22 patients with microalbuminuria than in the remaining patients with normoalbuminuria, although urinary DA levels did not differ between the groups. The 24-hour systolic and diastolic blood pressures were also higher in the microalbuminuric group than in the normoalbuminuric group. Multiple regression analysis revealed that urinary 5-HT excretion and 24-hour systolic blood pressure were independently associated with urinary albumin excretion. Furthermore, urinary 5-HT excretion was positively correlated with creatinine clearance as well as blood pressure but tended to be negatively correlated with fractional excretion of sodium.

CONClusions
Renal production of 5-HT is enhanced compared with that of DA in essential hypertensives with microalbuminuria. This imbalance may contribute to the genesis of hypertensive glomerular damage.

Keywords: blood pressure; dopamine; essential hypertension; 5-hydroxytryptamine; microalbuminuria.

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5-Hydroxytryptamine (5-HT) (serotonin) constricts renal vessels via activation of the 5-HT1 receptor in vascular smooth muscle cells and causes antinatriuresis via stimulation of the Na+-Pi cotransporter and Na+-K+-ATPase in tubular cells.1–4 Dopamine (DA) has the opposite effects on renal function, i.e., dilation of renal vessels and natriuresis.5–10 In addition, recent experimental studies have demonstrated that 5-HT promotes cell proliferation and synthesis of type IV collagen in mesangial cells,11–13 but DA inhibits mesangial cell proliferation.14 Thus, 5-HT and DA exert opposing effects on renal hemodynamics, tubular function, and glomerular structure.

Both 5-HT and DA are synthesized via decarboxylation of their precursors, 5-hydroxytryptophan and l-3,4-dihydroxyphenylalanine (l-DOPA), respectively, in the epithelial cells of proximal convoluted tubules of kidneys.8–10,15–20 5-hydroxytryptophan and l-DOPA are taken up with the same transporter of the proximal tubular cells21 and are decarboxylated by the same enzyme, l-aromatic amino acid decarboxylase (l-AADC) to produce 5-HT and DA. Therefore, 5-HT and DA could inhibit the production of either amine competitively at the uptake or decarboxylation process of their precursors in the tubular cells. Actually, both an in vitro experiment using isolated rat proximal tubules and an in vivo study in rats have shown that the administration of l-DOPA increases DA production and concomitantly decreases 5-HT production, leading to an imbalance of renal production between these 2 amines.9,10 Moreover, the alterations in the balance of renal production between 5-HT and DA could affect renal function and glomerular structure, thereby possibly leading to the occurrence of hypertension and renal damage.1,2,10,19,22

Our previous study demonstrated that renal production of 5-HT and renal vasoconstriction induced by 5-HT were greater in essential hypertensives than in normotensive controls.3 Therefore, in essential hypertensives, enhanced renal
production of 5-HT, greater renovascular responses to 5-HT or both could affect renal sodium excretion and renal structures, and lead to elevated systemic blood pressure and renal damage. By contrast, an experiment using isolated, perfused kidneys demonstrates that renal formation of DA as well as that of 5-HT is enhanced in the kidneys of spontaneously hypertensive rats. However, renal production of DA in relationship to 5-HT and clinical implications of the balance between 5-HT and DA have not been explored thoroughly in human hypertension.

In the present study, therefore, the balance of renal production between 5-HT and DA was compared between essential hypertensives with or without microalbuminuria, a surrogate marker for glomerular damage. Attention was also paid to the associations of these 2 amines with systemic and renal hemodynamics as well as urinary albumin excretion in patients with essential hypertension.

METHODS

Subjects

The study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association and after approval by the Ethics Committee at the University of Toyama. Eighty-two consecutive, untreated outpatients (45 men; 54 ± 11 years old) with essential hypertension were enrolled into the study after giving informed consent. Hypertension was diagnosed when the patient had a sitting diastolic blood pressure of >90 mm Hg and/or systolic blood pressure >140 mm Hg on 3 separate occasions during the 4-week period before the study began; blood pressures were measured with a sphygmomanometer.

Study protocol

Venous blood samples were obtained in the morning. Patients were instructed not to consume food, alcohol, or caffeine or smoke cigarettes within 12 hours before blood sampling. After patients rested for 30 minutes in a supine position, blood was collected via a venous catheter for measurements of biochemical indices, including plasma renin activity and plasma concentrations of aldosterone, noradrenaline, adrenaline, 5-HT, and DA. Thereafter, 24-hour ambulatory blood pressure monitoring was performed using a TM-2421 ambulatory blood pressure monitor and TM-2021 data recorder (A&D Co, Tokyo, Japan). In accordance with the method of Okubo et al., blood pressure was recorded every 30 minutes, and mean values were calculated for the entire 24 hours. Urine was collected during 24-hour blood pressure monitoring for measurements of the biochemical markers albumin, 5-HT, and DA.

Biochemical markers were measured by using conventional laboratory techniques: urinary albumin concentrations with immuno turbidimetry, plasma renin activity and plasma aldosterone concentration with radioimmunoassay techniques, and plasma levels of noradrenaline, adrenaline, 5-HT, and DA and urinary levels of 5-HT and DA with high-performance liquid chromatography and electrochemical detection.

Data analyses

Urinary excretion of 5-HT and DA was used to reflect production of these 2 amines within the kidney. Creatinine clearance was corrected for 1.73 m² body surface area. Fractional excretion of sodium or potassium was calculated as the percentage of the respective filtered electrolyte appearing in urine. Urinary albumin excretion was natural log-transformed to account for the skewed distribution of this variable.

Based on urinary albumin excretion, subjects were divided into 2 groups, those with microalbuminuria (n = 22; albuminuria 30–300 mg/d) and normoalbuminuria (n = 60; albuminuria < 30 mg/d). Variables were compared between the groups using the 2-tailed t test or the χ² test. Correlations between urinary 5-HT excretion, urinary DA excretion, or the ratio of urinary 5-HT to DA and urinary albumin excretion, systemic hemodynamics, or renal functions were determined using Pearson's correlation. The regression lines of these relationships were assessed with the ordinary least-squares regression method. Because the relationships had the experimental errors in both independent and dependent variables, the slopes of the regression lines were corrected by dividing with repression dilution, that is, 1 + (σ²/x²). Multiple regression analysis was performed to identify factors associated independently with urinary albumin excretion, using variables with a significant Pearson's correlation coefficient as explanatory variables. Data are presented as means ± SDs. Differences were considered statistically significant at P < 0.05.

RESULTS

The mean systolic/diastolic blood pressure and pulse rate at the outpatient clinic were 166 ± 19/102 ± 12 mm Hg and 72 ± 11 beats/min, respectively. None of the patients exhibited overt proteinuria (i.e., urinary protein > 0.3 g/d), decreased renal function (i.e., creatinine clearance <60 ml/min/1.73 m²), secondary hypertension, diabetes mellitus, or clinically evident cardiovascular diseases. Furthermore, there were no patients with microhematuria, in which 5-HT released from blood cells could easily increase urinary 5-HT excretion.

There were no significant differences in age, sex, and anthropometric variables between the normoalbuminuric and microalbuminuric groups (Table 1). The blood pressure and pulse rate at the outpatient clinic did not differ between the groups (Table 1). In contrast, 24-hour systolic and diastolic blood pressures were significantly higher in the microalbuminuric group than in the normoalbuminuric group, although the 24-hour pulse rate did not differ between the groups.

Creatinine clearance and fractional excretions of electrolytes did not differ between the groups (Table 2). Metabolic indices also did not differ between the groups, except for blood glucose levels. Plasma renin activity, plasma aldosterone concentration and plasma levels of epinephrine, norepinephrine, 5-HT, and DA were similar in the 2 groups. However, urinary 5-HT excretion and the ratio of urinary 5-HT to DA were significantly greater in the microalbuminuric group than in the normoalbuminuric group, although urinary DA excretion did not differ between the groups. This
indicates that the renal production of 5-HT was enhanced in the microalbuminuric group.

In the whole group, urinary albumin excretion was positively correlated with both urinary 5-HT excretion ($r = 0.42; P = 0.001$) and the ratio of urinary 5-HT to DA ($r = 0.26; P = 0.011$) (Figure 1). In contrast, no such relation was observed between the urinary excretion of DA and albumin. Urinary albumin excretion was also associated with 24-hour systolic ($r = 0.36; P = 0.001$) and diastolic ($r = 0.31; P = 0.005$) blood pressure, body mass index ($r = 0.25; P = 0.033$), high-density lipoprotein cholesterol ($r = -0.26; P = 0.021$) and plasma aldosterone concentration ($r = 0.26; P = 0.024$). Multiple regression analysis revealed that urinary 5-HT excretion and 24-hour systolic blood pressure were independently associated with urinary albumin excretion ($R^2 = 0.255; P = 0.001$). Urinary 5-HT excretion was

Table 1. Clinical Features in Normoalbuminuric and Microalbuminuric Patient Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Normoalbuminuric group (n = 60)</th>
<th>Microalbuminuric group (n = 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>...</td>
<td>54 ± 11</td>
<td>54 ± 11</td>
<td>0.900</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>...</td>
<td>31/29</td>
<td>14/8</td>
<td>0.334</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>...</td>
<td>162 ± 9</td>
<td>163 ± 8</td>
<td>0.507</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>...</td>
<td>64 ± 12</td>
<td>69 ± 12</td>
<td>0.088</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20–24</td>
<td>24.2 ± 3.5</td>
<td>25.8 ± 3.6</td>
<td>0.075</td>
</tr>
<tr>
<td>Clinic systolic blood pressure (mm Hg)</td>
<td>&lt;130</td>
<td>166 ± 19</td>
<td>168 ± 21</td>
<td>0.665</td>
</tr>
<tr>
<td>Clinic diastolic blood pressure (mm Hg)</td>
<td>&lt;85</td>
<td>102 ± 12</td>
<td>103 ± 14</td>
<td>0.766</td>
</tr>
<tr>
<td>Clinic pulse rate (beats/min)</td>
<td>50–90</td>
<td>72 ± 12</td>
<td>72 ± 9</td>
<td>0.834</td>
</tr>
<tr>
<td>24-hour systolic blood pressure (mm Hg)</td>
<td>&lt;120</td>
<td>143 ± 13</td>
<td>154 ± 14</td>
<td>0.001</td>
</tr>
<tr>
<td>24-hour diastolic blood pressure (mm Hg)</td>
<td>&lt;75</td>
<td>88 ± 8</td>
<td>96 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td>24-hour pulse rate (beats/min)</td>
<td>50–90</td>
<td>71 ± 8</td>
<td>75 ± 9</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Values are means ± SDs.

Table 2. Urinary Albumin Excretion, Renal Function, Biochemical Indices, 5-Hydroxytryptamine (5-HT), and Dopamine (DA) in Normoalbuminuric and Microalbuminuric Patient Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Normoalbuminuric group (n = 60)</th>
<th>Microalbuminuric group (n = 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary albumin excretion (mg/d)</td>
<td>&lt;30</td>
<td>12 ± 8</td>
<td>67 ± 69</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²)</td>
<td>70–130</td>
<td>125 ± 33</td>
<td>136 ± 34</td>
<td>0.178</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>1–2</td>
<td>1.0 ± 1.4</td>
<td>0.8 ± 0.4</td>
<td>0.665</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>8–18</td>
<td>8.4 ± 2.9</td>
<td>7.5 ± 2.9</td>
<td>0.208</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>70–110</td>
<td>90 ± 17</td>
<td>100 ± 18</td>
<td>0.021</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>150–219</td>
<td>192 ± 34</td>
<td>196 ± 33</td>
<td>0.610</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>50–149</td>
<td>109 ± 69</td>
<td>126 ± 88</td>
<td>0.364</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>40–90</td>
<td>55 ± 14</td>
<td>49 ± 12</td>
<td>0.054</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>0.4–1.8</td>
<td>0.73 ± 0.73</td>
<td>1.05 ± 0.81</td>
<td>0.094</td>
</tr>
<tr>
<td>Plasma aldosterone concentration (ng/dl)</td>
<td>3.0–10.0</td>
<td>6.9 ± 3.9</td>
<td>6.7 ± 3.2</td>
<td>0.924</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>30–100</td>
<td>31 ± 25</td>
<td>24 ± 14</td>
<td>0.227</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>100–450</td>
<td>226 ± 93</td>
<td>229 ± 61</td>
<td>0.866</td>
</tr>
<tr>
<td>Plasma 5-HT (ng/ml)</td>
<td>1.25 ± 0.46</td>
<td>1.50 ± 0.70</td>
<td></td>
<td>0.545</td>
</tr>
<tr>
<td>Plasma DA (ng/ml)</td>
<td>8.88 ± 3.76</td>
<td>8.00 ± 4.24</td>
<td></td>
<td>0.780</td>
</tr>
<tr>
<td>Urinary 5-HT excretion (µg/d)</td>
<td>61 ± 31</td>
<td>77 ± 35</td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td>Urinary DA excretion (µg/d)</td>
<td>1073 ± 856</td>
<td>1076 ± 1042</td>
<td></td>
<td>0.990</td>
</tr>
<tr>
<td>Ratio of urinary 5-HT to DA</td>
<td>0.072 ± 0.042</td>
<td>0.097 ± 0.042</td>
<td></td>
<td>0.024</td>
</tr>
</tbody>
</table>

Values are means ± SDs.

Abbreviations: FENa, fractional excretion of sodium; FENa, fractional excretion of potassium; HDL, high-density lipoprotein.
positively correlated with 24-hour systolic blood pressure ($r = 0.24; P = 0.039$) and tended to be positively correlated with 24-hour diastolic blood pressure ($r = 0.22; P = 0.054$) (Figure 2). However, there was no relationship between urinary 5-HT excretion and 24-hour pulse rate. Urinary 5-HT excretion was also positively correlated with creatinine clearance ($r = 0.44; P = 0.001$) but tended to be negatively correlated with fractional excretion of sodium ($r = -0.22; P = 0.054$) (Figure 3). In contrast, there was no relationship between urinary 5-HT excretion and fractional excretion of potassium. Neither urinary excretion of 5-HT nor urinary excretion of DA was correlated with plasma renin activity or plasma aldosterone or catecholamine levels.

**DISCUSSION**

**Major findings of the present study**

The major findings of the present study are as follows. First, plasma levels of 5-HT and DA were similar between essential hypertensives with normoalbuminuria and those with microalbuminuria. In contrast, urinary 5-HT excretion and the ratio of urinary 5-HT to DA were greater in the microalbuminuric group than in the normoalbuminuric group, although urinary DA excretion did not differ between the 2 groups. Thus, our data supported the hypothesis that renal 5-HT production was augmented in essential hypertensives with microalbuminuria. Second, in the whole group, not only systolic blood pressure but also urinary 5-HT excretion was independently associated with urinary albumin excretion. Urinary 5-HT excretion was also positively correlated with glomerular filtration rate as well as blood pressure but tended to be negatively correlated with fractional sodium excretion.

**Implications of imbalances in renal production between 5-HT and DA in hypertension**

Both 5-HT and DA are generated from their respective precursors by the same enzyme, L-AADC, mainly in the proximal tubular cells of the kidney. $^{8,10,13,18}$ Thereafter, 5-HT and DA are secreted as autocrine or paracrine hormones into the tubular fluid or peritubular interstitial space. $^{7,10}$ The experiments using renal microdialysis techniques demonstrated that the levels of 5-HT and DA in basal renal interstitial fluid were 5.22 and 2.17 µg/ml, respectively. $^{7}$ Thus, the range of 5-HT and DA concentration in renal interstitial fluid could be much higher than that in circulating blood (i.e., in the range of nanograms per milliliter). Accordingly, renal production of 5-HT and DA seems to be independent of the extrarenal production of these 2 amines. After the secretion from proximal tubular cells, 5-HT and DA act as reciprocal regulators of mesangial cell proliferation as well as renal hemodynamics and tubular function. $^{8,14,18,20}$ That is, 5-HT induces renovasoconstriction, antinatriuresis, antidiuresis, and mesangial proliferation and fibrosis in the kidney, whereas DA has the opposite effects on renal function and glomerular morphology. Therefore, it seems likely that the inappropriate balance of amounts or actions between 5-HT and DA within the kidneys affects...
Figure 2. Correlations of urinary 5-hydroxytryptamine (5-HT) excretion with 24-hour systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate (PR). Urinary 5-HT excretion was correlated positively with 24-hour systolic blood pressure and tended to be correlated positively with 24-hour diastolic blood pressure. Abbreviation: NS, not significant.

Figure 3. Correlations of urinary 5-hydroxytryptamine (5-HT) excretion with creatinine clearance (CcG), fractional excretion of sodium (FE_{Na}), and fractional excretion of potassium (FE_{K}). Urinary 5-HT excretion was correlated positively with creatinine clearance but tended to be correlated negatively with fractional excretion of sodium. Abbreviation: NS, not significant.
renal excretory capability, glomerular cell proliferation and fibrosis, possibly leading to the development of hypertension and renal damage.\textsuperscript{1,2,3,16,19} Furthermore, proximal tubular impairment secondary to the occurrence of renal damage might aggravate the imbalance of renal formation or secretion between 5-HT and DA.

The present study demonstrated that in essential hypertensives, urinary excretion of 5-HT, but not DA, could be associated positively with albuminuria, and also with both glomerular filtration rate and systemic blood pressure. The positive association of urinary 5-HT excretion with glomerular filtration rate seems consistent with our previous observation that efferent arterioles were more sensitive to the vasoconstrictive stimulus of 5-HT within the kidney in essential hypertensives.\textsuperscript{4} Accordingly, enhancement of intra-renal production of 5-HT could contribute to the occurrence of microalbuminuria in essential hypertensives via 2 distinct mechanisms: direct actions of 5-HT, including cell proliferation and production of type IV collagen in mesangial cells, and 5-HT-induced alterations in intrarenal hemodynamics. Furthermore, the alterations in the balance of renal production between 5-HT and DA could contribute to the genesis of microalbuminuria because of less inhibitory actions of DA on 5-HT. Alternatively, up- or down-regulation of receptors for 5-HT and DA\textsuperscript{2,5} could affect renal function and albuminuria in essential hypertensives. Thus, enhanced renal production of 5-HT might be associated with microalbuminuria, a marker or risk factor for future occurrence of cardiovascular diseases and renal failure.\textsuperscript{26–28}

**Possible mechanisms of imbalance in renal production between 5-HT and DA in hypertension**

The renal production of 5-HT and DA comprises uptake of their respective precursors, decarboxylation of precursors by l-AADC, and secretion or degradation of the 2 amines, mainly in the proximal tubular cells.\textsuperscript{7–10,15–18} Therefore, the difference of delivery of precursors to l-AADC between 5-HT and DA might lead to the imbalance in renal production between these 2 amines. This notion is supported by an in vivo experiment demonstrating that increased delivery of l-DOPA (i.e., precursor for DA) increased renal DA excretion and concomitantly decreased renal 5-HT excretion in normal rats.\textsuperscript{10} Because 5-HT and DA are secreted into the interstitial space other than the renal tubular lumen,\textsuperscript{7} the degree of secretion of these amines into the tubular lumen could affect urinary content of 5-HT and DA. It is also possible that microalbuminuria induced proximal tubular damage\textsuperscript{29} possibly leading to the impairment in renal capacity for synthesis or secretion of 5-HT and DA. Intratubular albumin might bind to 5-HT, DA, or both and protect these amines from their degradation. Further studies are needed to explore the precise mechanisms of the imbalance of renal production between 5-HT and DA in essential hypertensives with microalbuminuria.

The renal production of 5-HT and DA may also be influenced physiologically by renal sympathetic nerves or the renin-angiotensin system; sympathetic nerves innervate the renal tubules abundantly, and angiotensin II acts on the tubular cells.\textsuperscript{30–32} In the present study, neither urinary excretion of 5-HT nor urinary excretion of DA was correlated with plasma renin activity, plasma aldosterone concentration, or plasma catecholamine levels. Therefore, it seems unlikely that systemic activity of the renin-angiotensin-aldosterone system or the sympathetic nervous system affected renal production of 5-HT or DA, but it remains possible that regional activity in these kidney systems may play a role.

**Study limitations**

The present study has several limitations. First, it was a cross-sectional study, and longitudinal studies will be required to explore whether an imbalance of production between 5-HT and DA is a cause or effect of systemic pressure elevation or glomerular damage in patients with essential hypertension. Second, interventional studies using 5-HT receptor antagonists or DA agonists are useful to determine the pathophysiologic significance of this imbalance on albuminuria, as has been reported in diabetic nephropathy.\textsuperscript{33–35} Finally, the present study did not include normotensive subjects with or without microalbuminuria. These 2 groups should have been included to allow a definitive conclusion to be drawn concerning the imbalance of renal formation between 5-HT and DA in essential hypertensives with microalbuminuria. Despite its limitations, the present study showed that the imbalance of renal production between 5-HT and DA may contribute to the genesis of hypertensive glomerular damage in essential hypertensives.

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**DISCLOSURE**

The authors declared no conflict of interest.

**REFERENCES**


