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Whole Blood Serotonin and Tryptophan in Autism: Temporal Stability and the Effects of Medication[1]

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Whole blood serotonin (5-HT) was significantly increased in a drug-free autistic group (n = 17) compared to age- and sex-matched normal control (n = 20). Blood tryptophan (TRP) values and platelet counts were similar in unmedicated autistics and normal subjects; but whole blood concentrations of TRP were significantly lower, and 5-HT values tended to be lower in the medicated group compared to unmedicated autistics. Highly significant intraclass correlation coefficients and low mean percentage differences were found for repeated measures over a year's period of whole blood 5-HT and

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the platelet count in the unmedicated but not in the medicated group. Blood TRP values were highly variable over time in both the medicated and drug-free autistic groups.

INTRODUCTION

Schain and Freedman (1961) were the first to describe increased group mean values of whole blood serotonin (5-HT) in autism. Since then, this finding has been confirmed by many investigators (Anderson et al., 1987; Goldstein, Mahanand, Lee, & Coleman, 1976; Hanley, Stahl, & Freedman, 1977; Hoshino, Kumashiro, & Kaneko, 1979; Hoshino et al., 1984; Ritvo et al., 1970; Takahashi, Kanai, & Miyamoto, 1976; Yuwiler et al., 1975). The cause and significance of the elevation is unclear.

Assessment of tryptophan (TRP) levels in blood or TRP metabolism (Anderson et al., 1987; Heeley & Roberts, 1965; Hoshino et al., 1979; Hoshino et al., 1984; Minderaa et al., 1987; Schain & Freedman, 1961; Shaw, Lucas, & Rabinovitch, 1959), assessment of 5-HT uptake and release and other membrane properties of blood platelets (Anderson, Minderaa, Benthem, Volkmar, & Cohen, 1984; Boullin, Coleman, O'Brien, & Rimland, 1971; Katsui, Usuda, & Koizumi, 1986; Lucas, Warner, & Gottlieb, 1971; Yuwiler et al., 1975), and studies of 5-HT catabolism (Campbell, Friedman, Green, Small, & Burdoch, 1976; Cohen, Young, & Roth, 1977) have not resulted in a clear insight into the causes of increased blood 5-HT values in autistics.

Several other issues require further clarification. An important point considered in several reports in whether platelet count is increased in autistic patients and whether hyperserotonemia could be due to elevated platelet counts (Anderson et al., 1987; Hanley et al., 1977; Ritvo, 1977; Ritvo et al., 1970).

Furthermore there is some disagreement as to the effect of different kinds of antiepileptic or neuroleptic medication on mean values of whole blood 5-HT (Anderson et al., 1987; Hoshino et al., 1979; Jackman, Luchins, & Meltzer, 1983; Schain et al., 1961; Stahl, Woo, Mefford, Berger, & Ciaranello, 1983).

Only a few studies have dealt with the stability of 5-HT values over time (Ritvo et al., 1970; Wirz-Justice, Lichtensteiner, & Feer, 1977; Wirz-Justice & Puhringer, 1978; Wirz-Justice & Richter, 1979; Yuwiler, Ritvo, Bald, Kipper, & Koper, 1971). The stability of whole blood 5-HT over a longer period of time and the effect of medication on this stability in autistic children has not been previously assessed.

SUBJECTS AND METHODS

The autistic group comprised 40 students enrolled in a school for autistic individuals (28 boys, 12 girls; mean age ± *SD*: 19.4 years ± 4.9. A

diagnosis was made by several child psychiatrists and confirmed for this study by another child psychiatrist (R.M.) based on psychiatric evaluation, anamnestic information, and observations by the school staff before the data were known. Thirty-eight individuals received the diagnosis of autism, full syndrome present, according to DSM-III criteria (299.00). Two received the diagnosis of infantile autism, residual state (299.01). All autistic subjects were moderately to severely retarded, as determined by standard intelligence tests (Stanford-Binet, WAIS).

Seventeen of the autistic subjects were unmedicated for at least 6 months before the blood drawing. Of the 23 medicated autistics, 7 used phenothiazines, 7 used haldol, and 9 used anticonvulsant medication. These medicated subjects used their medication in a constant manner for more than 3 months before the blood was drawn. A control group comprised 20 high school students, teachers, and hospital employees, comparable in sex (15 boys, 5 girls) and age (mean age ± *SD*: 22.0 years ± 7.5). All reported being in good physical health and using no medication.

In a subgroup ($n = 26$) of the autistic subjects two blood samples were obtained approximately 1 year apart in order to test long-term stability. These subjects were divided into further subgroups according to their medication history. Ten were unmedicated for at least 6 months before the first blood drawing and remained unmedicated throughout the study period. Another 10 were medicated with a fixed dose for the period extending from at least 3 months before the first blood drawing through the time of the second drawing. A final subgroup comprised six autistic subjects who started or increased the dose of the medication between the first and the second blood drawing.

The mean time span (± *SD*) between the two blood drawings for the unmedicated group, the medicated (fixed dose) group, and the group with changed medication was 12.7 ± 0.7 months, 11.4 ± 4.9 months, and 12.3 ± 1.6 months, respectively.

Blood was drawn from all subjects at 10 a.m.–11 a.m. Whole blood 5-HT and TRP were analyzed using a HPLC-fluorometric method described elsewhere (Anderson, Young, Cohen, Schlicht, & Patel, 1981).

RESULTS AND DISCUSSION

Group Means and Correlations of Blood 5-HT, TRP, and Platelet Count

Serotonin values in the unmedicated autistic individuals were significantly higher compared to the normal controls if expressed as ng/ml ($p = .01$) as well as expressed as ng/10^9 platelets ($p = .02$) (Table I).

No significant differences were found for whole blood tryptophan levels or platelet count between unmedicated autistics and normal controls.

Table I. Whole Blood Serotonin and Tryptophan in Autistic and Normal Subjects[a] (Mean ± *SD*)

Subjects	*n*	Serotonin (5-HT) ng/ml	Serotonin (5-HT) ng/10^9 platelets	Platelet count per nl	Trytophan (TRP) μg/ml
Normal controls	20	116 ± 35.0$_a$	465 ± 162$_b$	258 ± 47.7	5.73 ± 1.18
Autistics unmedicated	17	163 ± 81.7$_{a,g}$	620 ± 303$_b$	267 ± 67.8$_{e,g}$	5.38 ± 1.13$_{c,d,f}$
Autistics medicated	23	146 ± 53.2	569 ± 192	265 ± 72.7	4.71 ± 1.14$_d$
Phenothiazines	7	128 ± 45.8$_g$	561 ± 223	234 ± 42.8$_g$	5.21 ± 1.25
Haloperidol	7	176 ± 75.5	550 ± 197	324 ± 73.5$_e$	4.52 ± 1.19$_f$
Anticonvulsants	9	136 ± 26.9	591 ± 163	242 ± 68.0	4.48 ± 1.02$_c$

[a]One tailed *t* test unmedicated autistics versus normal controls: $_a$: $p = .01$; $_b$: $p = .02$. One tailed *t* test medicated groups versus unmedicated autistics: $_c$: $p = .03$; $_d$: $p = .04$; $_e$: $p = .05$; $_f$: $p = .07$; $_g$: $p = .09$.

In the group of normal controls no correlation was found between 5-HT ng/ml and platelet count. Furthermore a significant negative correlation was found between 5-HT ng/10^9 platelets and platelet count ($r = -.45$, $p = .04$). On the contrary, in the unmedicated autistics 5-HT ng/ml values were significantly correlated with platelet count ($r = .49$, $p = .04$). In this group no correlation was found between 5-HT ng/10^9platelets and platelet count.

No correlation has been observed between age and any of the measures except for a negative correlation in the total medicated group between platelet count and age ($r = -.52$, $p = .01$). A t test did not show significant differences between male and female for any of the measures in the control group or the group of unmedicated autistics.

In general, these findings are in agreement with previous studies of 5-HT and TRP levels and platelet counts in autistic individuals, and tend to suggest that group differences in 5-HT levels are not due to differences in TRP levels or platelet counts. However, the group differences in correlations seen between 5-HT measures (ng/ml and ng/10^9 platelet) and platelet count, indicate other altered aspects of blood 5-HT in autism. It is not clear whether these additional differences are related to the basic finding of increased blood 5-HT in autism.

Effect of Medication

No significant differences of 5-HT values were found between the medicated and unmedicated subgroups of the total ($n = 40$) autistic group, although a tendency toward lower 5-HT values was observed in the combined medicated group and in two of the three medicated subgroups (see Table I). However, the differences were not as marked or as consistent as seen in the previous study of Anderson et al. (1987). Several other studies have not observed an effect of neuroleptic or anticonvulsant medication on blood 5-HT levels in retarded (Partington, Tu, & Wong, 1973), autistic (Hoshino et al., 1979; Schain & Freedman, 1961), or schizophrenic (Jackman et al., 1983; Stahl et al., 1983) subjects. The effect of medication is discussed further in the section on long-term stability.

The finding of significantly lower TRP levels in medicated autistic subjects (see Table I) is consistent with a previous report (Anderson et al., 1987), where a nonsignificant trend for lower TRP values in neuroleptic- and anticonvulsant-medicated autistics have been observed. These findings, together with the observed negative correlation between dose of neuroleptics and TRP values, strongly suggest that neuroleptics and anticonvulsant medication lower blood total TRP levels in autistics.

Long-Term Stability

No significant differences were observed between the 5-HT (ng/ml, and ng/10^9 platelets), the platelet count, and TRP μg/ml values of the first and the second blood drawing in the drug-free group of autistic individuals. In the medicated group ($n = 10$), values of 5-HT (ng/10^9 platelets) of the second blood drawing (562 $\pm$ 188) were significantly increased compared to the first drawing (431 $\pm$ 178, $p = .05$). With respect to 5-HT values expressed as ng/ml this difference was close to the level of significance ($p = .06$). In the group with initiated or increased medication TRP values of the second blood drawing were significantly lower compared to the first blood drawing ($p = .01$).

Significantly lower 5-HT values (ng/ml) were observed in the medicated group ($n = 10$, fixed dose) compared to the unmedicated group ($n = 10$) for the first blood drawing (104 $\pm$ 36.9 vs. 167 $\pm$ 81.8, $t = 2.22$, $p = .02$). For the second blood drawing, only a trend was found for lower 5-HT values (ng/ml) in the medicated group (fixed dose) compared to the unmedicated autistic subjects ($t = 1.34$, $p = .10$). However, using a MANOVA procedure, no significant group–time effect was found ($F = 1.21$, $p < .12$).

Highly significant intraclass correlations (Bartko, 1976) were found between the values of the first and the second blood drawing for 5-HT ng/ml ($r = .92$, $p < .01$), 5-HT ng/10^9 platelets ($r = .86$, $p < .01$) and the platelet count ($r = .93$, $p < .01$), but not for TRP, in the unmedicated group. No significant correlation for any of the measures was found in the medicated group, or in the group with changed medication. The highly significant intraclass correlation coefficients for the two observations of 5-HT (ng/ml and ng/10^9 platelets) and platelet count, and the relatively low mean intraindividual percentages of change (15, 14, and 7%, respectively) of these values show that these variables are stable in unmedicated autistics over a time span of more than a year. In the medicated group and the group with changed medication no significant correlations were found between any of the variables for the first and second blood drawing. Furthermore, rather high mean percentages of change of 5-HT values (ng/ml and ng/10^9 platelets) and platelet counts (54, 68, and 19%, respectively) were observed in the medicated groups. It appears that anticonvulsant, phenothiazine, and haloperidol medication have a large negative influence on the temporal stability of 5-HT levels and the platelet count. These findings are in agreement with those of Jackman et al. (1983) with schizophrenic patients. Neuroleptic medication in the group that started medication or increased the dose caused a decrease of 5-HT values in all cases ($n = 6$). For this subgroup the concentration of 5-HT (ng/ml) tended to be lower (201 $\pm$ 86.3 vs. 130 $\pm$ 50.2) and values of 5-HT per platelets (ng/10^9) were significantly lower (783 $\pm$ 337 vs. 533 $\pm$ 242, $p = .02$)

for the second drawing compared to the first drawing. Although no firm conclusions can be made on the basis of these data, they might indicate that the initiation of neuroleptic medication or a robust increase in dose of neuroleptics result in a drop of 5-HT values. This is in agreement with the lowering of 5-HT values in neuroleptic medicated groups previously reported (Anderson et al., 1987). No significant intraclass correlations were found between the TRP values of the first and second blood drawing for any of the three groups. Furthermore, relatively high mean percentages of change were seen in all groups. This means that TRP values are not very stable over a year's time in either unmedicated or medicated autistic subjects. A paired t test showed a significant intraindividual difference ($p = .01$) between the two TRP values for the group with changed medication, with the values of the second blood drawing being lower than the values of the first blood drawing. This gives further support to the conclusion that neuroleptic (and anticonvulsant) medication lowers blood TRP values in autistic subjects.

This study on the temporal stability and the effects of medication on whole blood 5-HT, TRP, and platelet counts has indicated to some extent what precautions and directions might be taken in research on the hyperserotonemia of autism.

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