

Increased neurotransmitter biosynthesis in phenylketonuria induced by phenylalanine restriction or by supplementation of unrestricted diet with large amounts of tyrosine

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Abstract. Seven phenylketonuria (PKU) patients aged 15-24 years were allowed unrestricted diet for 3 weeks. Three of these patients performed well on unrestricted diet according to visual reaction time variability (RTv 50-100 ms) and did not show significant changes when returning to the phenylalanine-restricted diet (RTv 70-100 ms). Neither did the concentrations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) change significantly. Four of the patients, however, performed rather poorly (RTv 120-220 ms) on unrestricted diet and improved significantly ($P < 0.03$) when the diet was restored (RTv 70-150 ms). The improvements were accompanied by significantly ($P < 0.01$ and $P < 0.02$) increases (mean 52% and 109%) in CSF levels of HVA and 5-HIAA. Five PKU patients aged 15-23 years were allowed unrestricted diet or unrestricted diet supplemented with various amounts of tyrosine (106-194 mg/kg per 24 h). Two of these patients performed very well on unrestricted diet (RTv 60 ms) and showed little change when the unrestricted diet was supplemented with tyrosine (RTv 70 ms and 80 ms). The three other patients, who performed rather poorly (RTv 120-220 ms), improved significantly ($P < 0.03$) when the unrestricted diet was supplemented with tyrosine (RTv 70-140 ms). HVA in CSF increased significantly ($P < 0.01$) with the tyrosine supplement when the amount exceeded a threshold of approximately 80 mg/kg per 24 h. The simultaneous increase in CSF level of 5-HIAA showed a positive correlation ($r = 0.90$; $P < 0.02$) with the increase in HVA concentration suggesting a functional interrelation between the dopaminergic and the serotonergic nervous systems.

Key words: Attention – Dopamine – PKU – Serotonin – Tyrosine¹

Introduction

Institution of phenylalanine-restricted diet during the first 2 weeks of life is effective in the prevention of severe mental retardation and neurological impairment in classic phenylketonuria (PKU) [4, 5, 17, 20]. Since a phenylalanine restricted diet may sometimes cause poor patient compliance the question of cessation of dietary treatment has been raised [13]. It has been proposed that the dietary treatment could be terminated when the central nervous system is almost fully developed. The function of the central nervous system, however, should also be considered especially with respect to the biosynthesis of dopamine, noradrenalin and serotonin since the biosynthesis of these neurotransmitters is inhibited by phenylalanine [1, 9, 12, 16, 18, 19, 29].

Waisbren et al. [42] have reviewed the psychological assessment of children with PKU after cessation of dietary treatment. Adverse results have been reported after diet termination at the age of 6-8 years when the patients were treated statistically as a homogeneous group. Some authors found a decrease in mean intelligence quotient (IQ), but in most cases IQ did not change significantly. As pointed out by Fuller and Shuman [15], however, some PKU children showed substantial loss in IQ after cessation of dietary treatment and this held especially true for those who had learning difficulties whilst on phenylalanine-restricted diet. Consequently Waisbren and co-workers [42] called for methods to differentiate between children who should remain on diet and those who may terminate. Investigation of such methods is one of the main objects of the present paper.

Later the results of the PKU Collaborative Study appeared. In this study, diet was discontinued at 6 years of age in 43 patients. Two years later the mean IQ was not significantly different from that of 38 children in whom dietary treatment had been continued [21].

It should be emphasized, however, that tests for IQ do not adequately describe neuropsychological functions such as attention. This implies that a slight or moderate neurotransmitter deficiency may escape detection. Increase in choice reaction time on other hand showed a positive correlation with the increase in plasma phenylalanine in ten PKU patients

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Abbreviations: PKU = phenylketonuria; RTv = reaction time variability; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindoleacetic acid; CSF = cerebrospinal fluid; IQ = intelligence quotient

in a double crossover protocol of dietary change [22]. Decrease in urinary excretion of homovanillic (HVA), a metabolite of dopamine, correlated with the increase in plasma phenylalanine and it was proposed that the increase in choice reaction time was due to decreased biosynthesis of dopamine [22].

In accordance with the results of Krause and co-workers [22] we found an increase in the median of the visual reaction time and/or in the variability of the reaction time in four PKU patients after cessation of dietary treatment [26]. In three of these patients increased reaction time was accompanied by decrease of HVA and 5-HIAA levels in CSF [26]. The present paper extends the study to seven PKU patients and discusses the biochemical mechanisms of the reduced neurotransmitter synthesis.

According to reaction time measurements, some patients seem to perform less well after termination of dietary treatment. It is preferable, therefore, to continue some kind of treatment at least in these cases. After an encouraging preliminary study [25, 27] we have investigated the effect of supplementation of unrestricted diet with large amounts of tyrosine, which is a precursor for the neurotransmitters dopamine and noradrenalin

Patients

Nine patients with classic PKU were divided into two partly overlapping groups. The first group, which consisted for four male and three female patients aged 15-24 years, was allowed unrestricted diet for 3 weeks. The second group had the unrestricted diet supplemented with tyrosine (106-194 mg/kg per 24 h) for another 3 weeks. The latter group consisted of three male and two female patients aged 15-23 years. The patients have been described in more details elsewhere [26, 27]. Tyrosine was administered as tablets each containing 0.5 g of tyrosine. One tablet was taken just before bedtime and the rest of the daily amount was divided into three equal doses and given with meals. Both the phenylalanine restricted and the unrestricted diet had approximately the following relative caloric composition protein 14%, carbohydrate 40% and fat 46%. Lumbar puncture and blood collection were carried out as described earlier [26]. This study was approved by the local ethical committee.

Fourteen healthy adolescents (10 female and 4 male) aged 15-24 years served as controls for the determination of reaction time variability. The best controls available for measurement of HVA and 5-HIAA in CSF were 12 patients (7 female and 5 male) aged 21-30 years and submitted to radiography on the suspicion of prolapsed lumbar disc.

Materials and methods

Vigilance was tested by continuous recording of visual reaction times. The patient was asked to extinguish a red lamp, which was lit at random intervals [26, 27]. One hundred reaction times were measured. The variability of the reaction time (RTv), defined as the 90-10 percentile difference, is a measure of the randomly occurring long reaction times and is considered an indicator of structural and biochemical derangement of the brain [7, 14].

HVA and 5-HIAA in CSF were measured quantitatively by HPLC. The chromatograph consisted of a solvent delivery pump (Waters 600 A; Millipore Waters, 34 Maple Street, Milford, MA 01757, US), automatic injection system (Waters WISP 710B), precolumn (Supelcosil LC-18-DB 2 cm × 4.6 mm 5 μm), analytical column (Supelcosil LC-18-DB 15 cm × 4.6 mm 3 μm, Supelco Inc., Supelco Park, Bellefonte, PA 16823, US) and electrochemical detector (Bioanalytical Systems LC-4B; Bioanalytical Systems Inc., 1205 Kent Ave., West Lafayette, IN 47906, US) using a potential at the working electrode of 0.7 volts versus a Ag/AgCl reference electrode. The precolumn, the analytical column, and the reservoir containing the eluent were immersed in a water bath at 30°C. The mobile phase was prepared by mixing a citrate buffer (0.1M, pH = 3.0) containing octanesulphonic acid (0.5mM) and ethylenediaminetetraacetic acid (0.1mM) with methanol (9+1 v/v). 100μl of unprocessed CSF were injected for the determination of HVA and 5-HIAA.

Tyrosine, tryptophan and phenylalanine were determined by means of their auto fluorescence using the chromatographic system described above except for the exchange of electrochemical detector with a fluorescence detector (Kontron SFM 23/B; Kontron Instruments AG, Bernstraße-Süd 169, CH-8048, Zürich, Schweiz). For the determination of tyrosine and tryptophan the excitation wavelength was 275nm and emission was 330 nm. The corresponding settings were 260/290 nm for the measurement of tyrosine and tryptophan 50 μl of unprocessed CSF was injected and the same amount was used for determination of phenylalanine.

The proteins in 50 μl of plasma were precipitated with 100μl of 8% (w/v) trichloroacetic acid. After centrifugation, 50 μl of the supernatant was injected for the measurement of phenylalanine. A further 50 μl was diluted with 100 μl of distilled water, and 50 μl of the latter sample was used for determination of tyrosine and tryptophan. The other amino acids were determined by an amino acid analyser (Chromaspek; Hilger Analytical, Westwood, Margate, Kent CT9 4JL, UK) with fluorescence detection.

Results

Seven PKU patients were allowed unrestricted diet for 3 weeks. The data obtained from this group were used to evaluate the inhibitory effect of phenylalanine on neurotransmitter biosynthesis. To achieve this, several relevant parameters were depicted as a function of plasma phenylalanine (Fig. 1A – D) Phenylalanine in CSF level in PKU diets was increased 16-fold in those on phenylalanine-restricted diet and 37-fold in those on unrestricted diet.

The mean CSF tyrosine level of PKU patients showed small (32% and 39%) but significant ($P < 0.03$ and $P < 0.08$) increases on phenylalanine-restricted diet and unrestricted diet, respectively, compared with controls (Fig. 1B). The mean CSF tryptophan concentration was increased somewhat more (86% and 89% on restricted and unrestricted diet respectively: $P < 3 \times 10^{-7}$ and $P < 5 \times 10^{-7}$) (see also Fig. 1B).

When compared to controls, the mean CSF concentration of 5-HIAA in PKU patients was significantly ($P < 0.03$ and $P < 0.00005$) decreased by 33% and by 65% on phenylalanine-restricted and unrestricted diet respectively. In the PKU patients, the CSF level of 5-HIAA showed an inverse correlation with plasma phenylalanine. The correlation coefficient and the level of significance were calculated using exponential regression. (Fig. 1C) The CSF level of HVA in PKU patients on phenylalanine-restricted diet (170 ± 21 nmol/l; mean \pm SD) was not significantly different from the control level (160 ± 47 nmol/l). On unrestricted diet, however, the mean concentration was decreased by 20% to 129 ± 20 nmol/l although the difference did not reach normal accepted levels of significance ($P < 0.1$). As illustrated in Fig. 1C, HVA in CSF showed a large inter-individual variation. When the decrease in HVA concentration for the PKU patients, was plotted against the increase in plasma phenylalanine, a good correlation ($r = 0.82$; $P < 0.04$) was obtained, indicating that in PKU patients, the HVA level actually decreased with increasing plasma phenylalanine. The decreased levels of HVA and 5-HIAA were considered reflections of decreased biosynthesis of both dopamine and serotonin.

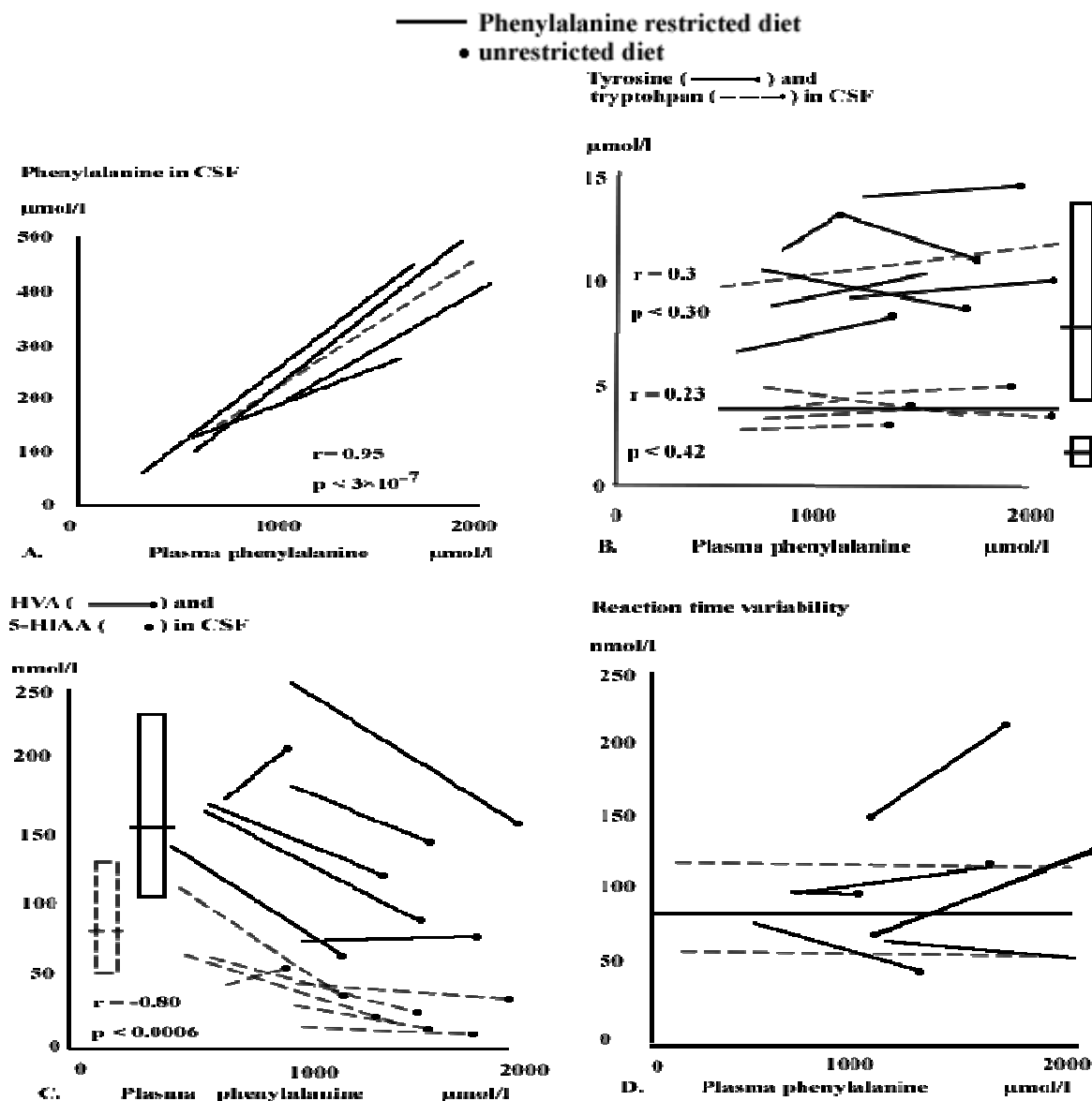


Fig. 1 A-D. Decreased vigilance was estimated from increased reaction time variability (D) and decreased neurotransmitter biosynthesis (C) in seven PKU patients after 3 weeks on unrestricted diet. Concentrations of phenylalanine (A), tyrosine and tryptophan (B) in CSF as a function of plasma phenylalanine. Bars indicate median and range of controls.

After cessation of dietary treatment, four out of seven PKU patients performed rather poorly according to RTv (120-220 ms), which was at or above the upper limit of the normal range (Fig. 1D). When the phenylalanine-restricted diet was restored, these patients improved ($P < 0.03$) as measured by RTv (70-150 ms). The improvements were accompanied by significant ($P < 0.01$ and $P < 0.02$) increases (mean 52% and 109%) in CSF levels of HVA and 5-HIAA

The decreases in RTv and the simultaneous increases in CSF concentrations of HVA and 5-HIAA for these four patients are illustrated in Fig. 2. Three patients performed well on unrestricted diet (RTv 50-200 ms) and did not show significant changes on returning to a phenylalanine-restricted diet (RTv 70-100 ms). Neither did the concentrations of HVA and 5-HIAA in CSF show consistent changes (Fig. 2).

Discontinuation of dietary treatment led to an almost three-fold increase in dietary phenylalanine intake. This resulted in a higher plasma phenylalanine (mean increase 102% $P < 0.0004$). The mean tyrosine intake was reduced by 49% and accompanied by a decrease of 33% in plasma tyrosine ($P < 0.007$). The CSF level of tyrosine showed small insignificant increases (Fig. 1B) resulting in a plasma/CSF ratio which was decreased by 40% ($P < 0.0002$). Dietary cessation led to a mean reduction of 33% in the intake of tryptophan accompanied by a relatively large and significant ($P < 0.001$) decrease (38%) in plasma tryptophan. Despite the decreased plasma level, the CSF concentration of tryptophan showed a small increase (Fig. 1B) resulting in a plasma/CSF ratio, which was reduced by 39% ($P < 0.0004$). A more elaborate report of the changes in plasma in out PKU patients after discontinuation of phenylalanine restricted diet was given by Nielsen [33].

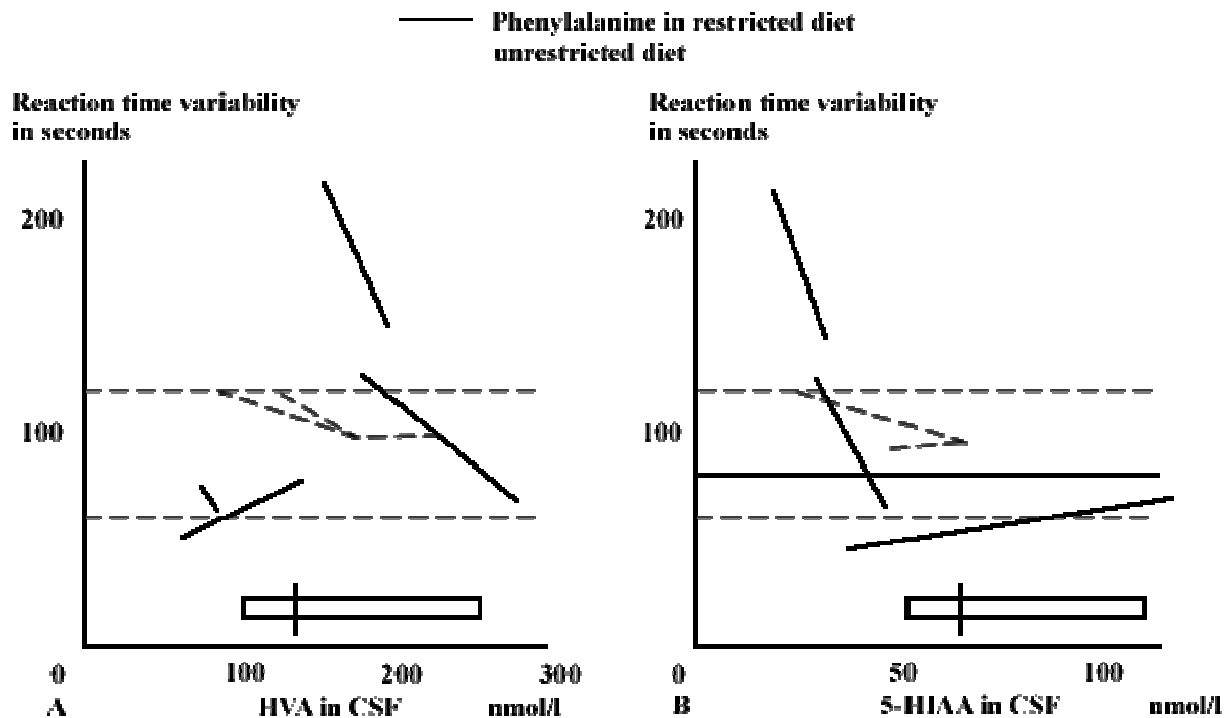
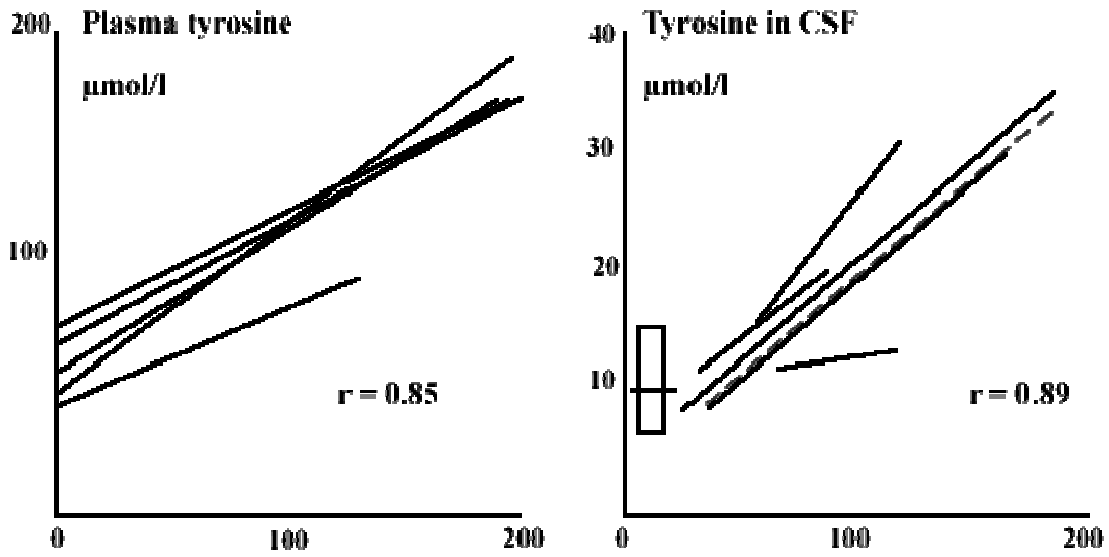


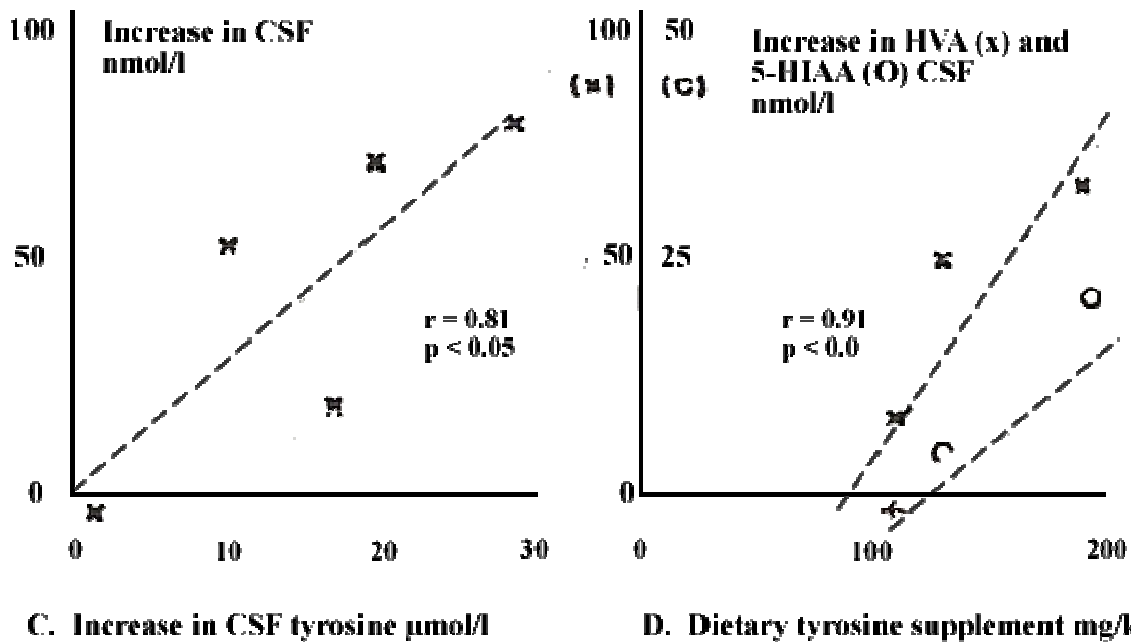
Fig. 2 A-B. Correlation between vigilance as estimated from reaction time variability and neurotransmitter biosynthesis as measured by HVA and 5-HIAA concentrations in CSF for seven PKU patients on phenylalanine restricted diet and after 3 weeks on unrestricted diet Median and range of controls are indicated.

Five PKU patients were allowed unrestricted diet or unrestricted diet supplemented with various amounts of tyrosine (106-194mg/kg per 24 h), which corresponded to a relative increase in tyrosine intake of 158%-342%. As expected, plasma tyrosine increased with increasing dietary tyrosine supplement (Fig. 3A) and CSF concentration of tyrosine correlated well with plasma tyrosine (Fig. 3B). The concentrations of HVA showed a large inter-individual variation. When the increase in HVA concentration was plotted as a function of the increase in CSF tyrosine, a good correlation was obtained (Fig. 3C). The same held true when increases in HVA were depicted as a function of increases in plasma tyrosine ($r = 0.83$; $P < 0.04$).

As a result of processes described above, HVA in CSF increased significantly with increasing tyrosine supplement when the amount exceeded a threshold of approximately 80 mg/kg per 24 h (Fig. 3D). The increase in HVA (76 ± 5 nmol/l; mean \pm SEM) in two patients who received the largest tyrosine supplement (190 and 194 mg/kg per 24 h) was not less than the corresponding increase (42 ± 19 nmol/l) observed in seven patients after returning to phenylalanine-restricted diet. The increase in HVA for the five tyrosine-treated patients varied with the tyrosine supplement ranging from -3% to +95% over pre-treatment levels. 5-HIAA in CSF also increased with tyrosine supplement (Fig. 3D) and the increases in 5-HIAA concentrations correlated ($r = 0.90$; $P < 0.02$) with the increase in HVA.



A. Dietary tyrosine supplement mg/kg/24 **B. Plasma tyrosine $\mu\text{mol/l}$**



C. Increase in CSF tyrosine $\mu\text{mol/l}$ **D. Dietary tyrosine supplement mg/kg/24**

Fig. 3 A-D. Increased neurotransmitter biosynthesis in five PKU patients after supplementations of unrestricted diet with tyrosine (A), which in turn increases CSF tyrosine (B). Enhancements of dopamine biosynthesis as estimated from HVA concentration in CSF correlate with CSF tyrosine (C). Increases in the biosynthesis of dopamine and serotonin as measured by increased CSF concentrations of HVA and 5-HIAA correlate with dietary tyrosine supplement (D). (O) unrestricted diet; (x) unrestricted diet supplemented with tyrosine.

The dopaminergic and the serotonergic nervous systems were considered as having antagonistic effects on vigilance. Consequently RTv, was depicted as a function of the molar ratio between HVA and 5-HIAA (Fig. 4). For two of the patients, who performed well according to RTv, no relation between RTv and HVA/5-HIAA was observed. The other three patients performed rather poorly on unrestricted diet having RTv at or above the upper limit of the normal range. During tyrosine treatment they showed decreases in RTv that were accompanied by increases in the molar ratio of HVA and 5-HIAA (Fig. 4)

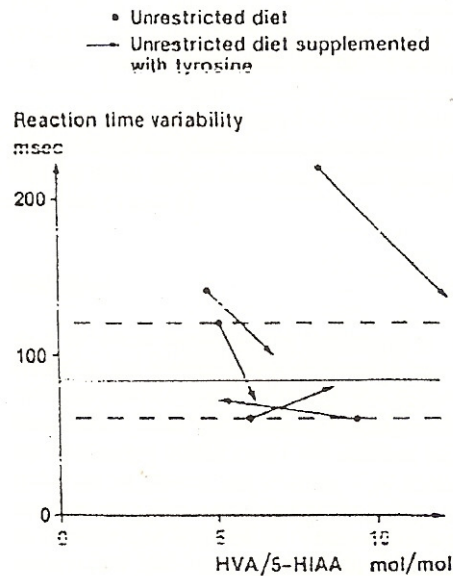


Fig. 4. Correlation between reaction time variability and molar ratio of HVA and 5-HIAA in CSF for five PKU patients before and after addition of tyrosine to unrestricted diet.

In an attempt to investigate the possible influence of tyrosine treatment on transport and metabolism of other amino acids in the brain, these amino acids were measured quantitatively in plasma and CSF samples obtained at the same occasion from each of the five tyrosine-treated patients. The results are shown in Table 1. During tyrosine treatment the CSF level of histidine increased by 18% ($P < 0.02$). Except for tyrosine, histidine and arginine the amino acids listed in Table 1 showed only small changes ($< 15\%$) in their CSF concentrations. Also in the plasma concentration and in the plasma/CSF ratio of these amino acids only small changes ($< 13\%$) were observed (Table 1).

Table 1. Amino acids ($\mu\text{mol/l}$) in plasma and cerebrospinal fluid of phenylketonuria patients on unrestricted diet without or with tyrosine supplementation

	Plasma		CSF		Relative change in the plasma/CSF ratio
	- Tyrosine	+ Tyrosine	- Tyrosine	+ Tyrosine	
Asx	54 \pm 20	52 \pm 17	7.3 \pm 3.0	6.2 \pm 1.8	12.1 %
Thr	113 \pm 20	98 \pm 17	22.8 \pm 6.4	20.8 \pm 4.3	- 4.1 %
Ser	103 \pm 21	98 \pm 35	25.0 \pm 2.3	26.2 \pm 6.2	- 4.3 %
Glx	504 \pm 204	518 \pm 83	495.6 \pm 28.9	533.9 \pm 74.2	- 2.7 %
Gly	241 \pm 82	238 \pm 93	4.6 \pm 1.4	4.2 \pm 1.0	10.4 %
Ala	402 \pm 94	387 \pm 59	28.0 \pm 8.8	25.0 \pm 7.4	9.3 %
Cit	27 \pm 10	26 \pm 8	2.4 \pm 1.0	2.4 \pm 0.9	- 4.2 %
Val	188 \pm 18	190 \pm 10	13.2 \pm 2.3	13.2 \pm 2.4	6.5 %
Met	74 \pm 4	24 \pm 5	2.6 \pm 0.3	2.5 \pm 0.5	3.8 %
Ile	53 \pm 6	53 \pm 9	3.7 \pm 0.5	3.7 \pm 0.6	- 2.9 %
Leu	94 \pm 12	92 \pm 9	8.8 \pm 1.3	8.7 \pm 1.2	- 1.3 %
Tyr	50 \pm 14	138 \pm 39 ^a	11.2 \pm 8.4	26.0 \pm 9.4 ^b	28.5 %
Phe	1466 \pm 334	1420 \pm 162	386.7 \pm 109.9	371.9 \pm 72.6	0.2 %
His	86 \pm 8	82 \pm 12	19.6 \pm 4.0	23.2 \pm 3.9 ^c	- 21.6 %
Trp	45 \pm 9	41 \pm 8	4.0 \pm 0.9	3.7 \pm 0.5	1.3 %
Orn	54 \pm 12	47 \pm 4	4.1 \pm 0.9	3.9 \pm 0.5	- 9.3 %
Lys	177 \pm 15	181 \pm 18	23.0 \pm 3.2	22.2 \pm 1.5	5.7 %
Arg	58 \pm 9	70 \pm 12	18.8 \pm 8.0	15.6 \pm 1.5	33.8 %

^a $P < 0.003$; ^b $P < 0.01$; ^c $P < 0.02$; ^d $P < 0.04$; ^e $P < 0.05$

Discussion

At least three factors might contribute to the observed decrease in the plasma levels of tyrosine and tryptophan after the cessation of dietary treatment. Firstly the dietary intake of these amino acids was reduced, but especially for tryptophan the diminished intake could not account for all the observed reduction in plasma concentration. A reduced intestinal absorption may also contribute to the decrease in plasma levels of tyrosine and tryptophan. This theory is supported by the observation that orally administered radioactively labeled amino acids (arginine and leucine) showed strongly impaired uptake into plasma in untreated PKU patients [24]. The normal intestinal absorption was restored when the

plasma level of phenylalanine was normalized by phenylalanine-restricted diet [24]. The high level of phenylalanine in the bile and the digestive fluids might be responsible for a competitive inhibition of the intestinal absorption of tyrosine, tryptophan and other amino acids [37, 50] resulting in loss of tyrosine and tryptophan in the stools of untreated PKU patients [50]. Using jejunal perfusion Wapnir and Lifshitz [44] found only slightly altered absorption rates for phenylalanine, tyrosine and tryptophan in an untreated PKU patient, and the uptake rates were almost normal when the patient was treated with a phenylalanine-restricted diet. Similar results were obtained for tyrosine and tryptophan in rats made hyperphenylalaninemic by adding p-chlorophenylalanine or large amounts (7%) of phenylalanine to the diet [45, 46]. The lack of major inhibition of intestinal absorption in these investigations may most likely be explained by an extensive flushing of intestinal phenylalanine by the perfusion fluid. When phenylalanine was added to the perfusion medium in a 10-fold molar excess, significant reductions in the intestinal absorption of tyrosine and tryptophan were observed [46].

Increased transamination of tyrosine [6, 10] and tryptophan [34] as measured by augmented excretion of their transamination products in urine may also contribute to the reduced plasma level of these aromatic amino acids. This theory is supported by the observation of increased activity of hepatic tryptophan aminotransferase in liver from hyperphenylalanine rats [51].

Before reaching the sites of protein and neurotransmitter biosynthesis in the brain the essential amino acids must pass the blood-barrier. This barrier actually consists of two barriers in series. The endothelial cells of the capillary walls are responsible for a highly selective transport of amino acids from plasma into the interstitial fluid of the brain. The second transport system is located at the neuronal cell membrane [37]. The actual concentration of amino acids in the interstitial fluid is unknown. It may be somewhat a higher than the concentration in CSF [38] and probably ranges somewhere between the concentrations in plasma and CSF. The plasma/CSF ratios for the nutritionally essential amino acids and tyrosine range between 4.3 and 39.0 [31, 35]. This means that the net transport through the endothelial cells of the capillaries is energetically favorable, whereas the transport across the cell membrane of the neurons is active and requires energy since the intracellular concentrations of the essential amino acids and tyrosine, as estimated from whole brain contents, are of the same order of magnitude as the plasma concentrations. For adult rat brain the ratios of brain contents to serum concentrations range from 0.35 to 1.6 for these amino acids [32]. In human brain obtained post mortem for retarded non-phenylketonuria patients the corresponding ratios for phenylalanine, tyrosine histidine and threonine ranged from 1 to 3 [31].

Transport across the endothelial cells is considered the rate-limiting process in the uptake of amino acids from plasma into the brain [37]. In PKU, however, it is tentatively proposed that the greatly increased level of phenylalanine in the interstitial fluid results in a more pronounced competitive inhibition of the transport of at least some of the neutral amino acids across the cell membrane of the neurons. Consistent with this hypothesis, tryptophan and tyrosine concentrations in CSF were increased in PKU patients compared to controls and a decrease in the plasma/CSF ratios of these amino acids were observed in the PKU patients after discontinuation of phenylalanine-restricted diet. In accordance with our results, McKean and coworkers found a 24% increase of tyrosine in CSF despite a 35% reduction in serum levels in untreated PKU patients compared with retarded patients without phenylketonuria [31]. Tyrosine and tryptophan levels in CSF also increased by 165% and 101% respectively when PKU patients had their phenylalanine-restricted diet supplemented with phenylalanine [30].

The tyrosine content of PKU brains obtained post mortem was decreased to 46% and 59% of control levels in grey and white matter respectively [31] and tryptophan in the cortex to 42% [29]. In infant rat brain a reduction in the content of tyrosine was observed after intraperitoneal administration of phenylalanine, The same was seen in adult rat brain when amethopterin, an inhibitor of phenylalanine hydroxylase, was administered simultaneously. In adult rat brain a depletion of tryptophan, histidine, valine, leucine, isoleucine, methionine and threonine was found both after dietary phenylalanine supplementation and intraperitoneal phenylalanine injection. [32, 39]. Since the intracellular concentrations of tyrosine and tryptophan in the neurons, as estimated from the brain contents, are most likely decreased when the brain is exposed to high levels of phenylalanine, the possibility that the absolute and relative accumulation in CSF of these amino acids was solely to reduced intracellular metabolism is ruled out. Direct evidence for an inhibitory effect of phenylalanine on tyrosine [1, 36] and tryptophan [18] uptake was obtained in vitro using synaptosomal plasma membrane vesicles isolated from rat brain.

The accumulation of tyrosine and tryptophan in CSF of PKU patients has been explained as a result of a competitive inhibition by phenylalanine of the re-uptake into the circulation mediated by the choroid plexus [31]. Inhibition of this transport may contribute to the increase in the CSF levels concurrent with the suggested reduced neuronal uptake discussed above. The existence, however, of increased interstitial concentrations of tyrosine and tryptophan as estimated by the CSF level and decreased intracellular concentrations judged from whole brain content still points to impaired neuronal uptake of these amino acids in PKU.

In PKU the intracellular concentration of phenylalanine in the neurons is elevated by 268% and 394% in white and grey matter respectively as estimated from the phenylalanine content of whole brain obtained post mortem from PKU patients [31]. The increased phenylalanine concentration probably inhibits dopamine and serotonin biosynthesis, since phenylalanine is a competitive inhibitor for both tyrosine and hydroxylase [19] and tryptophan hydroxylase [28], which are presumed to catalyze the rate-limiting processes in the biosynthesis of these neurotransmitters. Metabolites of phenylalanine are other possible inhibitors of neurotransmitter biosynthesis.

McKean [29] reported an inverse relationship between plasma phenylalanine and the biosynthesis of dopamine and serotonin as measured by HVA and 5-HIAA accumulation in CSF after probenecid administration. In necropsied

brains of untreated PKU patients the contents of dopamine, serotonin and noradrenalin were reduced to 30%-40% of normal values [29]. In accordance with McKean [29] we found that after dietary cessation the CSF concentrations of HVA and 5-HIAA decreased with increasing plasma and CSF phenylalanine when data from all seven patients were pooled.

After discontinuation of dietary treatment four out of seven PKU patients performed rather poorly according to RTv. When the phenylalanine-restricted diet was restored, these patients improved significantly and a large increase in CSF concentrations of HVA and 5-HIAA was observed. Three patients performed well on unrestricted diet and did not show consistent changes in RTv, HVA and 5-HIAA when returning to a phenylalanine-restricted diet. It is tentatively proposed that the former four patients lacked dopamine and serotonin when given an unrestricted diet, and that their performance improved due to increased biosynthesis of neurotransmitters. These patients ill most probably benefit from prolonged dietary treatment. The latter three patients that performed well according to RTv might not lack neurotransmitters even on an unrestricted diet. It is not possible, however, to decide whether they can safely terminate. More biochemical and neuropsychological investigations are needed to evaluate the effects of chronic severe elevation of phenylalanine levels in body fluids.

Dietary tyrosine supplement stimulated dopamine biosynthesis as estimated from the increase in CSF level of HVA (Fig. 3D). Simultaneously, an increase in serotonin synthesis was observed as judged by the increased concentration of 5-HIAA in CSF (Fig. 3D), and the increase in 5-HIAA level correlated with the increase in HVA concentration. If tyrosine has any effect on the transport of tryptophan or on biosynthesis of serotonin it may be expected to be inhibitory because of the structural similarities between tyrosine and tryptophan. Thus, tyrosine itself cannot account for the observed increase in serotonin biosynthesis.

It is tentatively proposed that the strongly reduced serotonin biosynthesis in PKU patients not receiving extra tyrosine and that the increase in serotonin biosynthesis after dietary tyrosine supplementation may be at least partly explained by a functional interrelation between the dopaminergic and the serotonergic nervous systems. It is assumed that the dopaminergic and the serotonergic nervous system have antagonistic effects on vigilance. This hypothesis is supported by the observation that dietary tyrosine supplement decreases RTv [27], whereas tryptophan supplementation increases RTv for PKU patients (Lou, unpublished results). In healthy individuals tryptophan increases self-reported fatigue, decreases self-rating of vigor and, relative to tyrosine, it increases auditory reaction time [23, 48]. Since vigilance is essential for the optimal function of the individual it may be speculated that the major reduction in serotonin biosynthesis observed in PKU patients not receiving extra tyrosine may be in part a response to sub-optimal dopamine biosynthesis. When dopamine biosynthesis is stimulated by dietary tyrosine supplementation the biosynthesis of the antagonistic neurotransmitters is probably allowed to increase, maintaining the relative balance between the two nervous systems.

Diets used for treatment of PKU are usually enriched in tyrosine to an extent which compensates for the enzymatic block in the conversion of dietary phenylalanine to tyrosine. In the present study reinstatement of phenylalanine-restricted diet resulted in a decrease in phenylalanine intake of 43 ± 10 mg/kg per 24 h (mean \pm SD) reaching the normal therapeutic level of 16 ± 9 mg/kg per 24 h. Simultaneously the dietary tyrosine consumption was increased by 37 ± 10 mg/kg per 24 h. It may be questionable whether this amount is sufficient to assure an optimal dopamine biosynthesis since even on a phenylalanine-restricted diet, the CSF concentration of phenylalanine is elevated 16 fold and the usual supplement is certainly insufficient on unrestricted diet in which case the CSF level of phenylalanine is increased 37-fold. Pratt [37] has proposed a further supplementation of the traditional phenylalanine-restricted diet with 50-100mg of tyrosine/kg per 24 h.

For rats on a diet containing 6% casein as the protein source, a relatively large decrease in brain content of several of essential amino acids including histidine was observed when the diet was supplemented with 5% tyrosine. Reduced protein biosynthesis and extensive poly-some disaggregation also occurred in the brain [11]. A less dramatic decrease (5%) in brain histidine was observed in rats fed a diet containing 7% tyrosine, but probably much more protein. Most of the other essential amino acids showed decreased but these were less pronounced than the corresponding reductions observed after feeding a diet supplemented with 7% phenylalanine [32]. It must be stressed that the tyrosine supplement (≤ 200 mg/kg per 24 h) used for treatment of PKU patients was much less ($< 15\%$ of calculated protein intake) than the supplement (83% of casein) added to the diet in the former experiment with rats.

Pratt has proposed that the traditional phenylalanine restricted diet should be supplemented with extra amounts (25-100 mg/kg per 24 h) of all of the amino acids, which are essential for the brain, i.e. the nutritionally essential amino acids plus tyrosine, because these amino acids are essential for the biosynthesis of proteins, neurotransmitters, myelin and lipids [37]. This supplement may be especially important for PKU mothers since in addition to the maternal intestine, the endothelial cells of the capillaries in the fetal brain and the neuronal cell membrane, the amino acids must pass one more barrier (the placenta) before reaching the sites of biosynthesis in the developing fetal brain. Besides their properties as essential amino acids, valine, leucine and isoleucine are also beneficial since they competitively exclude phenylalanine from the brain resulting in decreased CSF concentrations of phenylalanine [2, 3]. A case of maternal PKU with increased dietary tyrosine during pregnancy has been reported. The infant had head circumference and weight above the 50th percentile and length at the 50th percentile [40].

A negative correlation between plasma phenylalanine and performance on neuropsychological tests (full-scale IQ, steadiness, concept formation and tactile-motor problem solution) has been reported for PKU children aged 9.6 ± 3.6 years [8]. Using the patients as their own controls, Krause and coworkers [22] found a positive correlation between increase in plasma phenylalanine and increases in choice reaction times. These observations are in accordance with our

results on RTv measurements obtained with PKU patients who had RTv at or above the upper limit of the normal range when on an unrestricted diet (Fig. 1D). After plasma phenylalanine reduction in three previously untreated PKU patients, McKean [29] observed improvement in brain function as measured by shortening of the latency of the visual evoked responses. These improvements were accompanied by increased biosynthesis of dopamine and serotonin as measured by HVA and 5-HIAA accumulation in CSF after probenecid administration [29]. Krause and coworkers [22] found a negative correlation between changes in urinary excretion of dopamine, but no correlation between plasma phenylalanine and urinary serotonin was observed. The most likely reason for this discrepancy is that urinary excretion of dopamine and serotonin does not only reflect biosynthesis in the central nervous system but peripheral metabolism.

It may be concluded that discontinuation of dietary treatment of young adults with PKU may at least for some patients result in impaired mental function as measured by increased reaction time variability and decreased biosynthesis of dopamine and serotonin. When treatment with a phenylalanine-restricted diet is impractical, supplementation of an unrestricted diet with tyrosine (200 mg/kg per 24 h) may be beneficial since patients with elevated reaction time variability on an unrestricted diet do improve as measured by a decreased reaction time variability and by a relative increase in dopamine biosynthesis. It is tentatively proposed that the elevated level of phenylalanine in the interstitial fluid in the brain of PKU patients inhibits to an appreciable degree the neuronal uptake of at least some of the neutral amino acids including tyrosine and tryptophan. We tentatively suggest the existence of a functional interrelation between the dopaminergic and the serotonergic nervous systems.

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