

Increased Vigilance and Dopamine Synthesis by Large Doses of Tyrosine or Phenylalanine Restriction in Phenylketonuria

H. C. LOU,¹ C. LYKKELUND, A.-M. GERDES,¹ H. UDESEN¹ and P. BRUHN²

From the ¹John F. Kennedy Institute, Glostrup, and the ²Department of Neurology, Rigshospitalet, Copenhagen, Denmark

ABSTRACT. Lou, H. C., Lykkelund, C., Gerdes, A.-M., Udesen, H. and Bruhn P. (John F. Kennedy Institute, Glostrup and Department of Neurology, Rigshospitalet, Copenhagen, Denmark). Increased vigilance and dopamine synthesis by large doses of tyrosine or phenylalanine restriction in phenylketonuria. *Acta Paediatr Scand*, 76:560, 1987.

In a group of 9 patients with classical phenylketonuria (PKU) aged 15-24 years we examined the effect of phenylalanine restricted diet on vigilance, as judged by the continuous visual reaction times, and neurotransmitter synthesis, as judged by cerebrospinal fluid (CSF) homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) levels. HVA and 5-HIAA levels decreased significantly with increase in plasma phenylalanine concentration on free diet ($p < 0.01$ and $p < 0.00055$ respectively). Vigilance improved on phenylalanine restricted diet in 6 of the 7 patients with abnormally long reaction times on free diet. Addition to tyrosine (160 mg/kg/24 h) to the free diet resulted in an increased HVA/5-HIAA ratio in CSF in the six patients examined. In 14 patients on the free diet supplemented with tyrosine, an improvement in vigilance (reaction times at the 90 percentile) was seen in all 12 patients with values longer than normal mean (264 msec) ($p < 0.001$). Tyrosine treatment may be a therapeutic alternative when phenylalanine restriction is impractical. *Key words:* vigilance, dopamine, tyrosine, PKU

Early institution of a phenylalanine restricted diet is effective in the prevention of severe mental and neurological deficits in patients with phenylketonuria (1). The aim is usually to achieve plasma phenylalanine levels below $425 \mu\text{mol/l}$ during early childhood. Thereafter a gradual liberalisation of the diet is accepted.

A recent case report from our institution indicates that cessation of treatment even in adolescence may hamper mental function, at least in some patients. This has been demonstrated by decreased performance on tests of vigilance, such as continuous recording of reaction times (RT). These patients also developed impaired CNS synthesis of dopamine and serotonin as judged by decreased concentration of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (2). In a preliminary report we suggested that tyrosine and tryptophan supplementation may be useful in the treatment of PKU (3). The aim of the present study has been a preliminary evaluation of the possible role of high doses of tyrosine in the treatment of such patients.

PATIENTS AND METHODS

Vigilance. As a functional measure of cerebral efficiency vigilance was tested by recording continuous reaction times: A small red stimulus light was switched on at random time intervals (15 per min) for 6.5 min. The patient was required to extinguish the light as quickly as possible by pushing a button and a total of 100 reaction times were recorded. The reaction times were measured in 1/100sec. The 10, 50, and 90 percentiles were calculated. Reaction times and their variability (90-10 percentile difference) are regarded as valuable indicators of both structural and biochemical derangement of the brain (4). To measure the motor component, finger tapping tests were carried out and calculated in the same way.

Neurotransmitter metabolites and amino acids. CSF levels of HVA and 5-HIAA have been determined as they reflect the rate of synthesis of dopamine and serotonin in the central nervous system. Other factors, i.e. transport mechanisms, do also influence such levels. Lumbar puncture was done in a standardised way: always at 10 a.m., two hours after breakfast, while the patient was still in bed. The first 0.5 ml was discarded, and the next 1.5 ml used for analysis of HVA, 5-HIAA, phenylalanine, tyrosine and tryptophan, 2 ml of

venous blood was drawn simultaneously for amino acid analysis. The analyses were carried out by high performance liquid chromatography using electrochemical detection for the determination of HVA and 5-HIAA and fluorimetric detection for the quantitative measurement of the amino acids.

The following sets of examinations were performed:

A. In a sample of 9 patients, reaction times and levels of neurotransmitter metabolites and amino acids were determined after at least three weeks on the usual "relaxed" phenylalanine restricted diet (low protein) supplemented by an amino acid mixture without phenylalanine (Phenylidon[®], Aminogran[®]) or a low phenylalanine hydrolysates (Albumaid[®]). Plasma levels to about 1200 µmol/l were accepted. These examinations were also carried out after 3 weeks of unrestricted diet. The order of two regimens was random.

B. In 6 patients, who had abnormal reaction times on free diet, reaction time and finger tapping tests were carried out in a double blind crossover study with free diet and 160 mg tyrosine per kg (divided into three daily doses) or placebo tablets of similar appearance (calcium lactate) for three days.

C. In an additional sample of 8 patients, reaction times were measured on free diet and free diet supplemented with tyrosine (160 mg/kg divided into three daily doses). The two regimens were administered in this group lumbar puncture and determination of CSF transmitter metabolites and amino acids were carried out on the two regimens.

D. Finally, reaction time variability was determined double blindly in one patient on six occasions, using doses of tyrosine in the range 0-160 mg/kg/24 h in random order.

Table 1. Effect of cessation of dietary phenylalanine (phe) restriction on plasma phe, RT, and CSF neurotransmitter metabolites in adolescents with PKU

First line: phe restriction, second line no phe restriction

Pr.	Age/ sex	Age at dietary control	IQ	EEG	Neurol exam.	RT* (1/100 sec)			Plasma phe (µmol/l)	CSF	
						10 %	50 %	90 %		HVA (nmol/l)	5-HIAA (nmol/l)
1	19/M	3 months	71	Unprovoked normal FS 1½-3Hz +polyspikes	Slight dyscoordination	27	27	37	1011	189	31
						22	27	44	1664	149	18
2	23/M	22 months	85	Normal	Slight dyscoord. +pyr.signs	25	28	32	1008	264	48
						25	30	38	2050	165	29
3	24/F	4 years	70	Normal	Slight dyscoord.	22	25	32	576	171	69
						22	27	34	1613	93	28
4	15/F	5 months	96	Normal	Normal	20	23	30	606	174	61
						20	24	32	1431	126	25
5	16/M	14 months	100	3-5 Hz +sharpwaves	Slight dyscoord.	17	22	28	830	114	36
						17	22	32	1260	120	24
6	20/F	1½ months	57	4-7 Hz +sharpwaves	Normal	24	28	32	456	144	116
						24	26	31	1255	68	37
7	17/M	11 days	102	Unprov. normal FS: 5-7 Hz	Normal	22	25	30	605	118	39
						23	28	31	1160	119	29
8	16/M	3 days	85	3-5 Hz +sharpwaves	Normal	19	23	29	675	178	47
						18	22	28	980	216	58
15	16/M	1 months	82	2½-4 Hz +sharpwaves	Slight dyscoord.	20	23	27	1052	77	18
						20	23	26	1877	84	14
Normal controls (age range 15-24, n=14)						18.1±2.8		26.4±3.6			
										21.5±3.4	

* RT = reaction time

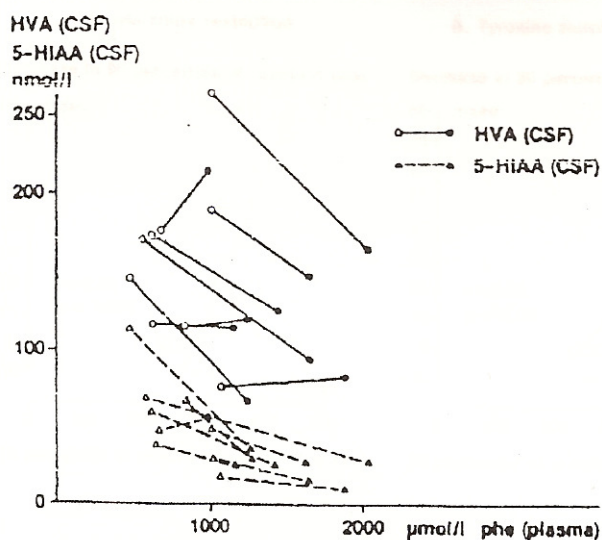


Fig. 1. Plasma phenylalanine and CSF HVA and 5-HIAA levels on phenylalanine restricted diet and free diet in random order.

RESULTS

Table 1 and Fig. 1 show a large inter-individual variation in CSF HVA concentration. HVA and 5-HIAA decrease significantly with increase in plasma phenylalanine on free diet ($p < 0.01$ and $p < 0.0005$ respectively, exponential regression). Tyrosine supplementation was found to increase the CSF HVA level in 4 of 6 cases and consistently raised the HVA/5-HIAA ratio (Table 2).

It is apparent from Table 1 that the five patients in whom dietary control was achieved late (at 3 months or later) had long and fluctuating reaction times.

Table 1 and 2, and Figs. 2, 3 and 4 show that both dietary phenylalanine restriction and

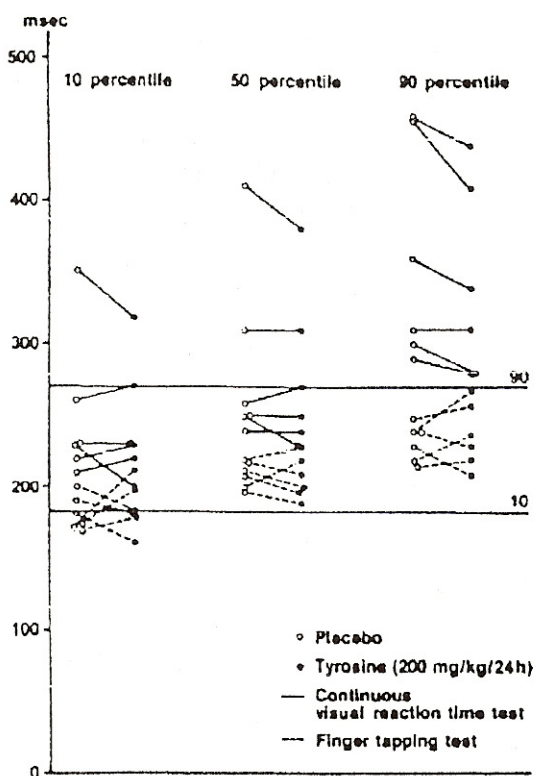


Fig. 2. In a double blind crossover study of six patients on a free diet, a significant decrease in the long RTs (90 percentile) is obtained by tyrosine supplementation (160 mg/kg/24 h) ($p < 0.02$). No effect was seen on the finger tapping test. The horizontal lines indicate the 10th and 90th percentiles of the control group (Table 1).

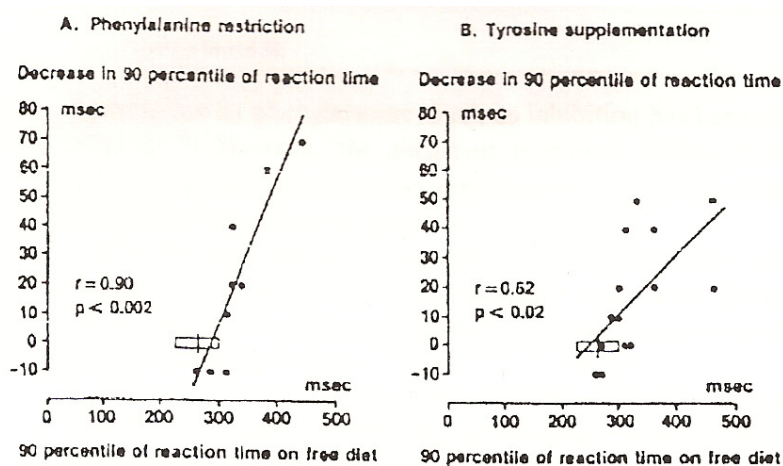


Fig. 3. Decrease in long RTs (90 percentile) on either phenylalanine restriction (A) or tyrosine supplementation (B) as a function of the RT (90 percentile) on unrestricted diet. Vertical bar indicates mean and one standard deviation of controls ($n=14$).

tyrosine supplementations have a normalizing effect on reaction times. This effect is particularly conspicuous in patients with very long reaction times (Fig. 3). Consequently, the inter-individual variations in reaction time were much larger without treatment, regardless of the order of the regimens.

The data from the double blind crossover test, on the effect of tyrosine supplementation to a free diet, is shown in Table 2 (the six patients above the double line), and in Fig. 2. The long reaction times are significantly shortened ($p < 0.02$, paired Student's *t*-test), whereas normal reaction time values (below the normal 90 percentile) were unaffected. Also the pure motor task of finger tapping is unaffected. Hence, tyrosine supplementation appears to shorten the abnormally long reaction times in PKU by facilitating attention and/or the initiation of motor activity as a response to the sensory stimulus. The effect is *not* due to increased speed of the finger tapping.

The effect of tyrosine on vigilance is confirmed by the results shown in Table 2, which includes 8 additional patients. At the reaction time test, a total of 12 patients out of 14 have 90 percentile values above the 90 percentile (mean) of 14 normal controls (Table 1). These abnormally long reaction times were reduced in all 12 patients by tyrosine ($p < 0.001$, paired Student's *t*-test).

Fig. 4 shows the effect of different doses of tyrosine on RT variability in one patient on unrestricted diet (patient 1). A linear relationship is apparent in the dose range used (0–160 mg/kg/h).

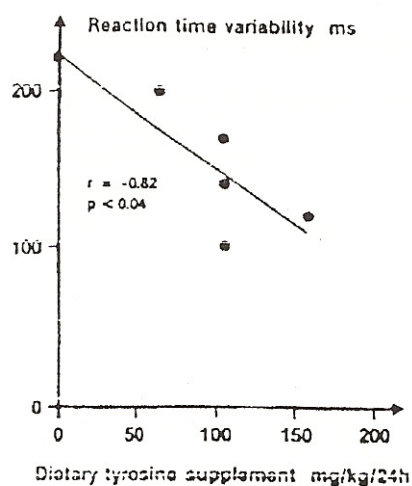


Fig. 4. An example (patient 1) of the dose-response effect of dietary supplementation with tyrosine on the continuous reaction time variability (90–10 percentile). The patient was on unrestricted diet, and the examinations carried out as a double blind study.

Table 2. Effect of tyrosine (tyr) (160 mg/kg) to free diet on plasma phe, tyr, RT and CSF HVA and HVA/5-HIAA
 First line: tyr, second line: +tyr

Pr.	Age/ sex	Age at dietary control	IQ	Neurol			Plasma		CSF				
				EEG Abn.	exam. abn.	RT* (1/100 sec)	phe (μ mol/l)	Tyr (μ mol/l)	HVA (nmol/l)	5-HIAA (nmol/l)	HVA/5 HIAA (nmol/l)		
1	19/M	3 months	71	+	+	22	26	36	1320	50			
						23	27	34	1360	72			
2	23/M	22 months	85	-	+	35	41	46	1760	46			
						32	38	44	1790	63			
3	24/F	4 years	70	-	+	23	25	30	1205	44			
						23	25	28	1300	51			
4	15/F	5 months	96	-	-	22	24	31	1431	49			
						21	24	31	1504	169			
9	17/M	10 months	59	+	+	26	31	46	1500	44			
						27	31	41	1375	68			
10	15/M	1 year	101	-	-	23	25	29	925	72			
						20	23	28	940	121			
11	21/M	3 months	83	-	+	22	27	46	1371	33	78	17	4.6
						22	26	32	1355	88	131	23	5.7
12	16/F	16 days	107	+	-	21	27	33	1385	23	116	27	4.3
						21	22	28	1220		154	30	5.2
13	16/M	25 days	100	+	+	17	22	32	1260	40	-		-
						16	22	32	1240	83	-		-
7	17/M	11 days	102	+	-	23	28	31	1492	51	119**	30	4
						21	22	27	1450	143	70	13	5.4
6	20/F	1½ months	57	+	-	24	26	29	1170	39	-		-
						21	26	28	1410	200	-		-
14	15/M	1 month	105	+	+	16	22	27	1205	35	455**	78	5.8
						16	22	27	1245	55	164	24	6.8
15	16/M	1 month	82	+	+	20	23	26	1877	60	84	14	6
						19	23	27	1657	123	104	14	7.4
16	19/F	18 months	77	-	-	20	22	26	989	38	85	16	5.2
						20	22	27	1242	187	166	18	9.4

*RT = reaction time.

** CSF did not flow freely on lumbar puncture, possibly accounting for the high concentrations of neurotransmitter metabolites.

DISCUSSION

The present study confirms an early case report suggesting that dietary discontinuation in patients with PKU may result in decreased neurotransmitter synthesis and impaired mental function, even in adolescence and young adulthood.

Increased plasma phenylalanine may in theory affect CNS synthesis of dopamine and serotonin through interference with the transport mechanisms of the transmitter precursors tyrosine and tryptophan (5). However, in an earlier study we did not find tyrosine and tryptophan consistency reduced in CSF (3). It is therefore unlikely that decreased tyrosine and tryptophan concentrations in the interstitial fluid in CNS are responsible for decreased neurotransmitter synthesis. If transport inhibition is involved in reduced neurotransmitter synthesis it is more likely due to impaired transport across the neuronal cell membrane. Another possible mechanism is

competitive inhibition of tyrosine-3-hydroxylase and tryptophan-5-hydroxylase by phenylalanine. Such an inhibition has been demonstrated both in vivo and in vitro (6, 7). However, the consistent increase in HVA/5-HIAA ratio after tyrosine administration suggests that dopamine synthesis may be enhanced by the intake of large amounts of its amino acid precursor tyrosine.

The finding that tyrosine administration consistently reduces prolonged reaction times without affecting the speed of the motor component (finger tapping) indicates that dopamine synthesis is of central importance in the regulation of attention and/or the initiation of motor activity in man.

It is our opinion that monitoring of HVA and 5-HIAA levels in CSF and tests of cerebral vigilance may assist in defining a group of young adult PKU-patients who will benefit from continued treatment. However, in many young patients a satisfactory compliance with a strict dietary regimen becomes increasingly difficult. It is therefore encouraging that administration of a naturally occurring neurotransmitter precursor such as tyrosine seems very effective in correcting the deficient synthesis of dopamine as well as the decreased vigilance. Supplementing the diet with a large dose of tyrosine may therefore be a new and important therapeutic alternative in cases where strict dietary control is impractical. The long term effects of such a regimen are not yet known and will require further investigations.

REFERENCES

1. Güttler F. Hyperphenylalaninemia. *Acta Paediatr Scand* 1980; Suppl 280: 1-80
2. Lou HC, Güttler F, Lykkelund C, Bruhn P, Niederwieser A. Decreased vigilance and neurotransmitter synthesis after discontinuation of dietary treatment for phenylketonuria (PKU) in adolescents. *Eur J Paediatr* 1985; 144; 17-20
3. Lou HC. Large doses of tryptophan and tyrosine as potential therapeutical alternative to dietary phenylalanine restriction in phenylketonuria. *Lancet* 1985; II: 150-51.
4. Elsass P, Christensen SE, Ranek L, Theilgaard A, Tygstrup N. Continuous reaction time in patients with hepatic encephalopathy. A quantitative measure of changes in consciousness. *Scand J Gastroenterol* 1985; 16: 441-47
5. Herrero E, Aragon MC, Gumenez C, Valdivieso F. Inhibition by L-phenylalanine of tryptophan transport by synaptosomal plasma membrane vesicles: Implications in the pathogenesis of phenylketonuria. *J Inherited Metab Dis* 1983; 6: 32-35
6. Curtis HC, Niederwieser A, Visconti M et al. Serotonin and dopamine synthesis in phenylketonuria. In: Haber B et al., eds. *Serotonin: Current aspects of neurochemistry and function*. New York: Plenum Publ Corp, 1982: 277-89.
7. Ikeda M, Levitt M, Udenfriend S. Phenylalanine as substrate and inhibitor of tyrosine hydroxylase. *Arch Biochem Biophys* 1967, 120: 420-27

Submitted Sept. 1, 1986. Accepted Dec. 13, 1986

(H.C.L) John F. Kennedy Institute
Gl. Landevej 7
DK-2600 Glostrup
Denmark