Tryptophan Metabolism in Children with Attentional Deficit Disorder

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The authors present the first report, to their knowledge, of hyperserotonemia in children with attentional deficit disorder who had normal intelligence. Hyperserotonemic children had significantly lower levels of plasma total and protein-bound tryptophan and a higher percentage of free tryptophan than those with normal serotonin levels. Plasma kynurenine did not differ, suggesting that the hyperserotonemia is not due to a blockade of the kynurenine pathway but may reflect an increase in tissue tryptophan uptake and use.

Although central serotonergic mechanisms have been implicated in arousal and motor reactivity (1), systems of primary importance in the attentional deficit disorders, there have been few studies of serotonin (5-HT) concentrations in children with such a disorder. Two studies reported decreased concentrations of blood 5-HT (2, 3) and one study (4) reported normal levels in children with attentional deficit disorder. Hyperserotonemia, a statistically significant elevation in platelet concentrations of 5-HT (5), had (apart from carcinoid and scleroderma) been found repeatedly and reliably in subgroups of autistic and severely retarded children (see reference 5 for review), but not in children with attentional deficit disorder who have, as a group, average intelligence.

As part of an ongoing study of tryptophan metabolism in neuropsychiatric disorders, we investigated hyperserotonemia and the correlations between 5-HT and other blood tryptophan metabolites, correlations between IQ and 5-HT, the effects of stimulants on 5-HT, and possible correlations between 5-HT and behavior in children with attentional deficit disorders.

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METHOD

We studied 55 children with attentional deficit disorder, with or without hyperactivity, from the private practice of a pediatrician (K.M.) specializing in such disorders. On the basis of history reported by parents and by school personnel and records, psychiatric interview, physical and neurological examination, speech and language evaluation, and psychoeducational testing, the child was diagnosed according to *DSM-III* criteria. The children were from 4 to 17 years of age (mean, 10.0±3.2 years). Their IQs ranged from 62 to 143 (mean, 105±26); 1 child had an IQ in the 60s and 2 had IQs in the 70s. Because, within the age range of our patients, there is no significant variation of 5-HT concentrations among normal children and adults (6), our control population consisted of 38 young adults with no known physical or emotional problems, ranging in age from 18 to 26 (mean, 23.6±2.1) (see Comment section for further discussion).

Specific Experiments

1. In all 55 children, platelet 5-HT, plasma tryptophan (total, free, and bound), and kynurenine were measured after 3 drug-free days.
2. Both to determine if a 3-day drug-free period was adequate and the stability of 5-HT levels, 13 children were randomly selected for 5-HT measures after a 10-day drug-free period.
3. The effect of stimulant medication on platelet 5-HT was investigated in 33 children (those willing to have another sample drawn). All had resumed taking their medication for at least 3 months; 20 were taking *d*-amphetamine and 13 were taking methylphenidate. According to clinical judgment (K.M.), all were receiving their optimal dose and showing their maximal therapeutic response.
4. A linear 5-point scale of overall severity was used to rate the children's behavior at the initial treatment intake; the same rater (K.M.), blind to the biochemical findings, evaluated behavior during the study.

All samples were drawn in the early morning, after an overnight fast and immediately before the morning medication was given. Procedures were explained and informed consent obtained from all patients, their parents, and controls.

Blood was collected and processed as previously

TABLE 1
Plasma Indole Measures in Children with Attentional Deficit Disorder Who Have Normal or Elevated Concentrations of Platelet Serotonin (5-HT)

	Children with Normal 5-HT (N=42)		Children with Elevated 5-HT (N=13)		Analysis[a]	
Measure	Mean	SD	Mean	SD	t	Significance
Platelet 5-HT ($\mu g/10^9$ platelets)	0.57	0.20	1.47	0.38	10.99	$p<.0005$
Tryptophan						
Total (μg/mg platelet-poor plasma)	12.78	5.81	8.28	2.60	2.70	$p<.005$
Bound (μg/ml platelet-poor plasma)	11.44	5.68	7.01	2.99	2.69	$p<.005$
Free (μg/ml platelet-poor plasma)	1.34	0.34	1.41	0.28	0.67	n.s.
Percent free	12.2	5.5	18.4	5.4	3.51	$p<.0005$
Kynurenine (μg/ml platelet-poor plasma)[b]	0.49	0.21	0.60	0.20	1.29	n.s.

[a]Student's two-tailed t test; df=53 except for kynurenine, where df=22.
[b]For this measure, the number of children with normal concentrations of platelet 5-HT is 13 and that for children with elevated concentrations of platelet 5-HT is 11.

described (7). Platelet concentrations of 5-HT were assayed according to the method of Belendiuk and associates (7), and plasma concentrations of total, free, and bound tryptophan were determined according to the method of Denckla and Dewey (8). Free typtophan was obtained according to the method of Knott and Curzon (9). Plasma concentrations of kynurenine were determined according to the method of Joseph and Risby (10). Hyperserotonemia was defined as a platelet concentration of 5-HT greater than 2 SD from the mean of controls.

P values were obtained with Student's two-tailed t test; correlations were performed with the Pearson product-moment statistic.

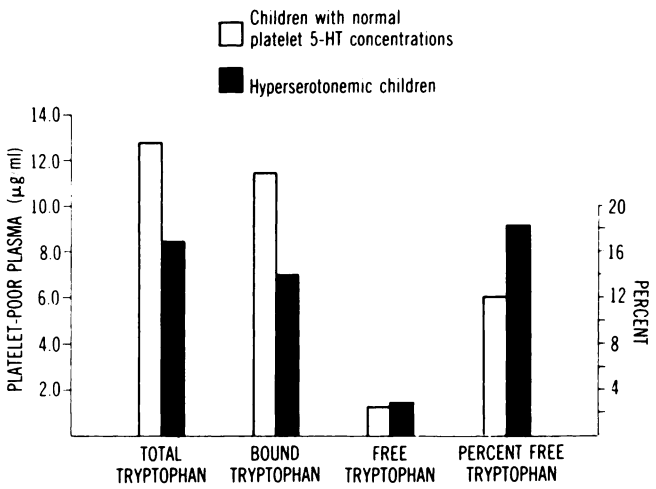
RESULTS

Children with attentional deficit disorder exhibited a slight increase in the mean concentration of platelet 5-HT in comparison with controls (.79±.46 versus .62±.22 $\mu g/10^9$ platelets; n.s.). However, 13 (24%) exhibited hyperserotonemia (5-HT greater than 1.06 $\mu g/10^9$ platelets). Serotonin concentrations could not be related systematically to age, sex, IQ, age at onset of the disorder, ratings of severity of the disorder, or presence, absence, or level of hyperactivity. All of the hyperserotonemic children had IQs of 90 or above.

Length of the drug-free period had no significant effects on 5-HT in the 13 hyperserotonemic children (.71±.27 versus .74±.23 $\mu g/10^9$ platelets; 3 versus 10 days). In addition, there was a significant correlation ($r=.75$, $p<.01$) between platelet 5-HT concentrations determined for the two blood drawings, performed 2 months apart.

Plasma concentrations of tryptophan and kynurenine in the 13 hyperserotonemic children were compared with those in the 42 children who exhibited normal 5-HT levels (see table 1 and figure 1). The total plasma tryptophan was significantly reduced in the hyperserotonemic children; this decrease was localized to the protein-bound pool (see table 1). The amount of free tryptophan did not distinguish the two groups; the percent of free tryptophan was therefore significantly increased. Plasma concentrations of kynurenine in hyperserotonemic children and those with normal 5-HT concentrations did not differ significantly.

FIGURE 1
Plasma Concentrations of Tryptophan in 13 Hyperserotonemic and 42 Nonhyperserotonemic Children with Attentional Deficit Disorder



In 33 children (including 12 of the original 13 with hyperserotonemia) who had resumed dextroamphetamine or methylphenidate regimens for at least 3 months, mean concentrations of 5-HT did not significantly change (dextroamphetamine, .92±.49 versus .92±.30; methylphenidate, 1.01±.53 versus 1.05±.23; with versus without medication). Although there was a regression of both very high and very low levels of 5-HT toward the mean, 10 of the 12 hyperserotonemic children not taking medications were still hyperserotonemic when taking medication. The 2 children who exhibited normal 5-HT values while taking medication were both receiving dextroamphetamine. Neither the levels of 5-HT with medication nor the changes in platelet concentrations of 5-HT following administra-

tion of drugs correlated with IQ, initial severity of the disorder, current severity of the disorder, drug dose, or clinical improvement.

COMMENT

There was a slight, not statistically significant increase in platelet concentrations of 5-HT in children with attentional deficit disorder. Approximately one-quarter (24%) of the group studied exhibited hyperserotonemia. Due to ethical considerations, our control group consisted of young adults. Data on platelet 5-HT concentrations in normal individuals (6) indicate that between the ages of 4 and 11 years blood 5-HT concentrations are approximately 25% greater than adult levels, decreasing to reach stable adult levels by about age 12 years. In the present study, hyperserotonemic children (mean age, 10.2±2.3 years) exhibited a 145% increase relative to adult controls. Therefore, hyperserotonemia could not be accounted for by age.

This report agrees with Rapoport and associates' findings (4) that mean concentrations of 5-HT in children with attentional deficit disorder did not differ signficantly from normal controls; it did not show the decreased 5-HT concentrations reported by Wender (2) and Coleman (3). Our findings differ in that our data indicate a large interindividual variability and a proportion of children with hyperserotonemia. Possibly the absence of hyperserotonemia in previous studies may be due to sample size or selection. Previous findings were based on smaller numbers of children (N=35, N=14, and N=25, respectively) than our current sample (N=55). A major reason for discrepancies may lie in many differences in methods. The two studies reporting decreased 5-HT levels, for example, measured total blood 5-hydroxyindoles and did not correct for platelet number.

Platelet concentrations of 5-HT appeared to be stable in our group of children while they were not taking medication. There was no significant difference in mean 5-HT concentrations between samples drawn on two separate occasions 2 months apart, the first after a 3-day drug-free period, the second after a 10-day drug-free period. In addition, individual values from the two drawings were significantly correlated ($r=.75$, $p<.01$). Other studies (5), and the experience of our laboratory with normal individuals, indicate the stability of blood 5-HT concentrations over long periods of time.

Mean platelet 5-HT concentrations did not change significantly with administration of either methylphenidate or dextroamphetamine, although there was a regression of both high and low 5-HT values toward the mean, as demonstrated by the decreased variance. However, 10 of the 12 hyperserotonemic children still exhibited values that were 2 SD above the mean; platelet 5-HT concentrations in the 2 remaining children decreased by approximately 50% following treatment with dextroamphetamine. The relevance of these findings is unclear, and no correlation with behavioral states could be discerned.

Our finding of no change in the mean 5-HT with methylphenidate treatment agrees with the findings of Rapoport and associates (4). The report of Wender and associates (11) that treatment with dextroamphetamine does not influence urinary excretion of 5-HIAA is not inconsistent with our findings of a lack of distinct effects of dextroamphetamine on platelet 5-HT levels.

Except for Coleman and Greenberg (3, 12), who found correlations between 5-hydroxyindole levels and behavioral change in a group described as aggressive and hyperactive children (most of whom were institutionalized for severe mental retardation), a direct relationship between 5-HT levels and behavior has, to our knowledge, never been demonstrated. In the present study, no relationship between platelet concentrations of 5-HT and a number of clinical or behavioral factors was found. Rapoport and associates (4) similarly were unable to find any such correlates.

Hyperserotonemia has been found in autism and in many conditions in which mental retardation is present (see reference 5). It appears to be roughly correlated with low IQ values (5, 13). To our knowledge, the present study is the first report of hyperserotonemia in a group of disturbed children who, on the average, had normal IQs (hyperserotonemic children with attentional deficit disorder, mean IQ=107, versus nonhyperserotonemic children with attentional deficit disorder, mean IQ=103).

Elevations in platelet 5-HT can occur as a result of quite *different* physiological mechanisms, ranging from differences in platelet number and characteristics (such as size, 5-HT uptake, binding, and efflux) to changes along the pathways for tryptophan metabolism. In the present study hyperserotonemic children exhibited increased concentrations of 5-HT expressed on a "per platelet" basis; platelet number was not accountable. Plasma total and protein-bound tryptophan were significantly decreased and percent of free tryptophan was significantly increased in comparison with clinically similar children with normal platelet 5-HT. However, there was no significant difference in the major metabolic product of tryptophan, kynurenine. Since plasma kynurenine was normal, this argues both against a major blockade in the kynurenine pathway as a causal mechanism in hyperserotonemia, and against an imbalance in the metabolic pathways for tryptophan leading to excessive hydroxylation (as in the carcinoid syndrome where we have observed that kynurenine is decreased). Rather, high 5-HT, low plasma total and protein-bound tryptophan, and a high percent of free tryptophan may be a consequence of an overall increased tissue uptake and use of tryptophan. We do not know the functional significance—if any—

of these observations. We do, however, know that plasma concentrations of tryptophan play an important role in the regulation of brain 5-HT synthesis, and that central serotonergic mechanisms have been implicated in arousal and motor reactivity, systems of primary importance in the attentional deficit disorder.

Basic knowledge of central serotonergic function and effects of central neuronal function on peripheral metabolism and measures is largely lacking, although "experiments of nature" in Huntington's disease (7) and motor neuron disease (1) (in which clinical severity and 5-HT elevations are related) indicate there may be some such link. Platelet 5-HT, in our experience, may thus reflect a state (1, 7) or, as in autism and retardation (5), a trait measure. Further animal, clinical (simultaneous measures of blood and urinary tryptophan metabolites), and familial studies could clarify the significance of these particular observations in attentional deficit disorder.

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