Effect of inhibition of MAO and COMT on intrarenal dopamine and serotonin and on renal function

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Wang, Yongqing, Theresa J. Berndt, Jennifer M. Gross, Michael A. Peterson, Mathew J. So, and Franklyn G. Knox. Effect of inhibition of MAO and COMT on intrarenal dopamine and serotonin and on renal function. Am J Physiol Regulatory Integrative Comp Physiol 280: R248–R254, 2001.—The purpose of the present investigation was to study the effects of inhibition of monoamine oxidase (MAO) and/or catechol-O-methyltransferase (COMT), enzymes involved in the degradation of dopamine (DA) and serotonin (5-HT), on intrarenal DA and 5-HT, as reflected in the renal interstitial fluid (RIF) microdialysate and urine, and on renal function. Inhibition of MAO selectively increased RIF 5-HT from 3.16 ± 0.38 to 8.03 ± 1.83 pg/min (n = 7, P < 0.05), concomitant with decreases in mean arterial blood pressure and glomerular filtration rate (2.09 ± 0.18 to 1.57 ± 0.22 mL/min, n = 7, P < 0.05). Inhibition of COMT significantly increased RIF DA (3.47 ± 0.70 to 8.68 ± 1.96 pg/min, n = 9, P < 0.05), urinary DA (2.00 ± 0.16 to 2.76 ± 0.26 ng/min, n = 9, P < 0.05), and absolute excretion of sodium (6.42 ± 2.00 to 9.82 ± 1.62 μmol/min, n = 10, P < 0.05). Combined inhibition of MAO and COMT significantly increased RIF DA, urinary DA, and urinary 5-HT, which was accompanied with increases in urine flow rate, and absolute (3.03 ± 0.59 to 8.40 ± 1.61 μmol/min, n = 9, P < 0.01) and fractional excretion of sodium. We conclude that inhibition of MAO selectively increases RIF 5-HT. COMT appears to be more important than MAO in the metabolism of intrarenal DA. Physiological increases in intrarenal DA/5-HT induced by inhibition of their degrading enzymes are accompanied with significant alterations of renal function.

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DA and 5-HT in the urine can be sampled by clearance study, DA and 5-HT in the RIF can be analyzed by the kidney microdialysis technique.

The present investigations were undertaken to study the effects of inhibition of MAO and/or COMT on DA and 5-HT in the RIF and urine and their effects on renal function.

METHODS

Animals

This study was performed on male rats weighing 260–450 g purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were fed normal rat chow and had free access to water.

Microdialysis and Clearance Studies

On the day of the experiments, rats were anesthetized with an intraperitoneal injection of 100 mg/kg body wt of 5-sec-butyl-ethyl-3-thioobarbituric acid (Inactin; Byk-Gulden Konstanz, Hamburg, Germany). The rats were placed on a heated table to maintain body temperature between 36 and 38°C. After the tracheostomy, one PE-50 catheter was placed in the left carotid artery to monitor mean arterial blood pressure (MAP) and to collect blood samples. Another catheter was placed in the left jugular vein for intravenous infusion of 2% inulin in 0.9% NaCl at a rate that provided 2 ml/100 g body wt volume expansion per hour and for drug administration. A PE-90 catheter was placed in the bladder for urine collection.

The left kidney was exposed, and a 5-mm microdialysis probe (30 kDa; Bioanalytical Systems, West Lafayette, IN) was placed in the renal cortex and infused with Ringer solution at a rate of 1 μl/min.

Five protocols were used.

Group 1: Time control (n = 6). After a 1.5-h recovery period, one 75-min urine clearance and microdialysate sample was taken. Two percent sodium metabisulfite (10 μl) and 4% cysteine (10 μl) were added to the microdialysate, and 33% acetic acid (0.2 ml) was added to the urine collection to prevent catecholamine degradation. Microdialysate and urine samples were immediately stored at -20°C after the collection for subsequent analysis. After a 30-min stabilization period, a second 75-min urine clearance and microdialysate sample was taken. A blood sample was taken at the midpoint during each clearance period.

Group 2: Combined inhibition of MAO-A and MAO-B (n = 7). This protocol is identical to that of group 1 except that a combination of MAO-A (Clorgyline, 5 mg/kg; Research Biochemicals International, Natick, MA; see Refs. 24 and 25) and MAO-B (Pargyline, 20 mg/kg; Research Biochemicals International; see Ref. 33) inhibitors was administered intravenously after the first clearance. Clorgyline and pargyline were dissolved in 0.5 ml of isotonic saline. After a 30-min equilibration period, a second clearance was taken.

Group 3: PBS control (n = 7). This protocol is identical to that of group 2 except that 3 ml PBS, which is used as the vehicle for the COMT inhibitor, were administered intravenously after the first clearance.

Group 4: Inhibition of COMT (n = 10). This protocol is identical to that of group 2 except that after the first clearance the inhibitor of COMT (15 mg/kg 3,5-dinitroctethol; Research Biochemicals International; see Ref. 8) dissolved in 3 ml of PBS was administered intravenously. After a 30-min equilibration period, a second clearance was taken.

Group 5: Combined inhibition of MAO-A, MAO-B, and COMT (n = 9). This protocol is identical to that of group 2 except that the inhibitors of MAO-A (5 mg/kg Clorgyline), MAO-B (20 mg/kg Pargyline), and COMT (15 mg/kg 3,5-dinitroctethol) were administered intravenously.

Analysis

The plasma and urine samples were analyzed to determine sodium, potassium, inulin, and phosphate concentrations. Sodium and potassium concentrations in plasma and urine were measured by flame photometry (Instrumentation Laboratory, Wilmington, MA). Phosphate concentrations were measured by the Chen et al. (4) method, and inulin concentrations were determined by the anthrone method (11).

DA and 5-HT in RIF and urine were measured by HPLC (free catecholamines and 5-HT in urine and dialysate). For measurements of free (nonconjugated) DA and 5-HT, urine samples and amines were absorbed on a weak cation exchange resin (Ambertite CG-50) at pH 6.1 and eluted with 5 ml of 1 M acetic acid. Separation of DA and 5-HT in the eluates was achieved by reverse-phase chromatography using 4.6 mm × 25 cm ultrasphere (Beckman Instruments, Fullerton, CA) columns. The mobile phase for separation was composed of 0.07 M PBS, 0.2 mM disodium EDTA, 0.7 mM heptane sulfonate, and 4% acetonitrile (vol/vol) at pH 3.65. Amperometric detection was performed using Bioanalytical Systems electrodes at 0.65 V relative to silver/silver chloride electrodes. Elution of DA and 5-HT occurred typically at 9 and 50 min, respectively. Quantitation of the amines was by HPLC with electrochemical detection. Dialysates were diluted 1:4 in 0.01 N HCl and injected onto HPLC. Analysis of RIF DA and 5-HT was prepared simultaneously in the same sample. Thus differences in RIF DA and 5-HT concentrations between treatments or experiments were due to differences in their peak heights.

Statistics

Values are expressed as means ± SE. Comparisons within a group were performed with a paired t-test. Comparisons between the time control and inhibition of MAO groups were made using an unpaired t-test. Comparisons between the time control, COMT inhibition, and COMT and MAO inhibition were made using one-way ANOVA followed by the Student-Newman-Keuls test. A P value <0.05 was considered to be significant.

RESULTS

The effects of inhibition of MAO on DA/5-HT in the RIF and urine are summarized in Table 1. In the time control group, RIF DA, RIF 5-HT, urinary DA, and 5-HT were stable throughout the experiments. Inhibition of MAO significantly increased RIF 5-HT from 3.16 ± 0.38 to 8.03 ± 1.83 pg/min (n = 5, P < 0.05).

The effects of inhibition of COMT alone or the combined inhibition of MAO and COMT on DA/5-HT in the RIF and urine are summarized in Table 2. In the PBS control group, DA and 5-HT in RIF and urine were similar in the two clearance periods. Inhibition of COMT significantly increased DA in both RIF (3.47 ± 0.70 to 8.68 ± 1.96 pg/min, n = 9, P < 0.05) and urine (2.00 ± 0.16 to 2.76 ± 0.26 pg/min, n = 10, P < 0.05). In the group with combined inhibition of MAO and COMT, RIF DA (1.52 ± 0.19 to 2.99 ± 0.62 pg/min, n =
8, $P < 0.05$) and urinary DA ($2.18 \pm 0.16$ to $3.30 \pm 0.25$ ng/min, $n = 9$, $P < 0.001$) significantly increased. Urinary 5-HT was also significantly increased from $0.09 \pm 0.17$ to $3.52 \pm 0.27$ ng/min ($n = 9$, $P < 0.01$). However, RIF 5-HT did not change. Inspection of Tables 1 and 2 shows that, although basal RIF DA values were similar between experimental groups, basal RIF 5-HT values exhibited more variability between the experimental groups. Because RIF DA and RIF 5-HT analysis is performed simultaneously in the same sample, it appears that RIF 5-HT values are far more variable. Because RIF 5-HT levels have never been determined previously, little is known regarding other factors that affect RIF 5-HT. Nonetheless, it is important to note that the changes within the groups are the most important aspect of these studies.

The effects of inhibition of MAO on renal function are summarized in Table 3. In the time control group, MAP, glomerular filtration rate (GFR), urine flow rate (Uv), plasma phosphate concentration ($P_{Pi}$), absolute phosphate excretion ($U_{Pi}$), fractional excretion of phosphate ($F_{Pi}$), absolute sodium excretion ($U_{Na}$), and fractional excretion of sodium ($F_{Na}$) were stable throughout the experiments. Inhibition of MAO significantly decreased MAP from $120.4 \pm 6.5$ to $96.0 \pm 7.5$ mmHg ($n = 7$, $P < 0.001$) and GFR from $2.09 \pm 0.18$ to $1.57 \pm 0.22$ ml/min ($n = 7$, $P < 0.05$). $F_{Pi}$ significantly increased from $22.0 \pm 2.1$ to $31.5 \pm 2.1\%$ ($n = 7$, $P < 0.05$), but $U_{Pi}$ was not significantly changed. $U_{V}$, $U_{Na}$, $F_{Na}$, and $P_{Pi}$ did not change.

The effects of inhibition of COMT alone or the combined inhibition of MAO and COMT on renal function are summarized in Table 4. In the PBS control group, GFR, $U_{V}$, $U_{Na}$, $F_{Na}$, and $P_{Pi}$ did not change, whereas MAP modestly decreased ($123.3 \pm 6.0$ to $115.1 \pm 4.4$ mmHg, $n = 7$, $P < 0.05$). $U_{Pi}$ modestly increased from $0.98 \pm 0.08$ to $1.23 \pm 0.09$ μmol/min ($n = 7$, $P < 0.05$), and $F_{Pi}$ modestly increased ($25.1 \pm 4.5$ to $31.0 \pm 3.5\%$, $n = 7$, $P < 0.05$). Inhibition of COMT significantly increased $U_{Na}$ from $6.42 \pm 2.00$ to $9.82 \pm 1.62$ μmol/min ($n = 10$, $P < 0.05$). $U_{Pi}$ ($0.87 \pm 0.12$ to $1.32 \pm 0.18$ μmol/min, $n = 10$, $P < 0.05$) and $F_{Pi}$ ($16.5 \pm 2.2$ to $25.1 \pm 2.8\%$, $n = 10$, $P < 0.05$) significantly increased. Other parameters were unchanged. The combined inhibition of MAO and COMT markedly increased $U_{V}$ from $34.2 \pm 7.8$ to $105.9 \pm 17.1$ μ/min ($n = 9$, $P < 0.001$), although MAP significantly decreased from $132.8 \pm 4.1$ to $101.8 \pm 2.5$ mmHg ($n = 9$, $P < 0.001$). $U_{Na}$ and $F_{Na}$ significantly increased from $0.9 \pm 0.3$ to $1.7 \pm 0.3\%$ ($n = 9$, $P < 0.01$), respectively. In addition, $U_{Pi}$ ($0.81 \pm 0.07$ to $1.50 \pm 0.30$ μmol/min, $n = 9$, $P < 0.05$) and $F_{Pi}$ ($14.4 \pm 0.9$ to $24.0 \pm 3.0\%$, $n = 9$, $P < 0.05$) significantly increased. GFR and $P_{Pi}$ did not change.

### Discussion

The present study demonstrates that 1) inhibition of MAO selectively increased RIF 5-HT ~2.5-fold, accompanied with significant decreases in MAP and GFR; 2) inhibition of COMT significantly increased RIF DA and urinary DA, concomitant with significant increases in $U_{Na}$; and 3) combined inhibition of MAO and COMT

### Table 1. Effect of inhibition of MAO-A and -B on DA/5-HT in the RIF and urine

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Period</th>
<th>RIF DA, pg/min</th>
<th>RIF 5-HT, pg/min</th>
<th>Urinary DA, ng/min</th>
<th>Urinary 5-HT, ng/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time control</td>
<td>5</td>
<td>Control</td>
<td>1.65 ± 0.27</td>
<td>11.63 ± 3.96</td>
<td>1.41 ± 0.34</td>
<td>1.00 ± 0.28</td>
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<tr>
<td></td>
<td>5</td>
<td>Saline</td>
<td>2.22 ± 0.64</td>
<td>11.32 ± 3.65</td>
<td>0.67 ± 0.55</td>
<td>-0.30 ± 1.59</td>
</tr>
<tr>
<td>MAO</td>
<td>5</td>
<td>Control</td>
<td>1.46 ± 0.43</td>
<td>3.16 ± 0.38</td>
<td>1.25 ± 0.13</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Inhibitors</td>
<td>1.11 ± 0.29</td>
<td>8.03 ± 1.83*</td>
<td>2.32 ± 0.82</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Δ</td>
<td>-0.36 ± 0.36</td>
<td>5.28 ± 1.79†</td>
<td>1.07 ± 0.90</td>
<td>-0.04 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of experiments. MAO, monoamine oxidase; RIF, renal interstitial fluid; DA, dopamine; 5-HT, serotonin; Δ, change. *$P < 0.05$ vs. control periods by paired t-test. †Significant difference using unpaired t-test.

### Table 2. Effect of inhibition of COMT or combined inhibition of MAO and COMT on DA/5-HT in the RIF and urine

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Period</th>
<th>RIF DA, pg/min</th>
<th>RIF 5-HT, pg/min</th>
<th>Urinary DA, ng/min</th>
<th>Urinary 5-HT, ng/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>6</td>
<td>Control</td>
<td>3.84 ± 0.98</td>
<td>14.56 ± 3.35</td>
<td>1.84 ± 0.21</td>
<td>1.30 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PBS</td>
<td>3.30 ± 0.51</td>
<td>14.70 ± 1.30</td>
<td>1.73 ± 0.28</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Δ</td>
<td>0.54 ± 1.06</td>
<td>11.04 ± 2.21</td>
<td>-0.11 ± 0.31</td>
<td>-0.29 ± 0.21</td>
</tr>
<tr>
<td>COMT</td>
<td>9</td>
<td>Control</td>
<td>3.47 ± 0.70</td>
<td>11.39 ± 2.88</td>
<td>2.00 ± 0.16</td>
<td>1.43 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Inhibitor</td>
<td>8.68 ± 1.96*</td>
<td>7.65 ± 2.24</td>
<td>2.76 ± 0.26*</td>
<td>1.27 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Δ</td>
<td>5.21 ± 1.89†</td>
<td>-3.73 ± 3.26</td>
<td>0.75 ± 0.29†</td>
<td>-0.17 ± 0.21</td>
</tr>
<tr>
<td>MAO + COMT</td>
<td>8</td>
<td>Control</td>
<td>1.52 ± 0.19</td>
<td>3.88 ± 1.58</td>
<td>2.18 ± 0.16</td>
<td>2.45 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Inhibitors</td>
<td>2.99 ± 0.62*</td>
<td>3.86 ± 0.91</td>
<td>3.30 ± 0.25*</td>
<td>3.52 ± 0.27*</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Δ</td>
<td>1.47 ± 0.52</td>
<td>-0.02 ± 1.44</td>
<td>1.12 ± 0.29†</td>
<td>1.07 ± 0.31††</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of experiments. COMT, catechol-O-methyltransferase. *$P < 0.05$ vs. control periods. †Significant difference compared with control. ‡Significant difference compared with COMT inhibition alone.
significantly increased RIF DA, urinary DA, and urinary 5-HT, which was associated with increases in Uv, UvNaN, and FENa and decreases in MAP.

Proximal tubules both synthesize and degrade DA and 5-HT. In general, DA is degraded by MAO and COMT, whereas 5-HT is deaminated via MAO (10, 41). When MAO inhibitors were administered, the degradation of 5-HT was blocked, whereas DA could still be metabolized by COMT. This can explain the observation that 5-HT increased, whereas DA did not change with inhibition of MAO. It is noteworthy that 5-HT increased 2.5-fold in the RIF but not in urine in the group with inhibition of MAO. This is likely due to the fact that 5-HT preferentially exits the basolateral membrane of the proximal tubules, which is consistent with our previous studies showing that RIF (microporous) 5-HT is higher than DA, whereas urinary 5-HT is lower than DA (2).

Based on previous studies (40, 41), we had expected that 5-HT excretion would increase with inhibition of MAO; however, acute inhibition of MAO resulted in significant increases in 5-HT only in the dialysate. The reason for the differing observations between these two studies may be related to the differing time course (acute vs. chronic administration of MAO inhibitors) of the experiments, since significant increases in 5-HT excretion were observed on the second and third days of treatment (40).

DA in the RIF and urine were both increased when the COMT inhibitor was administered. This is in contrast to our observations that DA did not change in the inhibition of the MAO group. Thus COMT appears to be more important than MAO in the degradation of intrarenal DA. This is in contrast to previous studies in which inhibition of COMT using tolcapone or entacapone did not increase DA or norepinephrine in microdialysis studies in brain tissue (26). However, because two different COMT transcripts have been described and their abundance varies between tissues (brain vs. kidney) and because tyrosine hydroxylase is not present in the proximal tubules of the kidney (43, 44), it is unlikely that administration of COMT inhibitors would have identical effects of DA metabolism in these tissues. It is interesting to note that inhibition of COMT increased RIF DA about 1.5-fold higher than its control period; however, urinary DA was only ~0.4-fold higher than its control period. In a study by Wang et al. (48), it was shown that both chronic sodium loading and acute gludopa administration stimulated DA release predominantly into the tubule lumen rather than the RIF. However, in the present study, inhibition of DA metabolism increased DA in RIF to a greater extent than in urine.

It is unclear why RIF 5-HT did not increase during the combined inhibition of MAO and COMT. We speculate that there might be competition between 5-HT and DA with regard to the pathway through which they exit the basolateral membrane of proximal tubular cells into RIF. Thus, when there is a significant increase in the transport of DA into RIF, RIF 5-HT may be prevented from being increased further. Previous studies demonstrate that the organic cation transporters (OCT1 and OCT2) are present on the apical and basolateral membranes of proximal tubule cells (14, 15, R251 INTRARENAL DOPAMINE AND SEROTONIN

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Period</th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>UV, μM/min</th>
<th>P&lt;sub&gt;Na&lt;/sub&gt;, mM</th>
<th>UV&lt;sub&gt;Na&lt;/sub&gt;, μmol/min</th>
<th>FE&lt;sub&gt;Na&lt;/sub&gt;, %</th>
<th>UvNaN, μmol/min</th>
<th>FE&lt;sub&gt;NaN&lt;/sub&gt;, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>7</td>
<td>Control</td>
<td>123.3 ± 6.0</td>
<td>2.39 ± 0.36</td>
<td>43.5 ± 12.0</td>
<td>1.90 ± 0.04</td>
<td>0.98 ± 0.08</td>
<td>25.1 ± 4.5</td>
<td>5.80 ± 1.25</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>PBS</td>
<td>7</td>
<td>Inhibitors</td>
<td>115.1 ± 4.4*</td>
<td>2.38 ± 0.21</td>
<td>48.8 ± 12.2</td>
<td>1.87 ± 0.08</td>
<td>1.23 ± 0.09*</td>
<td>31.0 ± 3.5</td>
<td>8.18 ± 1.47</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>COMT</td>
<td>10</td>
<td>Control</td>
<td>112.9 ± 4.6</td>
<td>2.88 ± 0.23</td>
<td>52.0 ± 14.3</td>
<td>1.94 ± 0.10</td>
<td>0.87 ± 0.12</td>
<td>16.5 ± 2.2</td>
<td>6.42 ± 2.00</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>10</td>
<td>Control</td>
<td>111.8 ± 5.3</td>
<td>2.76 ± 0.21</td>
<td>68.0 ± 13.3</td>
<td>1.96 ± 0.16</td>
<td>1.32 ± 0.18*</td>
<td>25.1 ± 2.8*</td>
<td>9.82 ± 1.62*</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>MAO + COMT</td>
<td>9</td>
<td>Control</td>
<td>132.8 ± 4.1</td>
<td>3.14 ± 0.31</td>
<td>34.2 ± 7.8</td>
<td>1.86 ± 0.11</td>
<td>0.81 ± 0.07</td>
<td>14.4 ± 0.9</td>
<td>3.05 ± 0.59</td>
<td>0.9 ± 0.3</td>
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<tr>
<td>Inhibitors</td>
<td>9</td>
<td>Control</td>
<td>101.8 ± 2.5*</td>
<td>3.28 ± 0.33</td>
<td>105.9 ± 17.1*</td>
<td>1.91 ± 0.14</td>
<td>1.50 ± 0.30*</td>
<td>24.0 ± 3.0*</td>
<td>8.40 ± 1.61*</td>
<td>1.7 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. *P < 0.05 vs. control periods. †Significant difference compared with control. ‡Significant difference compared with COMT inhibition alone.
brane of the thick ascending limb. The marked vaso-

kidney were localized only to the basolateral mem-

ber because the MAP was still within the autoregu-

tory range. Instead, it is more likely due to a rather

selective constriction of the afferent glomerular arte-

rioles caused by the increase of RIF 5-HT (7). This

possibility is further supported by the results of the

MAO plus COMT inhibition group. In this group, MAP

also decreased significantly, but GFR did not change,

perhaps because the intrarenal DA levels were in-

creased concomitantly under this situation, which may

have offset the vasoconstrictive effect of the increased

intrafusal 5-HT. Alternatively, because the increase of

5-HT in the MAO plus COMT inhibition group is pre-

dominantly in the urine, 5-HT might not exert a sig-

nificant vasoconstrictive effect on the afferent arte-

riole. The significant increase of FE\textsubscript{P}, observed with

MAO inhibition was unexpected, since 5-HT has been

reported to enhance phosphate reabsorption (10, 17).

This increase might be related to the concomitant de-

case of GFR because U\textsubscript{V}P\textsubscript{D} did not change signifi-


cantly. Although 5-HT has been reported to be an

antinatriuretic and antiphosphaturic substance (10,

17, 20, 41), with moderate increase in RIF 5-HT we did

not observe changes in U\textsubscript{V}P\textsubscript{D}, U\textsubscript{N}A, and FE\textsubscript{Na} in the

MAO inhibition group. This might be due to the fact

that the 1.5-fold increase of RIF 5-HT is not sufficient

to induce these changes. Alternatively, a previous

study demonstrated that the antinatriuretic effect of

increased intrarenal 5-HT was demonstrable only in

animals fed a high sodium intake (40).

The presence of 5-HT receptors in the kidney has not

been studied as extensively as DA. However, immuno-
histochemical studies performed in the rat kidney (38)
demonstrated that 5-HT\textsubscript{1A} receptors present in the

kidney were localized only to the basolateral mem-

brane of the thick ascending limb. The marked vaso-

constrictive effect of 5-HT has been reported to be

mediated by 5-HT and 5-HT\textsubscript{2} receptors (42, 45). Thus

the concentrations of 5-HT in the RIF might be ex-

pected to have effects on the renal tubules and the

vasculature.

DA is generally considered to be an intrarenal di-
uretic, natriuretic, and phosphaturic hormone (10, 17,

19, 20, 27). However, previous studies usually used DA

precursors or DA itself, which resulted in large, phar-

macological increases in intrarenal DA. With inhibi-

tion of COMT, when where there were approximately

one- to twofold increases in intrarenal DA, a significant

increase of UNa\textsubscript{D} was observed. This is consistent with

a study by Eklof et al. (8). There was a tendency for Uv

and FE\textsubscript{Na\textsubscript{D}} to increase in this group, although it did not

reach statistical significance. Uv\textsubscript{P\textsubscript{D}} and FE\textsubscript{P\textsubscript{D}} also signif-

icantly increased in the COMT inhibition group; how-

ever, these increases were not statistically significant

compared with the PBS control group. With MAO plus

COMT inhibition, there were increases in RIF DA,

urinary DA, and urinary 5-HT. However, the effect of

DA seems to predominate over 5-HT, since significant

diuresis and natriuresis was observed in this group,

both of which are typical effects of DA. Two factors

might account for the predominant role of DA over

5-HT seen in this group. First, RIF 5-HT might be more

important than urinary 5-HT in the regulation of fluid

and sodium reabsorption, similar to the effect of RIF

5-HT on GFR. Despite an increase of urinary 5-HT, RIF

5-HT did not increase in the MAO plus COMT

inhibition group. Second, the total increase of DA was

greater than that of 5-HT.

Other studies suggest that DA and sodium excretion

can be dissociated (31). In another study performed in

rats, inhibition of COMT did not increase DA excretion

but nevertheless produced a natriuresis (46). Because

the natriuresis produced by nitecapone was attenuated

by administration of a DA receptor antagonist, it was

suggested that the natriuresis produced by nitecapone

was due to stimulation of DA\textsubscript{1} receptors. Conversely,

other studies demonstrate that increased DA excretion

was not always associated with increased sodium ex-

cretion (3, 5, 12, 31). The natriuretic effect of DA is

clearly more consistently demonstrated in sodium-replete

or volume-expanded conditions (18). The ultimate

expression of the effect of increased intrarenal DA on renal

function is likely influenced by DA concentrations on

receptors in the apical and basolateral membranes and

by the local concentrations of substances that have

opposite effects on tubular transport, such as 5-HT or

angiotensin (6, 13). Furthermore, the nephron site of

action may be important; increased delivery from the

proximal tubule may be offset by increased reabsorp-

tion by more distal nephron segments.

Several challenges arise from the findings of the

present study. For example, the cellular mechanisms

underlying the differential delivery of DA/5-HT into

the RIF and the tubular lumen need to be further

delineated. This is particularly important because RIF

DA/5-HT and tubular luminal DA/5-HT may have dis-

tinct effects on the kidney. It is also critical to further

understand the relative importance of MAO and

COMT in the metabolism of DA and 5-HT, specifically

in the kidney, which is especially relevant in clinical

situations where MAO and/or COMT inhibitors are

used.

In conclusion, 1) inhibition of MAO selectively in-

creases RIF 5-HT; 2) COMT appears to be more impor-

tant than MAO in the degradation of intrarenal DA; and

3) physiological increases in intrarenal DA/5-HT

induced by inhibition of their degrading enzymes are
associated with significant alterations of renal function.

**Perspectives**

The activities of the enzymes for the synthesis (L-aromatic amino acid decarboxylase) and metabolism (MAO and COMT) of DA and 5-HT in the body are highest in brain and kidney tissue. The synthesis and metabolism of DA and 5-HT in neuronal tissue and their role as neurotransmitters are studied extensively. However, the physiological significance of the intrarenal synthesis on DA and 5-HT as intrarenal paracrine factors affecting renal function is not as well defined. The present in vivo study demonstrates that DA and 5-HT are distributed differentially between the lumen and the interstitium. This asymmetry of their distribution suggests that 5-HT may preferentially affect the renal vasculature, whereas luminal DA may affect tubular transport (1). The systemic administration of inhibitors of MAO and COMT modestly increased intrarenal DA and 5-HT levels, which altered GFR and tubular transport. The complex interactions and balance between DA and 5-HT synthesis, metabolism, and distribution in the kidney and their subsequent effects likely represent paracrine factors that alter the renal vasculature and contribute to sodium and phosphate homeostasis.

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