Autistic Children and Their First-Degree Relatives: Relationships Between Serotonin and Norepinephrine Levels and Intelligence

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Whole-blood serotonin (5-HT) and plasma norepinephrine (NE) were studied in 16 autistic children, 21 siblings of autistic children, and 53 parents of autistic children. Both plasma NE and whole-blood 5-HT were negatively correlated with vocabulary performance. Whole-blood 5-HT and plasma NE did not differ between autistic children with or without histories of self-injurious behavior or decreased pain sensitivity. Eighteen subjects were hyperserotonemic (whole-blood 5-HT >270 ng/ml). For these subjects, plasma NE was significantly higher than for subjects without hyperserotonemia. Seven of 10 families with one hyperserotonemic member had two or more hyperserotonemic members. Observations of familiality of whole-blood 5-HT suggest that larger-scale and more focused study of whole-blood 5-HT as a possible genetic marker may be productive.

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Whole-blood serotonin (5-HT) has been consistently shown to be elevated in groups of autistic children relative to normal controls.[1–3] Severely retarded children also have been shown to have equal or greater elevations in whole-blood 5-HT.[1,4,5] In addition, about 30% of autistic children and 50% of severely mentally retarded children have been found to have hyperserotonemia (whole-blood 5-HT greater than 1.67 standard deviations above the mean of normal control subjects).[3,5]

Other studies have found elevated plasma norepinephrine (NE) levels in autistic children compared with controls.[6] The mechanisms of these peripheral neurotransmitter elevations have not yet been identified. The search for specific dimensions, traits, or attributes that might be linked to these elevations has not yet yielded any reliable or distinctive correlations.

A previous study showed a negative correlation of full-scale IQ, verbal IQ, and developmental quotient with platelet 5-HT in psychotic children.[7] Another study found that severely mentally retarded children had higher whole-blood 5-HT than did mildly retarded children, who in turn had higher whole-blood 5-HT than did normal children.[5] Although these findings have not been observed uniformly, these studies led us to suspect a link between cognitive function and blood 5-HT, at least in these populations.

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Consequently, we conducted a study to determine whether whole-blood 5-HT or plasma NE were related to measures of intelligence in 16 autistic children, 21 siblings of autistic children, and 53 parents of autistic children. In addition, we studied these peripheral neurotransmitter levels in relation to presence or absence of a history of decreased pain sensitivity or of self-injurious behavior in the autistic children. Family correlations of whole-blood 5-HT and plasma NE were studied because a separate study conducted in 1982 and 1983 using a different method for analysis of whole-blood 5-HT[8] revealed significant correlations of the 5-HT and plasma NE among autistic children and their first-degree relatives (Leventhal et al., submitted for publication).

METHODS

Subjects

One hundred nineteen subjects were recruited from local chapters of the National Society for Autistic Citizens for various studies in 1987 and 1988. Both autistic children and all available first-degree relatives were considered subjects after they gave informed consent. Plasma ultrafiltrate 5-HT[9] and platelet 5-HT_2 binding data (Perry et al., submitted for publication) were reported previously for less than one-half of this sample. Autistic children had been diagnosed previously by other clinicians, and *Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised* (DSM-III-R)[10] diagnosis of autistic disorder was confirmed by two child psychiatrists (E.C. and B.L.).

Subjects described here were selected from the larger sample of 119 subjects based on two criteria: being medication-free (except for aspirin, acetaminophen, or over-the-counter antihistamines) and being able to give scorable responses on either the Peabody Picture Vocabulary Test–Revised (PPVT-R),[11] the vocabulary subtests of the Wechsler Intelligence Scale for Children–Revised (WISC-R),[12] or on the Wechsler Adult Intelligence Scale–Revised (WAIS-R).[13] First-degree relatives included on the basis of the above criteria were not excluded if the autistic proband was excluded. Analysis of relationships of whole-blood 5-HT and plasma NE among family members included subjects who could not give scorable responses to vocabulary testing. Demographic information is presented in Table 1.

TABLE 1. Demographic and neurochemical data for autistic subjects and family members

Characteristic	Autistic Subject	Sibling	Parent
Sex			
Male	16	12	24
Female	0	9	29
Mean±SD age	9.0±3.5	11.9±5.4	37.3±5.1
Mean±SD Vocabulary Quotient[a]	5.5±2.6	11.1±3.0	10.4±2.4
Mean±SD WAIS-R[b] Block Design Score	9.4±3.4 (n=9)	11.1±3.6 (n=20)	10.1±2.2 (n=53)
Mean±SD whole-blood 5-HT[c] (ng/ml)	266±122	226±95	182±64
Mean±SD plasma NE[d] (pg/ml)	330±151	287±102	252±85

[a]F=27.22, p<.00005; autistic < parent, sibling, p<.001
[b]Wechsler Adult Intelligence Scale–Revised
[c]F=6.85, p<.002; autistic > parent, p<.005
[d]F=3.68, p<.03; autistic > parent, p<.05

Cognitive Testing

Adult subjects completed the WAIS-R Vocabulary and Block Design subtests, and age-adjusted scaled scores were determined.[13] Subjects between the ages of 6 and 16 completed the WISC-R Vocabulary and Block Design subtests.[12] Subjects between the ages of 4 and 6 completed subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI).[14] The PPVT-R[11] was administered to autistic children and siblings less than 10 years old. Seven autistic children and one of their siblings were unable to give scorable responses on the WISC-R Block Design subtest, even though they were able to complete one of the vocabulary tests.

Because eight of the autistic children and one of the younger siblings could not perform the WPPSI or WISC-R vocabulary subtest, a composite verbal quotient (VQ) was obtained by using Wechsler scaled scores or by dividing PPVT-R mental age scores by chronological age and multiplying the ratio by 10. If an individual subject had both PPVT-R and Wechsler vocabulary scores, the scores were averaged to determine VQ.

Neurochemical Analysis

Whole-blood 5-HT was measured by high pressure liquid chromatography (HPLC) with fluorometric detection.[15] Intraassay and interassay coefficients of variation were 2.9% and 3.9%, respectively. Plasma NE was measured by HPLC with electrochemical detection,[16] with an intraassay coefficient of variation of 2.9% and an interassay coefficient of variation of 4.0%.

Because the laboratory recently had developed a method for measuring plasma indoleacetic acid (IAA) as a part of studies of peripheral tryptophan metabolism in these disorders, it was measured in the presently reported samples. HPLC with fluorometric detection based on the assay of Martinez and colleagues[17] was used, except that the mobile phase consisted of methanol/water (3:7) and .05 M sodium acetate at pH 4.3. A flow rate of 1.5 ml/min was employed. Intraassay coefficient of variation was 1.8%, and interassay coefficient of variation was 2.4%. No significant correlation between

IAA and other measures was found. (Data are available from the authors on request.)

The preferred and standard method for measurement of plasma NE is to insert an intravenous catheter into a subject who rests in a supine position for 20 minutes before the experimenter draws a supine, resting sample and then stands for five minutes before the researcher draws the standing NE sample.

Because a majority of unselected autistic children resist allowing a catheter to remain in their forearm, we collected blood samples via catheter while the subject was sitting and in a nonfasting state, at the beginning of the study. We used the preferred method of collection for the last 21 parents, however, and sitting measures inadvertently were not collected for this group. Although the collection method for this latter group was not equivalent to the sitting measure, plasma NE data for them—based on their supine, resting blood draw—were analyzed for any differences this group might have contributed to the data, and no effects were observed. Samples were frozen at –2°C for up to 16 hours before transport to storage at –70°C until analysis.

Clinical Measures

History of decreased pain sensitivity and self-injurious behavior was based on a history obtained from the parents by researchers blind to the neurotransmitter or cognitive measures.

Data Analysis

Correlations are reported as two-tailed. Where analyses of variance revealed significant differences, differences between specific cells were determined by the Scheffé Test for multiple ranges. Means and standard deviations are reported. Hyperserotonemia was defined as whole-blood 5-HT greater than the mean plus 1.67 standard deviation of an adult control group.[18] Statistics were calculated on an IBM PC using SPSS/PC+, Version 2.0.

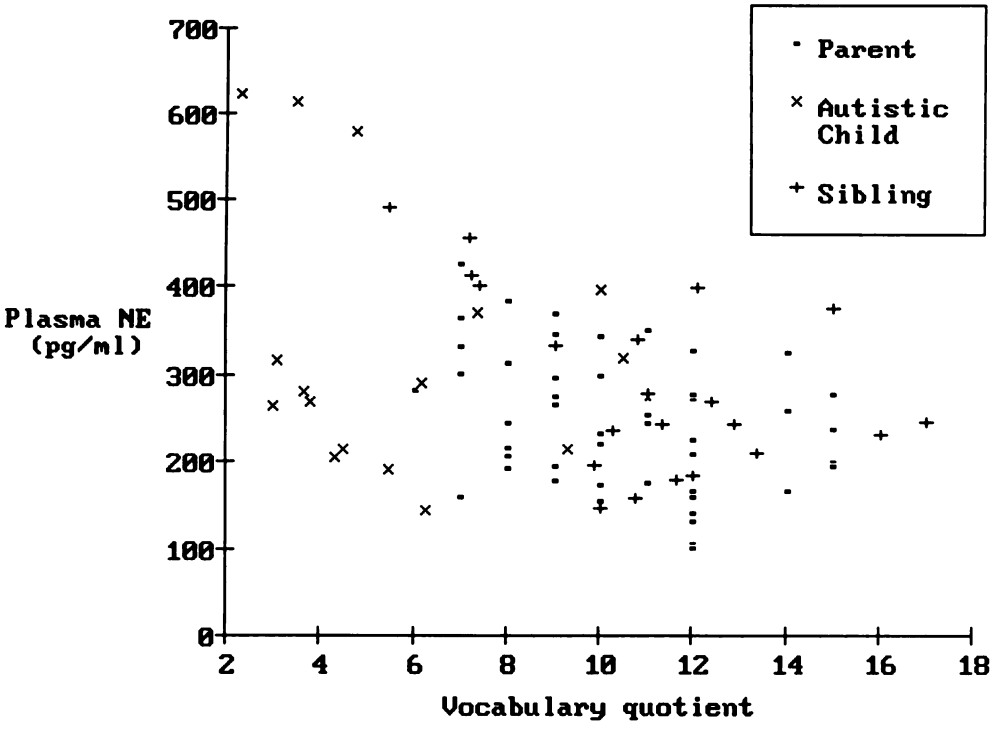
RESULTS

Plasma NE and whole-blood 5-HT were significantly increased in autistic subjects, relative to the parents of autistic children (see Table 1).

VQ was negatively correlated with plasma NE ($r=-.39$; $n=90$; $p<.0005$), and whole-blood 5-HT ($r=-.21$; $n=90$; $p<.05$) (see Figures 1 and 2). The relation between whole-blood 5-HT and VQ was not significant when autistic subjects were excluded ($r=-.11$; $n=74$; $p>.3$). There was not a significant correlation between VQ and whole-blood 5-HT when autistic children were analyzed separately ($r=.22$; $n=16$; $p>.4$).

A median split was performed to confirm the relation of VQ with NE and 5-HT. Only NE showed a statistically significant difference ($p<.01$) between subjects with VQ below or equal to the median of 10.0 (301±117; n=48) and above the median (243±84; n=42). To determine whether the relation between vocabulary performance and plasma NE might be caused by the lower VQ scores and higher plasma NE of autistic subjects, autistic subjects were excluded from the median split analysis. Plasma NE was higher for the parents and siblings whose VQ was less than 11.0 (284±93; n=36) than for parents and siblings whose VQ was greater than or equal to 11.0 (241±85; n=38; $p<.05$). To determine whether the six

FIGURE 1. Relationship between plasma NE levels and vocabulary quotients in autistic children and first-degree relatives



parents and siblings whose VQ was less than or equal to 7.0 contributed to this difference, we removed their data from the comparison. We found that plasma NE remained higher for the parents and siblings whose VQ was between 7.0 and 11.0 (276±91; n=36), compared with parents and siblings whose VQ was greater than 11.0 (227±75; n=30; p<.03). Analyses using only age-adjusted Wechsler vocabulary scaled scores (n=81) did not differ significantly from those using VQ (data available from authors on request).

In our study, 5-HT and NE were not significantly correlated with block design scores when all subjects were considered. However, whole-blood 5-HT (r=–.35; p<.01), but not NE, was significantly negatively correlated with Block Design scores when only parents were considered (n=53).

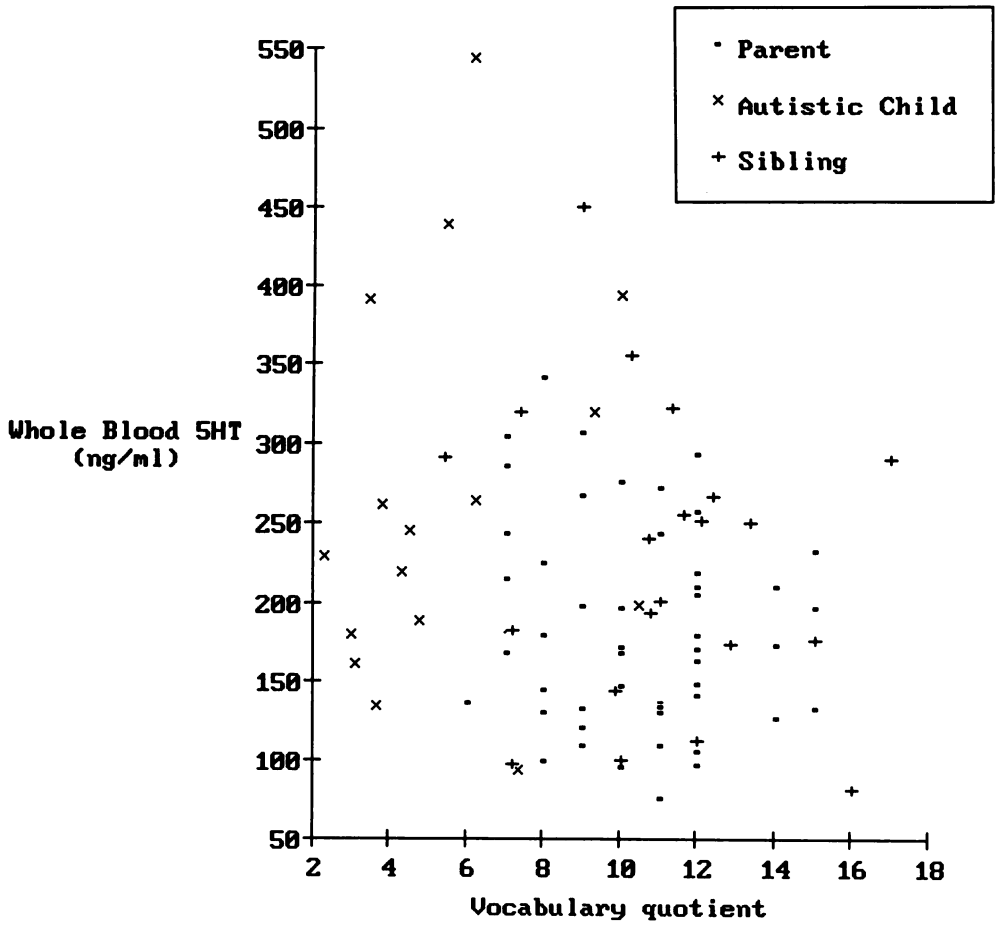
Five autistic children (31%), seven siblings (33%), and six parents (11.3%) had whole-blood 5-HT greater than 270 ng/ml. Plasma NE of these 18 hyperserotonemic subjects (324±117) was significantly greater than the plasma NE of 72 normoserotonemic subjects (261±101; p<.03). Hyperserotonemic autistic subjects (340±171) did not have significantly higher plasma NE than normoserotonemic autistic subjects (326±149). However, the 13 nonautistic hyperserotonemic subjects (318±97) had a higher mean plasma NE than did the 61 nonautistic normoserotonemic subjects (p<.02).

TABLE 2. Relationships between whole-blood serotonin (5-HT) levels of autistic subject and family members

Family Member	Mother	Father	Sibling
Autistic subject	.71** (n=18)	.32 (n=17)	.67* (n=10)
Mother		–.15 (n=22)	.40 (n=16)
Father			.57* (n=14)

*p<.05; **p<.001
n=number of subjects in pair-wise correlation

FIGURE 2. Relationship between whole-blood levels of 5-HT and vocabulary quotients in autistic children and first-degree relatives



Whole-blood 5-HT was significantly correlated between autistic children and their mothers and siblings and between fathers and siblings of autistic children (see Table 2). In seven of 10 families in which one family member was hyperserotonemic, another family member also was hyperserotonemic. There were no significant correlations of plasma NE among family members.

Whole-blood 5-HT and plasma NE were not different in subjects with or without a history of decreased pain sensitivity or self-injurious behavior (see Table 3).

Plasma NE drawn in different positions from 20 parents was robustly correlated. Plasma NE drawn when the subject was in the supine position at the time the catheter was inserted was correlated with plasma NE drawn with the subject in the supine position after resting 20 minutes (r=.86; p<.0005), and with plasma NE drawn after the

subject was standing for five minutes (r=.82; p<.0005). Plasma NE drawn from the subject lying in the supine position after resting 20 minutes after catheter insertion was correlated with plasma NE drawn after the subject had been standing for five minutes (r=.85; p<.0005).

Whole-blood 5-HT and plasma NE were both negatively correlated with age (5-HT: r=–.38, p<.0005; NE: r=–.24, p<.03).

DISCUSSION

Inverse correlations were found between plasma NE and whole-blood 5-HT with measured vocabulary ability in a group of autistic children and their first-degree relatives. For whole-blood 5-HT, this was accounted for by combining the autistic subjects with lower mean VQ and higher mean whole-blood 5-HT with the unaffected siblings and parents. For plasma NE, this relation was accounted for primarily by the unaffected family members. Only the correlation between plasma NE and vocabulary performance was confirmed by median split analysis. Wechsler age-adjusted Block Design scores were not correlated with the peripheral neurochemicals studied, except for whole-blood 5-HT in the parents of autistic children. This may have been due to the number of post hoc correlations.

Kuperman and colleagues[19] did not find a relation between intelligence of autistic children and whole-blood 5-HT. The present study also revealed a nonsignificant positive correlation for autistic children between vocabulary performance and whole-blood 5-HT. Therefore, there does not appear to be a significant correlation between whole-blood 5-HT levels and cognition in groups of autistic children or children with attention deficit disorder.[20] An earlier study conducted to determine whether whole-blood 5-HT was related to WAIS-R vocabulary performance in a group of normal control adults did not reveal a significant correlation.[18]

The present study failed to find a significant correlation between whole-blood 5-HT and vocabulary performance for first-degree relatives of autistic children considered separately. However, studies of mentally retarded children have revealed a relation between increasing levels of whole-blood 5-HT and increasing severity of mental retardation.[5,7] Although there is little evidence for a significant relation between whole-blood 5-HT and cognition in nonretarded populations, the relationship between cognition and whole-blood 5-HT in mentally retarded, nonautistic groups warrants further study.

Plasma NE of autistic children was elevated compared with that of their parents but not of their siblings. Autistic subjects had been shown to have higher NE values than age-matched control subjects.[6] Therefore, siblings also may have elevated plasma NE relative to normal, unrelated subjects, but tests of a normal, unrelated control group would be necessary to determine such a difference. Plasma NE drawn from subjects in the supine position at catheter insertion, supine after resting 20 minutes, and after standing 20 minutes was highly correlated. NE was higher in the samples taken from subjects in the standing position, as expected. The robust correlations of NE measures lend support for including the parents with NE measured in two different positions in this study, although the relation between VQ and NE was similar when these subjects were excluded from analysis. In addition, the NE correlation with VQ was stronger when the last 21 parents with supine, resting NE values were excluded. The correlation between NE and VQ remained significant when the autistic subjects were excluded. Moreover, the correlation remained significant when subjects with VQ less than or equal to 7.0 also were removed.

We know of no previous studies considering the relation between plasma NE and measured intelligence. There are few studies pertaining to the possible relation between catecholamines and cognition. Tennes and colleagues[21] found a positive correlation (r=.44) between urinary epinephrine excretion and intelligence, as measured by the Cognitive Abilities Test, in 14 normal second-grade girls, but found trends toward negative correlations of urinary epinephrine and NE with intelligence test scores in 16 boys. There were no significant differences in catecholamine excretion among subject groups with IQs between 86 and 105, 106 and 115, or 116 and 138. Raskind et al.[22] found elevated plasma and CSF NE and 3-methoxy-4-hydroxyphenylglycol (MHPG) in severely, but not moderately, impaired patients with Alzheimer's disease, although the relation between these findings and cognition is unclear.

These correlations—particularly between NE and vocabulary performance—are not sufficient to suggest a causal relation or mechanism for this relation. Collection

TABLE 3. Relationships of history of current or past self-injurious behavior (SIB) or decreased pain sensitivity (DPS) to peripheral levels of neurotransmitters in autistic children

Subject's Behavior	Whole-blood Serotonin (ng/ml)	Plasma Norepinephrine (pg/ml)
History of SIB		
no (n=8)	289±144	275±140
yes (n=8)	244±100	385±150
History of DPS		
no (n=9)	281±138	343±164
yes (n=7)	247±103	311±169

of NE in a standard manner from normal adults and children is necessary to determine whether the relations presented here are caused by the collection of NE under a stressful condition in most subjects (because of the insertion of the catheter), by response to orthostatic changes, by mechanisms specific to relatives of autistic subjects, or by a more general relation between plasma NE and verbal ability. Central measures of NE would be useful, although other investigators have found that plasma NE is highly correlated with both CSF NE and MHPG.[22–24]

Although whole-blood 5-HT and plasma NE were not significantly correlated for all subjects, hyperserotonemic subjects, whether autistic or not, had higher plasma NE than normoserotonemic subjects.

This study confirms previous studies that have shown significant correlations of platelet-rich plasma[25] and whole-blood 5-HT among family members (Leventhal et al., submitted for publication). Robust correlations were found between autistic subjects and their mothers and siblings, as well as between fathers of autistic subjects and siblings of autistic subjects. Furthermore, the finding that 70% of families with one hyperserotonemic member had at least one first-degree relative with hyperserotonemia is consistent with a study by Abramson and colleagues[26] that found that 64% of first-degree relatives of hyperserotonemic probands were hyperserotonemic, and a study conducted by our group (Leventhal et al., submitted for publication) that found that 30% of first-degree relatives of hyperserotonemic autistic probands were hyperserotonemic. These studies suggest that the trait of hyperserotonemia is familial, although not necessarily genetic. Although first-degree relatives were found to have an increased incidence of hyperserotonemia, this did not lead to an elevation of group mean whole-blood 5-HT in the parents. In contrast, no relationships within families of plasma NE were identified.

Decreased pain sensitivity and self-injurious behavior were not related to the peripheral neurochemicals studied. These data must be considered preliminary because of the small number of subjects and the absence of a standardized interview. In addition, studies of CNS measures of serotonergic function are needed to assess whether decreased CSF 5-HIAA found in other aggressive conditions[27,28] is present in mentally retarded or autistic subjects with self-injurious behavior.

In conclusion, this study suggests an inverse relationship between plasma NE and vocabulary performance that may be more fully elucidated with more direct and comprehensive measurement of both cognition and noradrenergic function. In addition, the study found that levels of whole-blood 5-HT and the trait of hyperserotonemia were familial. Focused study on a larger scale of hyperserotonemia as a possible genetic marker is suggested by these findings.

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