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 DA (3.47 ± 0.70 to 8.68 ± 1.96 pg/min, n = 9, P < 0.05), concomitant with increases 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.

microdialysis; renal interstitial fluid; phosphate; sodium; glomerular filtration rate; monoamine oxidase; catechol-O-methyltransferase

intrarenal dopamine (3,4-dihydroxyphenethylamine; DA) and serotonin (5-hydroxytryptamine; 5-HT) share the following two features in their synthesis: 1) they are synthesized abundantly by renal proximal tubular cells from L-3,4-dihydroxyphenylalanine and 5-hydroxy-L-tryptophan, respectively, and 2) the same enzyme, aromatic L-amino acid decarboxylase, catalyzes their synthesis (30, 37). DA can be metabolized both by deamination via monoamine oxidase (MAO) and by methylation via catechol-O-methyltransferase (COMT), whereas 5-HT is degraded primarily by MAO. The kidney contains one of the highest activities of MAO in the body (36). There are two molecular forms of the enzyme, MAO-A and MAO-B. MAO-A preferentially metabolizes 5-HT (36) and is selectively inhibited by clorgyline (22, 29, 32). MAO-B has a greater affinity for phenylethylamine (36) and is selectively inhibited by pargyline (22, 33, 36). COMT is also abundantly expressed in proximal tubular cells in the kidney (8) and is selectively inhibited by 3,5-dinitrocatechol (35, 47).

DA and 5-HT are important regulators of renal function (8, 9, 40, 41). Both dopaminergic and serotonergic receptors have been demonstrated in the kidney (38, 39). DA has been reported to dilate the renal vasculature and have diuretic, natriuretic, and phosphaturic effects, whereas 5-HT may have an opposite action (10, 16, 17, 19, 20, 27, 28). The effects of DA on renal function have been studied more extensively, whereas the effect of 5-HT on renal function is less clear.

A number of previous studies have used infusion of DA, 5-HT, or their precursors to investigate metabolism of DA and 5-HT in the kidney and their functional role. Few studies, however, have used inhibitors of the enzymes that metabolize DA or 5-HT to increase intrarenal DA and 5-HT (8, 41). Inhibitors for MAO and COMT have been used widely in the treatment of diseases in the nervous system (21, 29, 36), but the renal effects of these inhibitors are not clear.

It has been shown that the distribution of DA in the renal interstitial fluid (RIF) and the urine is differentially regulated (48), whereas the distribution of 5-HT between the urine and the RIF has not been extensively studied. Moreover, although the DA receptor DA1 is present on both the apical and the basolateral membranes of proximal tubules (34), the 5-HT receptor, 5-HT1A, has been predominantly localized to the basolateral membrane of renal tubules (38). Therefore, it is important to distinguish the distribution of DA and 5-HT in the RIF from those in the urine, since they might be expected to exert different effects. Although...
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-thioarbituric 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-dinitrocatechol; 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-dinitrocatechol) 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. For measurements of free (nonconjugated) DA and 5-HT, urine samples and amines were absorbed on a weak cation exchange resin (Amberlite 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 urinary 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 ± 0.16 to 3.30 ± 0.25 ng/min, n = 9, P < 0.001) significantly increased. Urinary 5-HT was also significantly increased from 2.45 ± 0.17 to 3.52 ± 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 (Uv_{Pi}), absolute glucuronide excretion (Uv_{Glu}), and fractional excretion of phosphate (FE_{Pi}), absolute sodium excretion (Uv_{Na}), and fractional excretion of sodium (FE_{Na}) were stable throughout the experiments. Inhibition of MAO significantly decreased MAP from 120.4 ± 6.5 to 96.0 ± 7.5 mmHg (n = 7, P < 0.001) and GFR from 2.09 ± 0.18 to 1.57 ± 0.22 ml/min (n = 7, P < 0.05). FE_{Pi} significantly increased from 22.0 ± 2.1 to 31.5 ± 2.1% (n = 7, P < 0.05), but Uv_{Pi} was not significantly changed. Uv, Uv_{Na}, Uv_{Glu}, 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, Uv, Uv_{Na}, FE_{Na}, and P_{Pi} did not change, whereas MAP modestly decreased (123.3 ± 6.0 to 115.1 ± 4.4 mmHg, n = 7, P < 0.05). Uv_{Pi} modestly increased from 0.88 ± 0.08 to 1.23 ± 0.09 μmol/min (n = 7, P < 0.05), and FE_{Pi} modestly increased (25.1 ± 4.5 to 31.0 ± 3.5%, n = 7, P < 0.05). Inhibition of COMT significantly increased Uv_{Na} from 6.42 ± 2.00 to 9.82 ± 1.62 μmol/min (n = 10, P < 0.05). Uv_{Pi} (0.87 ± 0.12 to 1.32 ± 0.18 μmol/min, n = 10, P < 0.05) and FE_{Pi} (16.5 ± 2.2 to 25.1 ± 2.8%, n = 10, P < 0.05) significantly increased. Other parameters were unchanged. The combined inhibition of MAO and COMT markedly increased Uv from 34.2 ± 7.8 to 105.9 ± 17.1 μl/min (n = 9, P < 0.001), although MAP significantly decreased from 132.8 ± 4.1 to 101.8 ± 2.5 mmHg (n = 9, P < 0.001). Uv_{Na} and FE_{Na} significantly increased from 3.03 ± 0.59 to 8.40 ± 1.61 μmol/min (n = 9, P < 0.01) and from 0.9 ± 0.3 to 1.7 ± 0.3% (n = 9, P < 0.01), respectively. In addition, Uv_{Pi} (0.81 ± 0.07 to 1.50 ± 0.30 μmol/min, n = 9, P < 0.05) and FE_{Pi} (14.4 ± 0.9 to 24.0 ± 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 Uv_{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>
</tr>
<tr>
<td>MAO</td>
<td>5</td>
<td>Control</td>
<td>2.32 ± 0.64</td>
<td>11.32 ± 3.65</td>
<td>1.25 ± 0.32</td>
<td>0.74 ± 0.20</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>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>
</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 Ur, UvNa, 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 (microdialysate) 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 interesting to note 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,

Table 3. Effect of inhibition of MAO-A and -B on renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Period</th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>Ur, μg/min</th>
<th>Pp, mM</th>
<th>UvU, μmol/min</th>
<th>FEp, %</th>
<th>UvNa, μmol/min</th>
<th>FENa, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time control</td>
<td>6</td>
<td>Control</td>
<td>109.7 ± 9.5</td>
<td>2.25 ± 0.30</td>
<td>32.0 ± 15.1</td>
<td>1.77 ± 0.26</td>
<td>0.83 ± 0.14</td>
<td>28.0 ± 9.1</td>
<td>3.82 ± 1.90</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saline</td>
<td>106.2 ± 13.0</td>
<td>2.06 ± 0.26</td>
<td>22.4 ± 4.2</td>
<td>1.76 ± 0.15</td>
<td>0.90 ± 0.28</td>
<td>26.3 ± 8.9</td>
<td>3.48 ± 1.24</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>MAO</td>
<td>7</td>
<td>Control</td>
<td>120.4 ± 6.5</td>
<td>2.09 ± 0.18</td>
<td>46.6 ± 6.8</td>
<td>1.50 ± 0.06</td>
<td>0.66 ± 0.06</td>
<td>22.0 ± 2.1</td>
<td>5.16 ± 0.94</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibitors</td>
<td>96.0 ± 7.5*</td>
<td>1.57 ± 0.22*</td>
<td>41.4 ± 7.3</td>
<td>1.66 ± 0.14</td>
<td>0.76 ± 0.08</td>
<td>31.5 ± 2.1*</td>
<td>5.07 ± 0.92</td>
<td>2.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of experiments. MAP, mean arterial blood pressure; GFR, glomerular filtration rate; Ur, urine flow rate; Pp, plasma phosphate concentration; UvU, absolute phosphate excretion; FEp, fractional excretion of phosphate; UvNa, absolute sodium excretion; FENa, fractional excretion of sodium. *P < 0.05 vs. control periods. †Significant difference between groups using unpaired t-test.

Table 4. Effect of inhibition of COMT or MAO + COMT on renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Period</th>
<th>MAP, mmHg</th>
<th>GFR, ml/min</th>
<th>Ur, μg/min</th>
<th>Pp, mM</th>
<th>UvU, μmol/min</th>
<th>FEp, %</th>
<th>UvNa, μmol/min</th>
<th>FENa, %</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>
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<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>
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<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>
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<td>1.8 ± 0.5</td>
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<tr>
<td></td>
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<td>Inhibitor</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></td>
<td></td>
<td>Δ</td>
<td>−1.1 ± 2.4</td>
<td>−0.12 ± 0.18</td>
<td>16.0 ± 13.5</td>
<td>0.02 ± 0.10</td>
<td>0.46 ± 0.17</td>
<td>8.6 ± 3.1</td>
<td>3.40 ± 1.42</td>
<td>0.8 ± 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></td>
<td></td>
<td>Inhibitors</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>
<tr>
<td></td>
<td></td>
<td>Δ</td>
<td>−90.9 ± 4.5†‡</td>
<td>0.14 ± 0.35</td>
<td>71.7 ± 13.2†‡</td>
<td>0.05 ± 0.17</td>
<td>0.69 ± 0.26</td>
<td>9.6 ± 3.5</td>
<td>5.36 ± 1.42</td>
<td>0.8 ± 0.2</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.
mediated by 5-HT and 5-HT2 receptors (42, 45). Thus, the constrictive effect of 5-HT has been reported to be studied in the rat kidney (38). However, immunohistochemical studies performed in the rat kidney (38) have demonstrated that the antinatriuretic and antiphosphaturic effect of increased intrarenal DA levels was increased concomitantly under this situation, which may have offset the vasoconstrictive effect of the increased intrarenal 5-HT. Alternatively, because the increase of 5-HT in the MAO plus COMT inhibition group is predominant in the urine, 5-HT might not exert a significant vasoconstrictive effect on the afferent arterioles. The significant increase of FE$_{Na}$ 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 decrease of GFR because Uv$_{Pi}$ did not change significantly. 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 Uv$_{Pi}$, Uv$_{Na}$, and FE$_{Na}$ 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, immunohistochemical studies performed in the rat kidney (38) demonstrated that 5-HT$_{1A}$ receptors present in the kidney were localized only to the basolateral membrane of the thick ascending limb. The marked vasoconstrictive effect of 5-HT has been reported to be mediated by 5-HT and 5-HT$_{2}$ receptors (42, 45). Thus, the concentrations of 5-HT in the RIF might be expected to have effects on the renal tubules and the vasculature.

DA is generally considered to be an intrarenal diuretic, natriuretic, and phosphaturic hormone (10, 17, 19, 20, 27). However, previous studies usually used DA precursors or DA itself, which resulted in large, pharmacological increases in intrarenal DA. With inhibition of COMT, when where there were approximately one- to twofold increases in intrarenal DA, a significant increase of Uv$_{Na}$ was observed. This is consistent with a study by Eklof et al. (8). There was a tendency for Uv and FE$_{Na}$ to increase in this group, although it did not reach statistical significance. Uv$_{Pi}$ and FE$_{Pi}$ also significantly increased in the COMT inhibition group; however, 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$_{1}$ receptors. Conversely, other studies demonstrate that increased DA excretion was not always associated with increased sodium excretion (3, 5, 12, 31). The natriuretic effect of DA is 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 reabsorption 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 distinct 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 increases RIF 5-HT; 2) COMT appears to be more important 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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