Urinary 5-Hydroxyindoleacetic Acid and Whole Blood Serotonin and Tryptophan in Autistic and Normal Subjects

Ruud B. Minderaa, George M. Anderson, Fred R. Volkmar, Grard W. Akkerhuis, and Donald J. Cohen

Urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion in two consecutive collection periods (5:00 PM-11:00 PM and 11:00 PM-8:00 AM) and whole blood serotonin (5-HT) and tryptophan (TRP) were measured in groups of unmedicated autistics (n = 16), medicated autistics (n = 20), and normal controls (n = 27). Whole blood 5-HT values were significantly higher in unmedicated autistics compared to normal controls. No significant differences were found in 5-HIAA excretion (µg/mg creatinine, mean ± SD) between unmedicated autistics (4.07 ± 1.52) and normal controls (3.50 ± 1.07), or between medicated (5.35 ± 2.93) and drug-free autistic individuals. No correlations were found between 5-HT values and urinary 5-HIAA excretion. Urinary 5-HIAA (µg/mg creatinine, mean ± SD) was significantly greater in hyperserotonemic autistic subjects (4.88 ± 0.87) compared to normal controls (3.50 ± 1.07, total collection period; p = 0.002). The relevance of these findings to the possibility that increased gut production of 5-HT might cause the elevated whole blood 5-HT levels seen in autism is discussed.

Introduction

In 1961, Schain and Freedman reported that the mean value of whole blood serotonin (5-HT) in a group of 23 autistic children was increased compared to a contrast group of mildly retarded children without autistic features. Since then, the finding of elevated serotonin levels in autistics has been replicated by a number of researchers (Ritvo et al. 1970; Yuwiler et al. 1971; Goldstein et al. 1976; Takahashi et al. 1976; Hanley et al. 1977; Hoshino et al. 1979, 1984; Anderson et al. 1987; Minderaa et al. 1986a). Group mean elevations ranging from 18% (Goldstein et al. 1976) to 137% (Hanley et al. 1977) above group means of normal controls have been observed. The cause of this hyperserotonemia in autistic children is unknown.
Most of the body's 5-HT is synthesized in the enterochromaffin cells of the intestine. After release into the plasma, 5-HT is either taken up by the platelet or cleared by the lung, liver, and other tissues. The three major possibilities investigated as potential causes of the hyperserotonemia of autism are increased synthesis, enhanced platelet uptake or storage, and decreased catabolism. The latter two possibilities have been well studied and, in general, neither the platelets handling of 5-HT (Lucas et al. 1971; Boullin et al. 1982; Yuwiler et al. 1975; Anderson et al. 1985) nor the functioning of the principle catabolic enzyme monoamine oxidase (MAO) (Boullin et al. 1976; Campbell et al. 1976; Cohen et al. 1977) appear to be altered in autism. However, recent work has indicated that the role of the platelet might bear reexamination (Rotman et al. 1980; Katsui et al. 1986; G. M. Anderson, unpublished data).

The studies of 5-HT synthesis in autism have focused on the measurement of urinary 5-hydroxyindoleacetic acid (5-HIAA) either with or without tryptophan loading. As most 5-HT is metabolized to 5-HIAA (Udenfriend et al. 1959), the urinary excretion rate of 5-HIAA can serve as a good index of the rate of 5-HT production, assuming catabolism is not greatly altered. Three studies of baseline urinary excretion of 5-HIAA have not found differences between autistic and normal subjects (Shaw et al. 1959; Schain and Freedman 1961; Partington et al. 1973). A report of higher 5-HIAA excretion in autistic subjects (Hanley et al. 1977) also indicated that autistic subjects excreted higher levels of 5-HIAA after a tryptophan load than did control subjects. Other studies of urinary 5-HIAA excretion following a tryptophan load have found lower (Sutton et al. 1958) or similar (Shaw et al. 1959; Schain and Freedman 1961) excretion rates in autistic subjects compared to normals.

In order to obtain a more definite answer to the question of whether or not the synthesis of 5-HT is increased in autism and to study relationships between urinary 5-HIAA and blood 5-HT, we have measured these variables in large groups of autistic and normal subjects.

Methods

The experimental group comprised 36 students enrolled in a school for autistic individuals. Written informed consent was obtained from the parents. A diagnosis was made by a child psychiatrist (R.M.) based on psychiatric evaluation, anamnestic information, and observations made by the school staff before the data were known. Thirty-four individuals were diagnosed as infantile autism, full syndrome present, according to DSM-III criteria (APA 1980: 299.00). Two subjects were diagnosed as infantile autism, residual state (299.01). Sixteen of the autistics (11 boys, 5 girls; mean age 20.6 ± 4.6 years, age range 14.3–28.7 years) were unmedicated for at least 6 months before the urine collection. Of a total of 20 medicated autistic subjects (16 boys, 4 girls; mean age 19.4 ± 4.1, age range 8.9–26.4 years), 6 used phenothiazines, 6 used haloperidol, and 8 used anticonvulsant medication. These subjects were medicated in a constant manner for more than 3 months before the urine collection. A control group consisted of 27 high school students, teachers, and hospital employees, comparable in age and sex (19 male, 8 female; mean age 20.3 ± 6.9, age range 9.1–36.1 years). Written informed consent was obtained from the adult control subjects, the control children, and their parents. All reported they were in good physical health and free of medication.

Urine was collected for two consecutive collection periods, namely, from 5:00 PM–11:00 PM and from 11:00 PM to 8:00 AM. During collection, the urine was kept at 3°C. Urine
was obtained from all 36 autistic subjects and 27 control subjects. Blood was drawn between 10:00 and 11:00 AM from a vein in the forearm. Blood was obtained from 33 of the autistic subjects (14 unmedicated, 19 medicated) and from 17 control subjects. For the autistics, the mean timespan between blood drawing and urine collection was 22 days (range 1–252 days). Within this timespan, there was no change in medication for any of the autistics. For the control group, the blood drawing was done the morning following the urine collection procedure. During the procedure, no specific recommendations were given in respect to diet, sleep, or physical exercise. Whole blood 5-HT and total TRP were analyzed using a high-pressure liquid chromatographic (HPLC)-fluorometric method, described previously (Anderson et al. 1981). Urinary 5-HIAA was analyzed using an HPLC method (Anderson et al. 1985).

Statistical Analysis and Results

**Urinary 5-HIAA Excretion**

Group means of urinary 5-HIAA for the separate and combined collection periods in the control group, the unmedicated autistic group, and the medicated autistic group are given in Table 1. Using a one-tailed $t$-test, 5-HIAA excretion rates in drug-free autistics and in normal controls were not significantly different. A nonsignificant trend ($p < 0.1$) was observed for slightly higher overnight 5-HIAA excretion ($\mu g/mg$ creatinine) in unmedicated autistics when compared to normal subjects. Two-tailed $t$-tests comparing 5-HIAA excretion in medicated autistics with unmedicated autistics did not show significant differences for any of the collection periods. Nonsignificant trends were observed for increased 5-HIAA excretion in several of the medicated subgroups examined (see Table 1). Several methodological issues of urinary 5-HIAA excretion were examined in detail.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>5:00 PM–11:00 PM</th>
<th>11:00 PM–8:00 AM</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>174 ± 117</td>
<td>175 ± 66.5</td>
<td>174 ± 72.5</td>
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<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
<td></td>
<td>3.53 ± 1.62</td>
<td>3.44 ± 1.06</td>
<td>3.50 ± 1.07</td>
</tr>
<tr>
<td>Autistics, unmedicated</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>203 ± 75.3</td>
<td>208 ± 89.9</td>
<td>205 ± 69.8</td>
</tr>
<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
<td></td>
<td>4.00 ± 1.58</td>
<td>4.16 ± 1.70*</td>
<td>4.07 ± 1.52*</td>
</tr>
<tr>
<td>Autistics, all medicated</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>333 ± 243*</td>
<td>225 ± 94.0</td>
<td>263 ± 125*</td>
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<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
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<td>5.77 ± 4.10</td>
<td>5.05 ± 2.58</td>
<td>5.35 ± 2.93*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>374 ± 224</td>
<td>238 ± 87.7</td>
<td>280 ± 87.0</td>
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<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
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<td>4.76 ± 1.98</td>
<td>4.92 ± 3.26</td>
<td>4.82 ± 2.39</td>
</tr>
<tr>
<td>Haloperidol-treated</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>370 ± 369</td>
<td>249 ± 76.3</td>
<td>294 ± 179</td>
</tr>
<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
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<td>7.91 ± 6.48</td>
<td>6.02 ± 2.25*</td>
<td>7.04 ± 3.85</td>
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<tr>
<td>Anticonvulsant-treated</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HIAA ($\mu g/hr$)</td>
<td></td>
<td>246 ± 120</td>
<td>201 ± 117</td>
<td>222 ± 103</td>
</tr>
<tr>
<td>5-HIAA ($\mu g/mg$ creatinine)</td>
<td></td>
<td>4.73 ± 2.74</td>
<td>4.19 ± 2.29</td>
<td>4.44 ± 2.10</td>
</tr>
</tbody>
</table>

*Unmedicated autistics versus normal controls, one-tailed $t$-test: $p < 0.1$.

'Medicated groups versus unmedicated autistics, two-tailed $t$-test: $p < 0.1$. 

Table 1. Urinary 5-HIAA Excretion during Evening (5:00 PM–11:00 PM) and Overnight (11:00 PM–8:00 AM) Collection Periods in Normal and Autistic Subjects
Highly significant correlations were found between urinary 5-HIAA and creatinine excretion rates at all collection periods in normals ($r = 0.61-0.74$) and at the overnight collection from unmedicated autistics ($r = 0.50$).

5-HIAA excretion rate did not significantly correlate with body surface area, collection volume, or age for any of the groups.

Groups did not significantly differ in urine volume or creatinine excretion; the sexes did not differ in 5-HIAA excretion; and first and second collection periods did not differ in 5-HIAA excretion rate or its concentration relative to creatinine.

Pearson product–moment correlation coefficients computed for the 5-HIAA values ($\mu$g/mg creatinine) of the first and second urine collection showed a significant correlation in the normal controls ($r = 0.38$, $p < 0.05$), the unmedicated autistics ($r = 0.66$, $p < 0.005$), the medicated autistics ($r = 0.66$, $p < 0.005$), and the total group ($r = 0.59$, $p < 0.0005$). However, if the urinary 5-HIAA excretion was expressed as micrograms per hour, no significant correlations were observed between the two collections in the normal controls or any of the autistic subgroups, although the relationship approaches significance ($r = 0.36$, $p < 0.1$) in the normal control group and was highly significant ($r = 0.34$, $p < 0.01$) in the total group.

5-HT and Total TRP Values in Blood

Group means of whole blood 5-HT, platelet count, and total TRP in the normal and autistic populations are given in Table 2. The values of 5-HT in nanograms per milliliter and 5-HT in nanograms per $10^9$ platelets for the unmedicated autistics were significantly higher than those for the controls ($p < 0.01$ and $p < 0.05$, respectively). No significant differences were found for the platelet count and TRP values between the unmedicated autistics and the normal controls.

Medicated and unmedicated autistics did not significantly differ in 5-HT values, platelet counts, or TRP values. However, platelet counts were significantly higher in the group of autistics on haloperidol compared to the unmedicated group ($p < 0.05$).

Relationship of Urinary 5-HIAA, Whole Blood 5-HT, and Blood TRP

No significant correlations between urinary 5-HIAA and blood 5-HT, blood 5-HT and blood TRP, or between blood TRP values and urinary 5-HIAA were found for any of

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Serotonin (5-HT)</th>
<th>Platelet count</th>
<th>Tryptophan (TRP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng/ml</td>
<td>ng/10^9 platelets</td>
<td>(per nl)</td>
</tr>
<tr>
<td>Normal controls</td>
<td>17</td>
<td>113 ± 24.6^a</td>
<td>443 ± 112^a</td>
<td>261 ± 50.4</td>
</tr>
<tr>
<td>Autistics, unmedicated</td>
<td>14</td>
<td>163 ± 86.3^a</td>
<td>630 ± 333^a</td>
<td>262 ± 58.7^c</td>
</tr>
<tr>
<td>Autistics, all medicated</td>
<td>19</td>
<td>147 ± 56.0</td>
<td>586 ± 191</td>
<td>261 ± 77.2</td>
</tr>
<tr>
<td>Phenothiazines</td>
<td>6</td>
<td>132 ± 48.7</td>
<td>599 ± 217</td>
<td>222 ± 29.3</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>6</td>
<td>185 ± 85.7</td>
<td>569 ± 227</td>
<td>332 ± 86.7^e</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>8</td>
<td>136 ± 28.7</td>
<td>586 ± 173</td>
<td>245 ± 72.1</td>
</tr>
</tbody>
</table>

^aOne-tailed t-test: $p < 0.05$.
^bOne-tailed t-test: $p < 0.01$.
^cTwo-tailed t-test: $p < 0.05$. 

Table 2. Whole Blood Serotonin and Tryptophan in Autistic and Normal Subjects (Mean ± SD)
the groups. When the control group (n = 20) is used to describe a normal range of 5-HT (ng/ml), an individual having a 5-HT value of greater than 174 ng/ml (mean ± 1.65 SD) would be in the upper 5% and could be termed hyperserotonemic. By this criteria, 25% of the unmedicated autistics (4 of 16) and 25% of the total group of medicated autistics (1 of 8 using anticonvulsive medication, 2 of 6 using haloperidol, and 2 of 6 using phenothiazines) could be judged to be hyperserotonemic. The urinary 5-HIAA values of the hyperserotonemic autistics were compared with those of the autistic subjects with normal whole blood 5-HT (ng/ml) values and with those of the normal controls. The unmedicated hyperserotonemic autistics showed significantly greater 5-HIAA excretion (µg/mg creatinine) compared to the normal controls (p < 0.05) (Table 3). In the unmedicated autistics, a nonsignificant trend was found for higher urinary 5-HIAA (µg/mg creatinine) in the hyperserotonemic autistics compared to the normoserotonemic autistics in the overnight collection period (p < 0.10) and in the combined collection period (p = 0.11). When 5-HIAA excretion was expressed as micrograms per hour, no differences were seen between the hyperserotonemic autistics and the normoserotonemic autistics or the normal controls. In the group of unmedicated hyperserotonemic autistics, a high correlation was observed (r = 0.86) between urinary 5-HIAA excretion (µg/mg creatinine) and blood 5-HT (ng/ml) that tended to be significant (p < 0.1).

In the medicated autistic subjects, significantly greater 5-HIAA excretion (µg/mg creatinine) was observed in the hyperserotonemic subgroup during the overnight collection period (p < 0.05) and the total collection period (p < 0.05) compared to the normoserotonemic autistic subgroup (data not shown). The medicated autistic subgroup also excreted significantly more 5-HIAA (µg/mg creatinine) compared to normal controls (p < 0.01).

Discussion

Although the normal control and unmedicated autistic groups were well matched with respect to sex and age, and the expected hyperserotonemia was clearly observed in the
group of unmedicated autistics, no significant differences were found in urinary 5-HIAA excretion (expressed as μg/hr or μg/mg creatinine). This is in agreement with the findings of several previous reports (Shaw et al. 1959; Schain and Freedman 1961; Partington et al. 1973).

We did observe greater urinary 5-HIAA excretion (μg/mg creatinine) in a subgroup of autistic individuals with increased blood 5-HT values compared to normoserotonemic autistic subjects or normal controls. The difference between hyperserotonemic autistics and normoserotonemic autistics was not significant, however, differences between hyperserotonemic autistics and normal controls were highly significant. These differences were more marked in medicated autistics, and medicated autistics excreted greater amounts of 5-HIAA than unmedicated autistics. These findings are parallel to those of Hanley et al. (1977), who reported increased urinary 5-HIAA excretion in four autistic subjects with increased whole blood serotonin values compared to four normoserotonemic mentally retarded children before and after oral tryptophan loading.

No significant correlation was found between the urinary 5-HIAA excretion and whole blood 5-HT values in the normal groups, nor in any of the autistic groups. However, in unmedicated hyperserotonemic subjects, a high correlation was found between these two measures that tended to be significant. These correlation data obtained in the autistic groups should be judged with caution because of the timespan between the blood drawing and the urine collection. However, whole blood 5-HT values have been found to be very stable measures over a year’s time in unmedicated autistics (Minderaa et al. 1986a) and in normal subjects (Yuwiler et al. 1971).

Some discussion of the large positive correlations observed between the hourly excretion of 5-HIAA and creatinine is called for. No significant correlations were observed between hourly 5-HIAA excretion and body surface area or urine collection volumes. The correlations between hourly rates of 5-HIAA and creatinine excretion remained significant after partialling out body surface area. These facts suggest that the correlation was not simply due to larger subjects excreting greater amounts of both compounds, or to incomplete collections. The puzzling correlations between 5-HIAA and creatinine excretion remain unexplained and warrant further investigation, particularly as similar correlations were seen between other catecholamines or their metabolites and creatinine (Hollister and Moore 1970; Minderaa et al. 1986b). In the meantime, it would appear to be advisable to express metabolite excretion using both units (per hour and per milligram of creatinine).

It is not clear which aspect of 5-HT physiology is most important in setting blood 5-HT levels in normal subjects. The rates of gut production, platelet uptake and storage, and catabolism by monoamine oxidase (MAO) might all play a role. In some pathological circumstances, like carcinoid syndrome (Crawford et al. 1967), excessively increased 5-HT production does lead to increased blood 5-HT values and increased urinary 5-HIAA excretion. However, it appears that under most circumstances, gut production of 5-HT, to the extent that it can be assessed by measuring urinary 5-HIAA, does not play a predominant role in setting blood 5-HT levels in either normal or autistic subjects.

Although the bulk of the data presented indicate similar gut production of 5-HT autistic and normal individuals, the increase of urinary 5-HIAA excretion and the high correlation between urinary 5-HIAA excretion and blood 5-HT seen in hyperserotonemic autistic subjects suggests that, in fact, some relationship between gut production and blood levels might exist in these subjects.

In conclusion, one can say fairly confidently that the increased whole blood 5-HT
values seen in autism are not due to a large increase in gut production of 5-HT. However, the possibility remains that the significant, but relatively small, increase in gut production seen in the hyperserotonemic autistic subjects might contribute to elevated whole blood 5-HT levels. Further assessment of urinary 5-HIAA excretion in larger groups of hyperserotonemic autistic subjects might throw more light on this issue.

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References


