Homovanillic Acid, Vanillylmandelic Acid and 5-Hydroxyindole-3-acetic Acid Are Undetectable in Urine of the Muskrat

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The muskrat (Suncus murinus) may be useful as a new experimental animal. In order to evaluate some biochemical characteristics of this animal, urinary excretion of homovanillic acid, vanillylmandelic acid and 5-hydroxyindole-3-acetic acid in the muskrat was determined by high-performance liquid chromatography (HPLC) with electrochemical detection. These acid metabolites of catecholamines and serotonin were not detectable or barely detectable (vanillylmandelic acid) in the urine of the muskrat in comparison with human, dog, rat or mouse. The secretion and/or metabolism of these neurotransmitter amines in the muskrat seem to be considerably different from those in other mammals.

Keywords—urinary excretion; HPLC; homovanillic acid; vanillylmandelic acid; 5-hydroxyindole-3-acetic acid; muskrat; Suncus murinus; dog; rat; mouse

The muskrat (Insectivora: Sorcidae: Corocidurinae: Suncus murinus) is found mainly in Southeast Asia including the southernmost part of Japan. Recently, it has been proposed that the muskrat may be useful as an experimental animal because it has many anatomical and physiological features in common with primates.1)

Homovanillic acid (HVA) and vanillylmandelic acid (VMA) are the main metabolic end-products of dopamine and norepinephrine or epinephrine, respectively. 5-Hydroxyindole-3-acetic acid (5-HIAA) is a main metabolite of serotonin. The levels of these acids in urine are of importance in obtaining information about the secretion of the above neurotransmitter amines. The urinary excretion of the three acids in humans has been determined by various techniques including high-performance liquid chromatography (HPLC), as summarized in our previous papers,2) and the values in normal subjects are known. On the other hand, the urinary excretion3)–11) of the acids in the dog, rat and mouse has not been determined by a reliable method such as HPLC, with a few exceptions4,8) where gas chromatography-mass spectrometry (GC-MS) was used, and that in the muskrat has never been determined by any means.

In this work we examined the urinary excretion of HVA, VMA and 5-HIAA in various animals including the muskrat by using HPLC with electrochemical detection.

Experimental

Animals—Rats (Wistar ST) and mice (ddY) were housed in a temperature- and humidity-controlled room (22 ± 3°C and 60 ± 10%, respectively). They were maintained on commercial laboratory food CE-2 (Nippon Cler Co., Tokyo, Japan). Beagle dogs were also housed in a temperature- and humidity-controlled room (22 ± 2°C and 55 ± 10%, respectively), and were maintained on CD-5 (Nippon Cler Co.). The muskrats were housed in a
temperature- and humidity-controlled room (25 ± 2°C and 60 ± 10%, respectively), and were maintained on CIEA-305 (Central Institute for Experimental Animals, Tokyo, Japan).

Urine Collection——Human urine was collected in polystyrene bottles. Rat and muskrat urines were collected in metabolic cages. Dog urine was obtained in a glass tube by using a cannula, and mouse urine was collected in a glass tube. These urine specimens were stored at −80°C until analysis.

Creatinine content in urine specimens was specifically determined by HPLC as described previously.12)

HPLC Analysis of HVA, VMA and 5-HIAA——HVA, VMA and 5-HIAA in urine were determined according to the method described previously.2) Urine (0.1—1.0 ml) was brought to a volume of 1.0 ml by adding distilled water. The solution was mixed with 4 ml of 0.03 N hydrochloric acid and applied to a column of QAE-Sephadex A-25 (acetate form; 28 × 9.5 mm i.d.). The column was washed with 8 ml of 0.01 N hydrochloric acid. The adsorbed HVA, VMA and 5-HIAA were eluted from the column with 10 ml of 0.2 M sodium chloride in 0.01 N hydrochloric acid. Then 3 g of sodium chloride and 1 ml of 1 M citric acid–sodium hydroxide buffer (pH 3.0) were added to the effluent. After the resulting mixture had been extracted with 10 ml of ethyl acetate by shaking for 1.5 min, 8 ml of the organic phase were taken and evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of methanol and 10 μl of the solution were injected into the liquid chromatograph.

The chromatographic conditions were as follows: HPLC apparatus, high-performance liquid chromatograph LC-3A (Shimadzu Seisakusho, Kyoto, Japan) equipped with an EC-8 electrochemical detector (Toyo Soda Manufacturing, Tokyo, Japan); column stainless-steel tubing (300 × 4.0 mm i.d.) packed with Lichrosorb RP-18 (particle diameter, 5 μm); eluent, 0.05 M potassium dihydrogen phosphate–phosphoric acid buffer (pH 2.2)–methanol (7:1, v/v); column temperature, 45°C; flow rate, 0.8 ml/min; detector oxidation potential, 0.750 V vs. Ag/AgCl; detector sensitivity, 80 nA at full scale.

Results and Discussion

Typical chromatograms of urine samples from a ddY mouse and a muskrat are shown in Figs. 1 and 2, respectively, and the analytical results for urine samples from human, dog, rat, mouse and muskrat are summarized in Table I. The excretion of these metabolites in dog, rat and mouse urine has been determined, but no simultaneous determination of the metabolites has been reported.

The data reported by other workers are summarized in Table II. The values for urinary excretion in rats obtained in this work are in broad agreement, especially with those measured by using GC-MS.4,8) The values in humans, which have been previously reported by us, are consistent with those reported in the literature.2)

Daily excretions of creatinine in dogs and mice were not determined, because urine of these animals was collected at arbitrary times. The urinary 5-HIAA levels in dog and mouse, converted into μg/kg/24 h by using the mean value of 25.7 μg/kg/24 h of daily creatinine excretion per kg body weight for human and,12) were 411 μg/kg/24 h and 159 μg/kg/16 h, respectively. The present value for the mouse is in the range reported by Bartlet.11) The value for the beagle dog, however, is somewhat larger than that reported by Erspeamer et al.3)
Table I. Urinary Excretion (µg/mg of Creatinine) of HVA, VMA and 5-HIAA in Human, Dog, Rat, Mouse and Muskrat

<table>
<thead>
<tr>
<th>Species</th>
<th>HVA</th>
<th>VMA</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (4 males and 4 females)</td>
<td>2.6±0.5</td>
<td>1.4±0.2</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>(1.6—4.9)</td>
<td>(0.7—2.2)</td>
<td>(0.8—3.3)</td>
<td></td>
</tr>
<tr>
<td>Beagle dog (5 males, 8 months,</td>
<td>0.9±0.2</td>
<td>0.3±0.2</td>
<td>1.6±0.9</td>
</tr>
<tr>
<td>10—12 kg)</td>
<td>(0.4—1.7)</td>
<td>(N.D.—1.1)</td>
<td>(3.4—50)</td>
</tr>
<tr>
<td>Wistar ST rat (5 males, 9 weeks,</td>
<td>4.6±0.4</td>
<td>0.6±0.2</td>
<td>8.3±0.6</td>
</tr>
<tr>
<td>210—260 g)</td>
<td>(3.6—5.7)</td>
<td>(N.D.—1.1)</td>
<td>(5.9—9.4)</td>
</tr>
<tr>
<td>ddY mouse (6 males, 7 weeks,</td>
<td>14±4</td>
<td>1.8±0.4</td>
<td>9.3±0.7</td>
</tr>
<tr>
<td>30—35 g)</td>
<td>(1.1—28)</td>
<td>(0.5—4.8)</td>
<td>(7.7—17)</td>
</tr>
<tr>
<td>Muskrat (3 males and 3 females,</td>
<td>N.D.</td>
<td>&lt;0.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>13—17 weeks, 40—80 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are the means ± S.E. with the range in parenthesis.

Table II. Comparable Data for Metabolite Excretions by Dog, Rat and Mouse

<table>
<thead>
<tr>
<th>Animal</th>
<th>HVA</th>
<th>VMA</th>
<th>5-HIAA</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>82 µg/kg/24 h</td>
<td>C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>Rat (Wistar)</td>
<td>31 µg/24 h&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.9 µg/24 h&lt;sup&gt;3&lt;/sup&gt;</td>
<td>55 µg/24 h&lt;sup&gt;3&lt;/sup&gt;</td>
<td>HPLC</td>
<td>This paper</td>
</tr>
<tr>
<td></td>
<td>(2.5 µg/ml&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>(17 µg/kg/24 h&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>(230 µg/kg/24 h&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.44 µg/ml</td>
<td></td>
<td></td>
<td>GC-MS</td>
<td>4</td>
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<tr>
<td>Rat (Wistar)</td>
<td>41 µg/24 h</td>
<td>65 µg/24 h</td>
<td></td>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>Rat (Albino)</td>
<td></td>
<td>49 µg/24 h</td>
<td></td>
<td>SP&lt;sup&gt;5&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>Rat (Albino)</td>
<td></td>
<td>0.940 µg/24 h</td>
<td></td>
<td>SP</td>
<td>7</td>
</tr>
<tr>
<td>Rat&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.4 µg/24 h</td>
<td></td>
<td></td>
<td>GC-MS</td>
<td>8</td>
</tr>
<tr>
<td>Rat&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>184 µg/kg/24 h</td>
<td>C</td>
<td>9</td>
</tr>
<tr>
<td>Rat (Wistar)</td>
<td></td>
<td></td>
<td>266 µg/kg/24 h</td>
<td>C</td>
<td>10</td>
</tr>
<tr>
<td>Mouse&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>129 µg/kg/16 h</td>
<td>C</td>
<td>11</td>
</tr>
</tbody>
</table>

Values are expressed as means. <sup>a</sup> The species was not designated. <sup>b</sup> C means colorimetry; SP, spectrophotometry. <sup>c</sup> These values were calculated by using 6.69 mg/24 h or 28.0 mg/kg/24 h as the mean values of urinary creatinine in rats (see reference 12). <sup>d</sup> This was converted by using a urine volume of 12.3 ml/24 h in rats.

On the other hand, the muskrat scarcely excreted these metabolites in the urine despite its apparent anatomical and physiological similarities with primates. The secretion and/or metabolism of catecholamines and serotonin in the muskrat seem to be considerably different from those not only in humans but also in dogs, rats and mice.

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References