Determination of Some L-3,4-Dihydroxyphenylalanine and Dopamine Metabolites in Urine by Means of Mass Fragmentography

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We describe a mass-fragmentographic method for determination in urine of the following metabolites of L-3,4-dihydroxyphenylalanine and dopamine: vanillactic acid, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylethanol, and 3,4-dihydroxyphenylethanol. Deuterated analogs were used as internal standards. The method is fast, reproducible, sensitive, and selective, and does not require the use of time-consuming clean-up procedures. Normal excretion values in terms of creatinine, expressed as a function of age, as well as values obtained for patients with neurogenic tumors, a patient during therapy with L-3,4-dihydroxyphenylalanine, and a patient receiving dopamine are presented and discussed.

Additional Keyphrases: values for normal persons, persons with neurogenic tumor, and persons receiving dopa or dopamine · age-related effects · gas chromatography/mass spectrometry · cancer

Major and minor metabolic pathways of catecholamine metabolism in health and disease are rather well understood (1-4). Measurements of catecholamines and their metabolites in urine provide an overall picture that is a net result of organ-to-organ (and even subarea-to-subarea of an organ) variation in catecholamine metabolism (2). This variation is due to local differences in enzyme concentrations, the existence of multiple forms of the enzymes (isoenzymes), and to other regulating mechanisms influencing this metabolism. Consequently, research of local and overall catecholamine metabolism may lead to a better understanding of diseases in which catecholamines play an important role and possibly may result in more specifically directed therapies. For instance, effort has been made to establish the mechanisms involved in catecholamine metabolism during parkinsonism (4), its treatment with L-dopa (5), neurogenic tumors (6), and states of abnormal behavior (4).

Here, we describe a method for determining some L-dopa and dopamine metabolites in urine by means of mass fragmentography with use of deuterated internal standards. Urinary concentrations of these metabolites—VLA, DOPAC, MOPET, and DOPET—for normal healthy persons, patients with neuroblastoma, and two patients receiving L-dopa and dopamine therapeutically are presented and discussed. The results demonstrate the potential usefulness of the method.

In addition, we determined HVA, VMA, and MOPEG.

Materials and Methods

Standards, Reagents, Samples, and Equipment

VLA, DOPAC, and HVA were purchased from Sigma Chemical Co., St. Louis, Mo. 63178; MOPET from Calbiochem, La Jolla, Calif. 92037; deuterium chloride (37% in deuterium oxide), deuterium oxide, bis(trimethylsilyl)trifluoroacetamide, and lithium aluminum deuteride were from Merck, Darmstadt, Germany. 3% OV-1 and 3% OV-225, both on Supelcoport 80-100 mesh, were from Supelco Inc., Bellefonte, Pa. 16823.

We collected 24-h urine specimens from normal individuals, patients with neuroblastoma, a parkinsonian patient receiving each day three tablets of "Sinemet" [containing 25 mg of carbi-dopa (α-methyl-dopa hydrazine) and 250 mg of L-dopa per tablet; Merck Sharp

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Received July 25, 1977; accepted Nov. 1, 1977.

3 Nonstandard abbreviations used: L-dopa, L-3,4-dihydroxyphenylalanine; VLA, vanillactic acid (4-hydroxy-3-methoxyphenylactic acid); DOPAC, 3,4-dihydroxyphenylacetic acid; MOPET, 3-methoxy-4-hydroxyphenylethanol; DOPET, 3,4-dihydroxyphenylethanol; HVA, homovanillic acid; VMA, vanilmandelic acid (4-hydroxy-3-methoxyphenylmandelic acid); MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol; TMS, trimethylsilyl; and d, deuterium (4H).
& Dohme, Haarlem, The Netherlands], and a patient receiving 170 mg of dopamine per day intravenously.

The specimens were acidified to pH 1.0 with concentrated hydrochloric acid and stored at −20 °C until analysis. Creatinine was measured according to Chasson et al. (7).

Combined gas chromatography/mass spectrometry was performed with a Varian Aerograph 1400 gas chromatograph, coupled to a Varian MAT 112 mass spectrometer equipped with a Brunnée type molecular separator and a four-channel selected-ion-monitoring device.

Procedures

2,5,6-Trimethyle- vanillactic acid (VLA-d3), 2,5,6-
trimethoprene-3,4-dihydroxyphenylacetic acid (DOPAC-
d3), 1,1-dimethyle-1-hydroxy-2-(2,5,6-trimethyle-cate-
chol)-ethane (DOPET-d5) and DOPET-d0 were pre-
pared as described elsewhere (8). 1,1,2,2-Tetra-
methyle-1-hydroxy-2-(2,5,6-trimethyle-vanil)-ethane
(MOPET-d7) was synthesized from 2,5,6-trimethyle-
3-methoxy-4-hydroxyphenyl-2,2-dimethyle-acetic acid
(HVA-d3) by reduction with LiAlH4 in tetrahydrofuran,
similarly as described for MOPET-d6 (8). HVA-d3 was
synthesized by heating 300 mg of HVA in 12 ml of 16%
DCI in D2O for 72 h at 80 °C in a heating block.

Portions of urine containing 0.1–1.0 mg of creatinine,
were processed separately for the determination of acidic (DOPAC, VLA) and alcoholic (MOPET, DOPET) metabolites as described elsewhere (9). Before extraction and after enzymatic hydrolysis of alcoholic metabolites, 1000 ng of DOPAC-d3, 500 ng of VLA-d3,
500 ng of MOPET-d3, and 25 ng of DOPET-d3 were added to the urine samples as internal standards. The evaporated extracts were derivatized with 100 or 50 μl of bis(trimethylsilyl)trifluoroacetamide. Standard solutions containing various amounts of the compounds to be measured and fixed amounts of internal standards were used to prepare calibration curves.

Gas chromatography/mass spectrometry. Volumes of 1–2 μl were injected into the gas chromatograph/mass spectrometer combination, equipped with a 2-m coiled-glass column (1.2 mm i.d.) packed with OV-1 or OV-225. Helium flow rate was 10 ml/min, ionization energy 70 eV, separator temperature 250 °C, injector temperature 270 °C, source temperature 275 °C, and interface temperature 300 °C.

Integration time in selected ion monitoring was 0.1 or 0.3 s per channel, depending on the chromatographic peak width. Column temperature was set at 160, 175, and 185 °C, as indicated below.

Determination of VLA, DOPAC, MOPET, and DOPET by means of mass fragmentography. Metabo-
lite concentrations were obtained by calculating the peak-height ratios of the fragment ions of labeled and unlabeled compounds and comparing them to those obtained from known amounts, plotted on a calibration curve.

Relative peak heights of VLA-d0 and VLA-d3 were determined on OV-1 (185 °C) at m/e = 428, 431 (M+) and on OV-225 (160 °C), both at m/e = 338, 341
(M+–HO-TMS) and m/e = 209, 212 (M+–
C2H5O2(TMS))2. DOPAC-d0 and -d1 peak heights were determined on OV-1 at 175 °C at m/e = 384, 387 (M+).

MOPET and DOPET were determined simultaneously in a single run on OV-1 (160 °C) at m/e = 312, 319 (M+) and m/e = 370, 375 (M+), respectively.

Gas chromatographic determinations. HVA, VMA,
MOPEG, and (when detectable) MOPET, DOPAC, and VLA were determined by gas chromatography with flame ionization detection as described before (9).

Results

Sensitivity, Reproducibility, and Specificity

There was a linear relationship between the amount of protium compound and the peak-height ratio of the d0 ions and the corresponding ions of the deuterated internal standard in the cases of VLA, DOPAC, MOPET, and DOPET (Figure 1).

The detection limit for the method (peak:background
= 2) was well below the concentrations of these metabolites in the urine of all subjects studied and varied between 0.5 and 1.0 μg/g of creatinine. The values
measured ranged from 0.05 (lowest normal value for MOPET) to seven times (the highest DOPET value observed, a patient with neuroblastoma) the amount of internal standard added.

The reproducibility of the analytical method, determined by analyzing 15 different samples for DOPAC, resulted in a day-to-day variation of less than 8% and a within-day variation of less than 4%.

Figure 2 shows typical mass fragmentograms, recordings of the derivatives of VLA, DOPAC, MOPET, and DOPET and their deuterated analogs in the urine of healthy control persons. Except for the measurement of DOPAC, no interfering peaks were observed under the experimental conditions used. The peak height of DOPAC might be slightly increased by a following peak with m/e = 384 (observed in three cases). The specificity of the assay was determined for VLA by measuring five urine samples at three different sets of ions, and with use of two different stationary phases (Table 1).

Excretion Values

Normal persons. Excretion values, in terms of creatinine, were determined for apparently healthy control persons ranging in age from 13 days to 64 years. The results, expressed as a function of age, are shown in Figure 3.

Patients. Excretion values determined for patients with neuroblastoma (no. 1–7), a patient receiving dopamine (no. 8), and a patient receiving L-dopa (no. 9) are shown in Table 2. The measurements were done by means of mass fragmentography and gas chromatography. Results of HVA, VMA, and MOPEG determinations for patients no. 8 and 9 are presented in Table 3.

MOPET excretion values compared with those of HVA. Results of measurements of MOPET (μg/g of creatinine) were compared with those of HVA (mg/g of creatinine). Figure 4 shows the comparison for both normals and patients. The regression line was y = 5.72x + 80 (y being the MOPET value and x the HVA value), with a correlation coefficient of 0.9708.

Mean urinary MOPET excretion was 1.08% (range: 0.21–3.12%) of HVA excretion. A decreasing excretion of MOPET relative to HVA with increasing age was observed for normal persons (Figure 5).

Discussion

The mass-fragmentographic method described for the determination of small amounts of VLA, DOPAC, MOPET, and DOPET in urinary extracts is fast, reproducible, sensitive, and selective, and requires no time-consuming sample clean-up procedures. We did
Table 2. Urinary Excretion of VLA, DOPAC, MOPET, and DOPET by Patients with Neuroblastoma and by Two Patients Receiving Dopamine or L-Dopa

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age</th>
<th>Sex</th>
<th>VLA, mg/g creat.</th>
<th>DOPAC, mg/g creat.</th>
<th>MOPET, µg/g creat.</th>
<th>DOPET, µg/g creat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 a 0 m</td>
<td>F</td>
<td>0.20 (16)</td>
<td>1034</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 a 1 m</td>
<td>F</td>
<td>0.45 (23)</td>
<td>683</td>
<td>354</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 a 7 m</td>
<td>F</td>
<td>3.00 (198)</td>
<td>3284</td>
<td>1718</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 a 7 m</td>
<td>F</td>
<td>16.00 (30)</td>
<td>669</td>
<td>662</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3 a 4 m</td>
<td>F</td>
<td>5.40 (8.1)</td>
<td>825</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3 a 10 m</td>
<td>M</td>
<td>n.d. (74)</td>
<td>2432</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7 a 6 m</td>
<td>M</td>
<td>0.20 (23)</td>
<td>603</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>60 a</td>
<td>M</td>
<td>0.40 0.8</td>
<td>344</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>63 a</td>
<td>M</td>
<td>5.00 11.0</td>
<td>306</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

* a, years; m, months. Values were determined by means of mass fragmentography, except those in parentheses, which were determined by gas chromatography. No. 1–7, patients with neuroblastoma; patient no. 8 received 170 mg of dopamine and no. 9 750 mg of L-dopa and 75 mg of carbi-dopa per day. F, female; M, male; n.d., not determined.

Table 3. Urinary Excretion of HVA, VMA, and MOPEG by a Patient Receiving Dopamine (No. 8) and a Patient Receiving L-Dopa (No. 9)

<table>
<thead>
<tr>
<th>No.</th>
<th>HVA</th>
<th>VMA</th>
<th>MOPEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>28.9</td>
<td>6.1</td>
<td>5.3</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>0.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Values are expressed in µg/mg of creatinine.

not estimate the accuracy of the method by enriching urines with known amounts of the compounds measured. However, we saw no re-exchange of deuterium labels in the internal standards under the conditions described, and, disregarding possible differences in behavior between protium and deuterium analogs during clean-up and analysis, the method can be considered as absolute (isotope dilution).

The normal values in terms of creatinine we determined for apparently healthy control persons and expressed as a function of age (Figure 3) resulted in typically normal excretion patterns for VLA and MOPET, as already described for HVA, VMA, and MOPEG (9). Normal DOPAC and DOPET excretions per milligram of creatinine showed a less-pronounced age dependency. Normal values for DOPAC for adults accorded with those previously reported by Weg et al. (10) (Weg et al., range: 0.33–1.85, mean ± 1 SD: 0.88 ± 0.32 mg/24 h; this paper, range: 0.6–2.0 mg/g of creatinine), whereas normal values determined for total MOPET were lower than those reported by Karoum et al. (11) (Karoum et al., range: 47–302, mean ± 1 SD: 151 ± 29 µg/g of creatinine; this paper, range: 27–37 µg/g of creatinine). To our knowledge, normal urinary excretion of VLA and DOPET has been only roughly estimated hitherto. Both VLA (5) and DOPET (12) excretions for adults were reported to be less than 100 µg/24 h. We found somewhat higher values for VLA (range: 80–400 µg/g of creatinine), whereas DOPET values were substantially lower (range: 5–26 µg/g of creatinine).

Patients suffering from neuroblastoma4 excreted greater amounts of DOPAC, MOPET, and DOPET (Table 2). In three cases, increased amounts of VLA were excreted. VLA reportedly may or may not be present in any given specimen of urine of patients with neuroblastoma (13, 15), its presence having been associated with serious malignancy (14) and an unfavorable course of the disease (15). If so, small increases in VLA excretion should be detected by a more sensitive and selective analytical method than gas chromatography with flame ionization detection. This is demonstrated for patient no. 3 (Table 2), who excreted a slightly increased amount of VLA, not clearly detectable by our routine gas-chromatographic method.

Except for the MOPET value determined for patient no. 2 (Table 2), the gas-chromatographic measurements that we had at hand correlated reasonably well with those obtained by mass fragmentography.

Fig. 4. Urinary excretion for MOPET as compared with HVA

Values determined by gas chromatography (HVA) and mass fragmentography (MOPET). a, normal persons; b, patients with neuroblastoma; c, patient receiving L-dopa. O, patient receiving dopamine.
A parkinsonian patient receiving L-dopa in combination with an extracerebral L-dopa decarboxylase (EC 4.1.1.28) inhibitor, showed increased excretion of VMA, DOPAC, MOPET, DOPET, and HVA, whereas VLA and MOPEG excretions were normal (Tables 2 and 3). The increase in urinary L-dopa and dopamine metabolites and the normal values for the epinephrine and norepinephrine metabolites after L-dopa ingestion are in agreement with the results reported by Calne et al. (5) and Routh et al. (16), but in conflict with the increased VMA excretion reported by Szpunar et al. (17). The ratio between the sum of the DOPAC and HVA excretions and the VLA excretion (a decarboxylase/transaminase index) obtained for this patient was higher than that reported by Sandler et al. (18). This can be explained by the fourfold higher dose of carbidopa (300 mg) administered to their patients, leading to a more efficient L-dopa decarboxylase inhibition.

Increased amounts of MOPET, DOPET, HVA, VMA, and MOPEG were measured for a patient receiving 170 mg of dopamine per day as a treatment for cardiogenic shock. The increased values for VMA and MOPEG could imply that at least part of the therapeutic effect of the administration of dopamine must be ascribed to its conversion to norepinephrine or epinephrine, or both. However, stress, leading to increased values for urinary VMA (13), must be taken into account for such patients. Metabolic tracer studies with administered deuterated dopamine will provide more insight.

Our MOPET values for normal persons and patients correlated well with those for HVA (Figure 4). The mean urinary excretion of MOPET relative to HVA for the entire group studied is in accord with that reported by Gjessing (19) for normal adults (1%). We did not observe differences between normal persons and patients with neuroblastoma (19), or between patients with neuroblastoma and the patient receiving L-dopa (5).

The observed tendency for younger normal persons to have higher urinary ratios of the alcoholic metabolite MOPET relative to the acidic metabolite HVA (Figure 5) has previously been established for the ratio of MOPEG (alcoholic) to VMA (acidic), both metabolites of epinephrine and norepinephrine (9). Hence it might be concluded that there are small alterations in overall catecholamine metabolism, which favor the formation of acidic rather than alcoholic vanil metabolites.

As a consequence of the relatively constant ratio between MOPET and HVA values observed (Figure 4), one may question whether measurements of MOPET can provide additional information.

Measurement of VLA, DOPAC, MOPET, and DOPET excretion in health, disease, and during therapy with dopamine or L-dopa has provided more insight into overall catecholamine metabolic routes. As our results show, quantification of these compounds could be of importance for the prognosis and follow-up of patients with neuroblastoma, the establishment of metabolic routes in parkinsonism with and without therapy, and for a better understanding of processes involved during the administration of catecholamines and drugs affecting catecholamine metabolism, to patients with cardiogenic diseases.

We thank Drs. A. W. Teelken and J. W. Viersma for providing urine samples and for their useful discussions, Mr. G. Nagel for the mass-spectrometric analysis, and Dr. A. Groen for his encouragement.

References


