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Effects of Diet Discontinuation and Dietary Tryptophan Supplementation on Neurotransmitter Metabolism in Phenylketonuria

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Abstract. Diet discontinuation in 10 young adults with phenylketonuria (PKU) resulted in an increase of 100% in the concentration of phenylalanine in plasma and cerebrospinal fluid (CSF), and a statistically significant decrease in the concentration of 7 essential plasma amino acids. In CSF the concentration of tyrosine and tryptophan was unchanged, and the concentration of homovanillic acid (HVA) and 5-hydroxyindoleacctic acid (5-HIAA) declined, but only the latter statistically significantly. The concentration of 5-HlAA was significantly lower in the PKU patients, both on and off diet, compared to normal controls. When II young adults with PKU received a normal diet supplemented with tryptophan, a 5-fold increase in the plasma concentration and a 3-fold increase in the spinal concentration of this amino acid were observed. Due to a 3-fold increase, the concentration of 5-HIAA reached normal levels. The possibility of an alternative to the phenyl nine-restrictive diet in PKU is discussed.

Introduction

Phenylketonuria (PKU) is an inborn error of phenylalanine metabolism, which untreated results in severe mental retardation. This is prevented by giving the child a phenylalanine restricted diet within the first month of life. It has been reported from other treatment centers [1-4] that diet discontinuation at the age of 8 years or earlier caused a decline in intellectual function and problems of behavioural changes. For some patients, however, the diet is inconvenient in puberty. The present study shows that termination of the dietary treatment at this (and probably at any) age causes disturbances in plasma amino acids and, more seriously, in the intracerebral metabolism of dopamine and serotonin. In an earlier study [5] we have investigated the effect of tyrosine supplementation on dopamine synthesis. Subsequently, we decided to examine whether tryptophan supplementation is capable of stimulating serotonin synthesis in spite of high concentrations of phenylalanine in plasma and cerebrospinal fluid (CSF).

Patients and Methods

Patient Group 1. Ten young adults with PKU, aged 15-24 years, were: studied on and off diet in a crossover design. The patients were on a low dietary phenylalanine intake for at least 3 weeks and off diet for another 3 weeks in random order. At the end of each 3-week period lumbar puncture was performed, and CSF amino acids and neurotransmitter metabolites determined. The normal diet contained 1.3 g protein/kg body weight, and excessive quantities of meat and milk products were avoided.

Patient Group 2. Eleven other young adults with PKU, aged 14-25 years, had the normal diet supplemented with 4.5 g tryptophan per day. Lumbar puncture was performed for CSF analysis after at 1cast 3 weeks on normal diet, and after l week a normal diet supplemented with tryptophan in arbitrary order .

Control Group. Eighteen young adults without PKU, aged 18-31 years, submitted for radiculography on suspicion of prolapsed lumbar disc, served as control group for measurement of homovanillic add (HVA), 5-hydroxyindoleacetic acid (5-HIAA), phenylalanine, tyrosine and tryptophan in CSF.

. *Lumbar Puncture.* This was done in a standardized way: 2 h after breakfast, while the patient was still in bed, the liquor was collected in 7 fractions, each consisting of 0.5 ml. The fractions were stored al -80°C until examination for HVA, 5-HIAA, phenylalanine, tyrosine and tryptophan by HPLC as described earlier [6].

Blood Samples. Immediately before lumbar puncture venous blood was drawn. The heparinized plasma was frozen in aliquots and stored at -20 °C, until amino acids were analysed according to Andersen et al. [7]. In addition phenylalanine, Tyrosine and Tryptophan were analysed by HPLC according to Lykkelund et al. [6].

The following statistical methods were used:

Pratt's rank sum test for matched-pairs for comparing the data for patient group l, on and off diet, and the data for patient group 2, off diet and off diet with tryptophan supplementation.

Mann- Whitney (Wilcoxon) rank sum test for comparing the two patient groups to the normal controls, and for checking the comparable data of the two patient groups off diet.

Spearman test for calculation of the rank correlation coefficient between the plasma phenylalanine concentration and the concentrations of the other essential amino acids.

In all instances, a 5% level of significance was used

Table I. Effect of diet discontinuation on plasma concentrations of essential amino acids in 10 young adults with PKU

	Medians and 95% confidence limits, µmol/l			
	on diet		off diet	
Phenylalanine	730	576-1,011	1462[a]	983-1,877
lsoleucine	95	65-146	52[a]	29-67
Leucine	154	123-174	82[a]	57-126
Lysine	236	195-271	176[a]	142-190
Methionine	34	21-37	22[b]	17-34
Threonine	219	172-258	113[a]	84-134
Tryptophan	74	60-89	50[a]	40-52
Tyrosine	80	65-106	54[a]	45-72
Valine	278	197-373	198[c]	145-348

[a] $p < 0.01$; [b] $p < 0.05$; [c] $0.2 < p < 0.4$.

Results

Diet Discontinuation, Patient Group 1

In table I the changes in plasma amino acids subsequent to diet discontinuation are listed. The median plasma phenylalanine concentration was twice as high on normal diet compared to phenylalanine-restricted diet. Median plasma concentrations of the other essential amino acids declined by 25-48% on normal diet. All changes were statistically significant, except the decrease by 29% in the concentration of valine. The concentrations of methionine, isoleucine, Lysine, threonine, tryptophan and tyrosine on and off diet were negatively correlated to the phenylalanine concentration. The higher the concentration of phenylalanine, the lower the concentration of the amino acids in question. The p values were ranging from $p<0.001$ to $p<0.05$ and the correlation coefficients from -0.75 to -0.45. For leucine and valine the correlation was not statistically significant.

Table II. Neurotransmitter metabolism in PKU, effect of diet discontinuation and of dietary supplement with tryptophan

	Median concentrations and 95% confidence limits (in parentheses)						
	controls (n = 18)	CSF, patients group 1 (n = 10)		CSF, patient group 2 (n = 11)		plasma, patient group 2	
		on diet	off diet	off diet	+ tryptophan	off diet	+tryptophan
Phenylalanine[a]	9.6 (8.3-10.3)	161 (104-245)	328 (240-461)	414 (258-539)	396 (228-487)	1,463 (1,205-1,864)	1,611 (1,014-1,852)
Tyrosine[a]	7.9 (6.3-8.9)	11.8 (8.9-14.1)	12.4 (9.0-14.9)	13.2 (12.0-16.4)	13.1 (11.6-14.8)	48 (33-60)	49 (38-60)
Tryptophan[a]	2.2 (2.1-2.5)	4.2 (3.6-4.6)	4.0 (3.8-4.5)	3.3 (2.3-4.4)	10.8 (9.4-17.0)	43 (38-47)	229 (159-453)
HVA[b]	168 (119-227)	172 (114-229)	123 (84-216)	162 (119-183)	155 (114-226)	-	-
5-HIAA[b]	92 (73-125)	48 (32-103)	28 (18-57)	33 (22-47)	92 (50-103)	-	-
HVA/5-HIAA	1.8	3.6	4.4	4.9	1.7	-	-

[a]μmol/l; [b]nmol/l.

The data for CSF appear from table II. Only the amino acids phenylalanine, tyrosine and tryptophan were determined. As in plasma, the concentration of phenylalanine increased twice subsequent to dietary termination, while tyrosine and tryptophan concentrations were unchanged. However, both on and off diet, the concentrations of the latter two amino acids were higher than observed in the control group (table II). The median tyrosine concentration was about 50% higher in the patients ($p < 0.005$). The median tryptophan concentration was about 85% higher in the patients ($p < 0.001$).

The median concentration of the dopamine metabolite HVA in the patients on diet was not different from the median of the control group. After diet discontinuation the median in the patient group was somewhat lower, but the change was not statistically significant.

The median concentration of the serotonin metabolite 5-HIAA in the patient group on diet was only 52% of the concentration in the control group ($p < 0.001$). When the patients were off diet, the 5-HIAA concentration was even lower, i.e. 30% of the concentration in the control group ($p < 0.001$). The difference on and off diet was statistically significant ($p < 0.001$).

Tryptophan Supplementation
Patient Group 2

The results are listed in table II. In plasma a more than 5-fold increase in the median tryptophan concentration was observed when this group received the normal diet supplemented with tryptophan ($p < 0.001$). The phenylalanine and tyrosine concentrations remained unchanged.

In CSF again the phenylalanine and tyrosine concentrations were unaltered. The median tryptophan concentration increased more than 3-fold on tryptophan supplementation ($p < 0.001$). Corresponding well to this, a little less than a 3-fold increase in the median 5-HIAA concentration ($p < 0.001$) was observed. The median HVA concentration did not change.

The median concentrations in CSF of phenylalanine, tyrosine tryptophan, HVA and 5-HIAA of the two patient groups off diet differ numerically from each other, but except for the tryptophan concentration ($p < 0.05$) the differences are not significant.

Discussion

Dietary Effects on Essential Amino Acid Concentrations

The present investigation shows that the increase in plasma phenylalanine after termination of dietary treatment in 10 young adults with PKU was followed by a significant decrease in most of the essential plasma amino acids. The concentrations of these amino acids were negatively correlated to the plasma phenylalanine concentration.

An earlier study [8] has demonstrated that patients with untreated PKU have lower plasma levels of many amino acids, compared to normal subjects and to mentally retarded institutionalized patients without PKU as well.

In the present study diet termination was followed by a reduced intake of seven of the essential amino acids. However, this fact can only partly account for the decrease in plasma amino acids. A possible explanation of the low plasma concentrations could be a reduced intestinal absorption. Oral administration of labeled amino acids to patients with PKU and control subjects revealed an impaired uptake into blood in patients with PKU. Furthermore, no difference in amino acid uptake could be demonstrated in treated patients with PKU, as compared to normal subjects [9].

The high levels of phenylalanine in the bile and digestive juices are likely to monopolize the transport carrier system in the intestinal wall, and thereby reduce the rate of absorption of the other neutral amino acids [10].

In CSF the increase in the concentration of phenylalanine subsequent to diet discontinuation was comparable to the increase in plasma. The concentrations of tyrosine and tryptophan remained unchanged, yet these concentrations were 1.5 and 1.9 times higher in the PKU patients compared to the normal control group, respectively. As the high plasma phenylalanine was thought to inhibit the transport of these two amino acids across the blood-brain barrier [Il], this observation was unexpected. In agreement with the present results, Ratzmann et al. [12] have demonstrated a CSF tyrosine concentration in PKU patients about 2.2 times higher as compared to the control group. These observations may reflect disturbances in the intracellular metabolism of tyrosine and tryptophan in brain cells and a reduced transport across the neuronal cell membrane. This hypothesis is supported by the finding that the content of tyrosine in PKU brain cortex, obtained postmortem, was decreased to 50% of control values and tryptophan to 42% of control values [13]. The conc1usion must be that the cerebrospinal concentrations of tyrosine and tryptophan do not reflect the intracerebral content of these amino acids in PKU.

Dietary Effects an Neurotransmitter Metabolism

The median concentration of HVA in CSF from patient group 1 does not differ from the control group. After diet termination a decrease was observed, but this was not statistically significant. The concentration of 5-HIAA was significantly 1ower in the PKU patients on diet, as compared to the control group, and 5-HIAA decreased further after diet termination. The high intracerebral concentration of phenylalanine not only inhibits the transport of tyrosine and tryptophan into neurons, but also causes a competitive inhibition of tyrosine hydroxylase and tryptophan hydroxylase , which catalyze the first, rate limiting step in the synthesis of dopamine and serotonin, respectively. The next step, catalyzed by L-aromatic amino acid decarboxylase, is inhibited by the phenylalanine metabolites phenyl lactic acid and phenyl acetic acid [14].

It is not readily understood why serotonin metabolism seems to be more disturbed than dopamine metabolism. Especially off diet, one should expect a certain limitation of tyrosine available for dopamine synthesis in PKU. An earlier study [15] demonstrated decreased vigilance and neurotransmitter synthesis after diet discontinuation in 4 patients with PKU.

A later study [5] showed that both vigilance and dopamine synthesis were increased when the normal diet was supplemented with large doses of tyrosine.

Effects of Tryptophan an Neurotransmitter Metabolism

The present study shows that a tryptophan supplementation to the normal diet normalizes, or at least increases, the spinal concentration of 5-HIAA in 11 young adults with PKU (table II). On the normal diet without tryptophan supplementation none of the 5-HIAA concentrations in the patients were within the *95%* confidence limits of the control group.

A competitive inhibition of an enzymatic reaction depends on the relative amounts of substrate and inhibitor. Instead of lowering the concentration of the inhibitor phenylalanine, by the restrictive PKU-diet, it is possible to neutralize the inhibition by increasing the concentration of substrate in this case tryptophan the precursor to serotonin. By doing this, an increase in CSF of the concentration of 5-HIAA was observed in all patients. The increase in 5-HIAA was almost equal to the increase in spinal tryptophan, which indicates that tryptophan hydroxylase was not saturated with its substrate.

The HVA/5-HIAA ratio in CSF of PKU patients on normal diet is 2.7 times higher compared to control individuals (table II). When the normal diet is supplemented with tryptophan, this ratio decreases to 0.9 times the ratio in the control group, because the median 5-HIAA concentration increases to normal levels, whereas the concentration of HVA is unaffected. So, supplementation with dietary tryptophan is able to normalize the HVA/5-HIAA ratio in PKU.

A tryptophan supplementation has a more striking effect on the biochemical changes in neurotransmitter synthesis in PKU than a tyrosine supplementation. But when tyrosine was given solely ,it could improve vigilance in 9 young adults with PKU [5]. The c1inical effect of tryptophan is apparently more doubtful. This will be debated later in another study.

It seems that a dietary supplement with both tryptophan and tyrosine may be an alternative to the standard treatment of PKU in adolescence, where compliance with the diet often is poor.

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