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Plasma precursor amino acids of central nervous system monoamines in children with coeliac disease

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Abstract

Some children with coeliac disease show behavioural disorders such as depression and other signs which have been correlated with reduced central monoamine metabolism. We have therefore investigated the brain availability of the monoamine precursors tryptophan and tyrosine in 15 untreated children with coeliac disease and 12 treated children with coeliac disease as well as in 12 control children. Significantly decreased plasma concentrations of tryptophan were found in untreated children (mean (SD) 13 (4) μmol/l, $p<0.001$) compared with treated children (31 (13) μmol/l), and in both groups of coeliac children when compared with control children (81 (22) μmol/l). A significantly lower ratio of plasma tryptophan to large neutral amino acids (tyrosine, valine, isoleucine, leucine, and phenylalanine) was also observed, which could indicate impaired brain availability of tryptophan in coeliac children and was more pronounced in untreated children. The impaired availability of tryptophan could produce decreased central serotonin synthesis and in turn behaviour disorders in children with coeliac disease.

Untreated coeliac disease is known to be associated with an abnormal intestinal mucosa,[1 2] which produces biochemical and immunological abnormalities.[3-5] However, coeliac disease also produces neurological and psychiatric complications in children. Behavioural disorders such as schizophrenia, depression, and obsessional neurosis have been described in adults[6-8] and rarely in children[9 10] with coeliac disease.

Affective disorders have long been related to monoamine production in the central nervous system.[8] Thus there is some evidence that depressed patients may have decreased plasma concentrations of tryptophan.[11 12] The biogenic amines are synthesised in neurones from precursor amino acids circulating in blood[13] and cross the blood-brain barrier by specific transport mechanisms.[14] Catecholamines are synthesised from tyrosine and serotonin from tryptophan (essential amino acid). However, concentrations of tyrosine and tryptophan in the brain depend not only on the plasma concentrations but also on the plasma concentration of large neutral amino acids competing for transport in this system.[15] Thus amino acid availability in plasma regulates the monoamine turnover in the central nervous system.[15]

There is little information available on precursor amino acids of monoamines in adult coeliac disease[16] and there is even less for children. In view of this, we have investigated amino acid concentrations in plasma in order to determine whether there is impaired brain availability of tyrosine or tryptophan, which in turn could impair central monoamine synthesis, thus causing behavioural disorders in children with coeliac disease.

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Methods

SUBJECTS

All children were studied after informed consent was obtained from their parents. The diagnosis of coeliac disease was made using the criteria formulated by the European Society for Paediatric Gastroenterology and Nutrition.[1 2] The two groups studied, treated and untreated coeliac disease, were composed of completely different patients.

The untreated group comprised 15 children with a mean age of 8·8 years (range 5·2–14·1 years), who had partial, subtotal, or severe partial villous atrophy of jejunal mucosa. The mean value of IgG class antigliadin antibodies in plasma was 21·1 (range 7·2–70·3) arbitrary units (Pharmacia). Nine children in this group presented signs of behavioural disturbances, four were irritable and five apathetic when examined for the study. After the study, three patients with behavioural disturbances improved with treatment of the coeliac disease.

The treated group comprised 12 children, mean age 8·9 years (range 7·1–14·2 years), who had a normal intestinal biopsy and were thriving. These children had received a gluten free diet for a mean period of one year. The mean plasma value of IgG class antigliadin antibodies was 2·7 arbitrary units (range 0·8–3·9 U), which was significantly different ($p<0.001$) from that of untreated coeliac children. None of the treated patients showed behavioural disturbance.

The control group comprised 12 children, mean age 10·3 years (range 6·4–13·9 years), with no clinical or biochemical signs of gastrointestinal, nutritional, metabolic, infective, or psychiatric diseases. The mean plasma value of IgG class antigliadin antibodies was 2·1 arbitrary units (range 0·8–3·2 U).

AMINO ACID ANALYSIS

Fasting (eight hours) blood samples were taken from all children with Na_2 ethylenediamine tetra-acetic acid (Merck) and plasma separated by centrifugation and stored at −25°C. The analysis of plasma amino acids was performed by adding 0·1 ml aliquots of plasma to 0·1 ml of 0·4 mmol/l methionine sulphone (Fluka) in 0·1N HCl as internal standard. Then the samples were applied to Ultrafree MC filters (Waters Assoc,

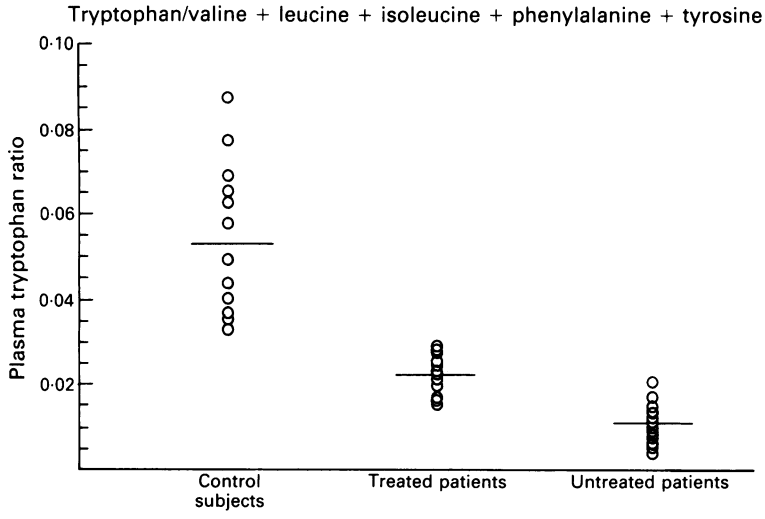

Figure 1: Plasma tryptophan ratio in 15 untreated children with coeliac disease, 12 treated children, and 12 control children. The horizontal bars indicate the mean value of the ratio. The differences between the three groups are significant ($p<0·001$).

Plasma amino acid concentrations (μmol/l) in children with coeliac disease (mean (SD))

	Control subjects (n=12)	*Treated patients (n=12)*	*p*	*Untreated patients (n=15)*
Aspartic acid	8 (2)	8 (6)	NS	5 (2)
Glutamic acid	63 (17)	72 (22)	<0·05	54 (14)
Serine	222 (33)	201 (44)	NS	189 (45)
Asparagine	132 (21)	119 (34)	NS	106 (18)*
Glycine	368 (60)	337 (93)	NS	275 (91)*
Glutamine	818 (136)	806 (145)	NS	664 (134)*
Taurine	101 (21)	106 (32)	<0·05	80 (23)*
Histidine	77 (12)	62 (16)	NS	54 (13)*
Citrulline	61 (20)	46 (14)*	<0·05	34 (10)‡
Threonine	210 (74)	249 (73)	NS	242 (68)
Alanine	524 (95)	630 (136)	NS	637 (173)
Arginine	105 (20)	83 (18)	NS	78 (23)*
Proline	462 (127)	369 (129)	NS	323 (66)†
Tyrosine	122 (33)	96 (22)*	NS	82 (20)†
Valine	336 (95)	254 (41)*	NS	248 (74)*
Methionine	36 (11)	31 (11)	NS	22 (7)‡
Cystine	113 (25)	129 (37)	NS	123 (31)
Isoleucine	93 (30)	71 (18)*	NS	68 (15)†
Leucine	174 (44)	131 (27)*	NS	133 (35)*
Phenylalanine	97 (22)	101 (18)	NS	114 (35)
Tryptophan	81 (22)	31 (13)‡	<0·001	13 (4)‡
Ornithine	133 (36)	106 (40)	NS	80 (37)*
Lysine	247 (43)	214 (36)	NS	202 (53)*

*p<0·05, †p<0·01, ‡p<0·001 with respect to controls. NS=not significant.

Milford, MA, USA) with a 10000 molecular weight cut off limit and ultrafiltered by centrifugation in a refrigerated centrifuge for 20 minutes at 2000 *g*. Aliquots of 25 μl were desiccated under vacuum and derivatised with methanol-water-triethylamine-phenylisothiocyanate (7:1:1:1). After 10 minutes at room temperature excess solvents and reagent were eliminated under vacuum and stored at −25°C until analysis. The phenylthiocarbamyl derivatives of amino acids[17] were assayed by reverse phase high performance liquid chromatography using the Pico-Tag method (Waters Assoc) for physiological amino acids[18] with minor modifications. The initial flow was 0·6 ml/min, reaching 1·0 ml/min in 12 minutes after a linear rise. After this, the flow was fixed at 1·0 ml/min. The column was an application-specified C18 Pico-Tag column (15 cm×3·9 mm, Waters Assoc) and the temperature controlled at 44°C. The chromatographic system was a liquid chromatograph (Waters Assoc), consisting of a 600E gradient system, a U6K injector, a 484 absorbance detector (268 nm) and a Baseline 810 chromatography data workstation.

Triethylamine (Pierce H), phenylisothiocyanate (Pierce H), amino acid standards (Sigma), and eluents were obtained from Waters. High performance liquid chromatography water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

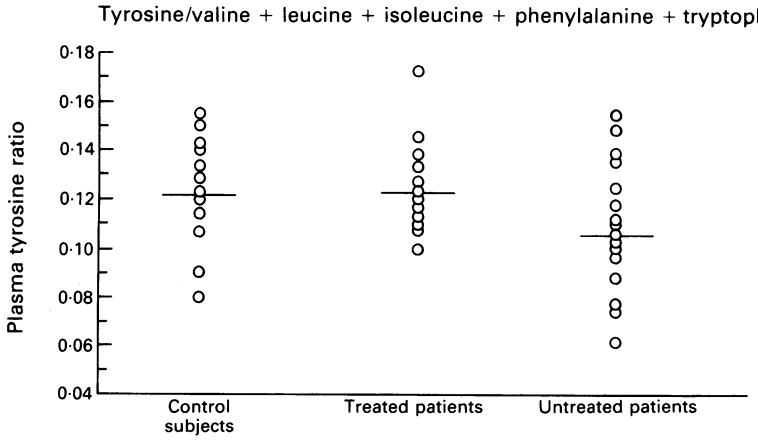

Figure 2: Plasma tyrosine ratio in 15 untreated children with coeliac disease, 12 treated children, and 12 control children. The horizontal bars indicate the mean value of the ratio. The differences between the three groups were not significant.

STATISTICAL ANALYSIS

Statistical analysis was carried out using either the two tailed Student's *t* test for parametric data, after checking the normality of the values investigated after the Kolmogorov-Smirnov test, or the Mann-Whitney U test for non-parametric data.

Results

The Table shows fasting amino acid concentrations in plasma from control children and treated and untreated children with coeliac disease. Glutamine, lysine, valine, glycine, alanine, and proline were the major components. A comparison of amino acid concentrations in treated and untreated children showed a significantly increased concentration of tryptophan in treated children ($p<0·001$). Moreover, plasma tryptophan concentrations were significantly higher in control children ($p<0·001$) when compared with both groups of coeliac patients. Glutamic acid, taurine, and citrulline concentrations were also significantly decreased ($p<0·05$) in untreated children compared with treated children.

On the other hand, both treated and untreated children had significantly decreased plasma concentrations of citrulline, tyrosine, valine, isoleucine, and leucine compared with control children. Moreover, asparagine, glycine, taurine, histidine, arginine, proline, methionine, ornithine, and lysine concentrations were significantly decreased in untreated children compared with control children.

The ratio of plasma tryptophan to the sum of large neutral amino acids (tyrosine, valine, isoleucine, leucine, and phenylalanine), called the 'plasma tryptophan ratio' (Fig 1), was significantly different between control children (0·055

(0·024) mean (SD)) and treated children (0·021 (0·003), $p<0·001$ compared with control children) and untreated children (0·010 (0·002), $p<0·001$ compared with control children) as well as between treated and untreated children ($p<0·001$). Moreover, this ratio was lower in untreated children with behavioural disturbances (0·008 (0·003), $p<0·05$) than in untreated children without these symptoms (0·012 (0·002)). However, the ratio of plasma tyrosine to the sum of valine, isoleucine, leucine, phenylalanine, and tryptophan, known as the 'plasma tyrosine ratio' (Fig 2), showed no difference between control children (0·121 (0·026)) and treated (0·122 (0·021)) and untreated (0·110 (0·029)) children.

Discussion

Our results show that children with treated and untreated coeliac disease have decreased tyrosine concentrations in plasma compared with control children. Also, plasma concentrations of other larger neutral amino acids such as valine, isoleucine, and leucine, but not phenylalanine, were significantly decreased in both treated and untreated children. These decreased levels of larger neutral amino acids could explain the normal plasma tyrosine ratio found in both coeliac groups compared with the control group. This ratio is thought to indicate the availability of tyrosine to the brain according to Fernstrom and Faller.[14]

Plasma tryptophan concentrations were significantly lower in untreated and treated children than in control children. Regardless of the existence of significantly decreased plasma concentrations of larger neutral amino acids other than phenylalanine, the tryptophan ratio was diminished in both the treated and untreated groups. In this case the decrease in the plasma tryptophan ratio is due to the considerable fall in plasma tryptophan concentrations. Thus the lower plasma tryptophan ratio might indicate that all children with coeliac disease have impaired availability of tryptophan in the brain, such impairment being greatest in untreated children with symptoms of behavioural disturbances, in which the tryptophan ratio was most significantly decreased (see Results).

The findings of this study regarding plasma tryptophan concentrations and the plasma tryptophan ratio to some extent do not agree with the observations reported by Hallert *et al*[16] in untreated adult patients with coeliac disease. Only four of 11 untreated adults showed a significant decrease in the plasma tryptophan ratio compared with adult controls,[16] and three of them had a psychiatric illness. In our study all untreated children had decreased plasma tryptophan concentrations, but only nine of 15 had signs of behavioural disturbances. In addition, the plasma tyrosine ratio was found to be normal in adult patients,[16] as in the present study.

The low tryptophan concentrations seen in both treated and untreated children could indicate the existence of metabolic reactions reducing transport of dietary tryptophan to the brain rather than impaired absorption,[16] since according to Hallert *et al*[16] untreated adults and control subjects showed a similar rise in plasma tryptophan after oral casein administration. Our findings also suggest that tryptophan was decreased in plasma metabolically rather than by reduced absorption because other essential amino acids such as valine, isoleucine, and leucine were also reduced in both treated and untreated groups independently of jejunal mucosa integrity. Moreover, the existence of normal plasma values of phenylalanine and threonine, which are essential amino acids and must be absorbed by the intestinal tract, supports the hypothesis of an altered metabolism to explain the greatly decreased tryptophan concentrations in the plasma of children with coeliac disease. We think, however, that these changes in tryptophan might not be specific to coeliac disease and that they might be altered in a similar way in other enteropathic diseases.

The impaired availability of tryptophan to the brain in the untreated children may explain signs of reduced serotonin synthesis in the central nervous system, such as depression[19] or other affective disorders, which were found[9] or described[10] in children with coeliac disease. These behavioural disturbances are frequently seen in our children at the onset of the disease. These patients can be irritable, querulous, depressive, apathetic, or negate, and they also may present stereotyped movements. An improvement in mood and mental activity has been seen in our patients after a period on a gluten free diet, and it has also been recognised by experienced physicians.

In our study, nine of 15 untreated children with coeliac disease presented signs of behavioural disturbances (irritability and apathy). These untreated children had significantly lower plasma tryptophan ratios than children without behavioural symptoms. Thus, there seems to be a relation between behavioural disturbances and greatly decreased tryptophan ratios. However, there was no relation between the severity of coeliac disease, determined by clinical, histological, or biochemical data, and the plasma tryptophan ratio.

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