
CLIN. CHEM. 30/2, 301–303 (1984)

Liquid-Chromatographic Evaluation of Age-Related Changes in the Urinary Excretion of Free Catecholamines in Pediatric Patients

Thomas G. Rosano

Pediatric patients (to age 18) without neuroblastoma show an age-related decrease in urinary excretion of each of the catecholamines—epinephrine, norepinephrine, and dopamine—in relation to creatinine excretion. From these data, I have developed reference intervals for pediatric age groups. Application of these ranges to seven patients with neuroblastoma and ganglioneuroblastoma indicated a high clinical sensitivity for the urinary dopamine determination but significant false-negative results for epinephrine and norepinephrine.

Additional Keyphrases: creatinine excretion, neuroblastoma, dopamine, epinephrine, norepinephrine, untimed urine specimens, cancer

Neuroblastoma, a tumor of neural crest origin, is more common in children than adults. Synthesis of catecholamines by these tumors is the basis for biochemical diagnosis and monitoring. Measurement of the catecholamine metabolites, homovanillic acid (HVA) and vanilmandelic acid (VMA), in urine has been advocated (1–3) as an aid to diagnosis of neuroblastoma. Supranormal urinary excretion of free catecholamines, especially dopamine, has also been reported in patients with neuroblastoma (4–7). Fluorometry of trihydroxyindole (8), widely used for measuring urinary epinephrine and norepinephrine, lacks precision, is subject to dietary interferences, and does not measure dopamine. These analytical problems limit the usefulness of free catecholamine measurement in the diagnosis of neuroblastoma. The availability of liquid-chromatographic methods (9–11) allows more specific and precise measurement of urinary epinephrine, norepinephrine, and dopamine. Application of determinations of urinary catecholamines to the diagnosis of neuroblastoma, however, requires an evaluation of pediatric reference ranges. I have, therefore, used these methods to evaluate age-related values for each of the urinary catecholamines, indexed to creatinine excretion. The pediatric values so obtained are used to interpret urinary catecholamine data on untimed samples from patients with a confirmed diagnosis of neuroblastoma or ganglioneuroblastoma.

Materials and Methods

I measured urinary free catecholamines by "high-pressure" liquid chromatography (9) modified as described in Application Note 15 (Bioanalytical Systems, Inc., West Lafayette, IN 47906), the only additional modification being substitution of Amberlite CG-50, 100–200 mesh (Mallinkrodt Inc., Paris, KY 40361), for BioRex 70 exchange resin in the pre-chromatography sample-preparation step. Chromatographic equipment used in the study included a Model 870 pump (Du Pont Instruments, Wilmington, DE 19898) with a µBondapak octadecyl silica column (Waters Associates, Milford, MA 01757). I used a glassy carbon electrode (BioAnalytical Systems, Inc.). The voltage regulator and current amplifier were assembled by our Biomedical Engineering Department, according to published schematics (12).

Departments of Clinical Chemistry and Biochemistry, Albany Medical Center, Albany, NY 12208.
Presented in part at the 35th national meeting of the AACC, July 1983, New York, NY.
Received September 21, 1983; accepted October 21, 1983.
To establish reference ranges for the urinary catecholamines, I obtained untimed specimens of urine from 85 pediatric (18 years and younger) and 31 adult patients. Patients with a discharge diagnosis indicating neoplasia, endocrinopathy, or muscular dystrophy were eliminated from this part of the study. All urines were obtained sometime between 1200 and 1700 hours. Creatinine was measured colorimetrically within 24 h of collection, by an alkaline picrate method, with an IL 508 Analyzer (Instrumentation Laboratory, Lexington, MA 02173). The pH of separate portions for catecholamine analysis was adjusted to 3.0; samples were then stored at −10 °C until assayed a week later.

Untimed urine specimens were also obtained, before treatment, from seven patients suspected of having neuroblastoma. This diagnosis was histologically confirmed in six of the patients. The tumor in patient 6 was classified as a transitional ganglioneuroblastoma. These specimens were assayed for catecholamines and creatinine as described above, and also for catecholamine metabolites. HVA was determined by the method of Rosano et al. (13), VMA by the method of Pisano et al. (14). I used the pediatric reference ranges determined by Gitlow et al. (15) in interpreting the metabolite data.

Results

Figure 1 shows the relation between age and excretion of epinephrine, norepinephrine, and dopamine. The regression analysis shows a decrease in the excretion of all three catecholamines during the first 18 years. The steepest negative slope and the best correlation between age and catecholamine excretion were seen for dopamine (correlation coefficient = 0.63). In Table 1 the data are evaluated according to pediatric age groups. Mean excretion (relative to creatinine) decreases with increasing age of the group, and the greatest variability is observed during the first few years. Data from 31 individuals older than 18 years show lower values and less variability than any of the pediatric age groups. There was no significant sex-related difference in catecholamine excretion in either the pediatric or adult population.

I used the age-related ranges determined above to evaluate catecholamine data from seven patients with confirmed diagnoses of neuroblastoma or ganglioneuroblastoma. Table 2 shows the catecholamine and metabolite data for these patients. For interpretation, I compared the data with the upper limit of the reference range as reported in Table 1 or as reported by Gitlow et al. (15) for VMA and HVA, and found that epinephrine and norepinephrine are not clinically sensitive in six and four of these patients, respectively. In contrast, dopamine concentrations exceeded the upper limit of the reference range in all seven patients. A similar treatment of metabolite data shows that HVA and VMA were clinically sensitive in six of the seven patients.

Two patients (13 and 14 years old) with Duchenne-type muscular dystrophy showed an apparent increase in urinary catecholamine excretion. The respective ranges for epinephrine, norepinephrine, and dopamine in these two patients were 0.038–0.133, 0.196–0.422, and 1.450–2.060 mg/g of creatinine. Even though there was no evidence of neural crest tumor, these results were not included in the determination of pediatric reference ranges because of the effect of muscular dystrophy on muscle mass and therefore on creatinine excretion.

Discussion

Recent liquid-chromatographic methods for determining urinary free catecholamines (10, 11) are more specific than the trihydroxyindole method. These reports have focused on analytical evaluation and on clinical studies of hypertensive adults, with and without pheochromocytoma, although Moyer et al. (10) cited reference ranges for a pediatric population based on 24-h urine specimens. Because of difficulties in obtaining reliable 24-h urine specimens from pediatric patients, other workers (15) have advocated the use of untimed urine samples with excretion expressed in terms of creatinine; hence, the present study. The decrease

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>n</th>
<th>Mean mg/g creatinine</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epinephrine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 1</td>
<td>18</td>
<td>0.066</td>
<td>0.100</td>
<td>0–0.375</td>
</tr>
<tr>
<td>1–4</td>
<td>24</td>
<td>0.026</td>
<td>0.020</td>
<td>0–0.062</td>
</tr>
<tr>
<td>4–10</td>
<td>22</td>
<td>0.022</td>
<td>0.021</td>
<td>0.005–0.093</td>
</tr>
<tr>
<td>10–18</td>
<td>20</td>
<td>0.013</td>
<td>0.013</td>
<td>0.003–0.058</td>
</tr>
<tr>
<td>&gt;18</td>
<td>31</td>
<td>0.008</td>
<td>0.009</td>
<td>0.001–0.044</td>
</tr>
<tr>
<td><strong>Norepinephrine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 1</td>
<td>18</td>
<td>0.121</td>
<td>0.065</td>
<td>0.025–0.310</td>
</tr>
<tr>
<td>1–4</td>
<td>24</td>
<td>0.079</td>
<td>0.056</td>
<td>0.025–0.290</td>
</tr>
<tr>
<td>4–10</td>
<td>23</td>
<td>0.058</td>
<td>0.022</td>
<td>0.027–0.108</td>
</tr>
<tr>
<td>10–18</td>
<td>20</td>
<td>0.046</td>
<td>0.023</td>
<td>0.004–0.105</td>
</tr>
<tr>
<td>&gt;18</td>
<td>31</td>
<td>0.040</td>
<td>0.021</td>
<td>0.009–0.112</td>
</tr>
<tr>
<td><strong>Dopamine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 1</td>
<td>18</td>
<td>0.294</td>
<td>0.272</td>
<td>0.24–1.29</td>
</tr>
<tr>
<td>1–4</td>
<td>24</td>
<td>0.583</td>
<td>0.302</td>
<td>0.08–1.22</td>
</tr>
<tr>
<td>4–10</td>
<td>23</td>
<td>0.449</td>
<td>0.142</td>
<td>0.22–0.72</td>
</tr>
<tr>
<td>10–18</td>
<td>20</td>
<td>0.265</td>
<td>0.098</td>
<td>0.12–0.45</td>
</tr>
<tr>
<td>&gt;18</td>
<td>31</td>
<td>0.188</td>
<td>0.075</td>
<td>0.03–0.35</td>
</tr>
</tbody>
</table>

Fig. 1. Relation between age and excretion of epinephrine, norepinephrine, and dopamine.

Regression line was determined for data from pediatric patients.

302 CLINICAL CHEMISTRY, Vol. 30, No. 2, 1984
in catecholamine excretion with age up to 18 years and the high interindividual variability during the first few years coincides with the excretion patterns of the major catecholamine metabolites, HVA and VMA (15).

These data on patients with neuroblastoma or ganglioneuroblastoma indicate the potential clinical usefulness of dopamine determinations. Several earlier studies are consistent with this finding of increased urinary dopamine in neuroblastoma. In a series of 29 patients with neuroblastoma or ganglioneuroblastoma, Hinterberger and Bartholomew (7) found that urinary dopamine and HVA were more frequently increased than VMA or epinephrine and norepinephrine. Kaser (6) reported increased dopamine excretion by 34 of 36 patients with neuroblastoma. Studies by Page and Jacoby (4) and by Greer et al. (5) also show a high clinical sensitivity of urinary dopamine in neuroblastoma. The recent finding (16) that dihydroxyphenylalanine may be the precursor of at least part of the urinary free dopamine does not diminish the potential clinical utility of this measurement, because this compound is also produced by neuroblastoma tumor tissue (4), a fact that may enhance the clinical sensitivity of the urinary dopamine measurement.

Evaluation of the diagnostic significance of the urinary dopamine measurement has been limited by the lack of available methodology or knowledge of normal excretion rates. I believe that the present method, along with an understanding of the age-related effect on excretion of the catecholamines, will facilitate diagnosis and studies of these tumors.

I thank Mr. John Barr for technical assistance throughout the course of this study.

References


