JCI The Journal of Clinical Investigation

Biochemical and neuropsychological effects of elevated plasma phenylalanine in patients with treated phenylketonuria. A model for the study of phenylalanine and brain function in man.

W Krause, … , D Freides, L Elsas

J Clin Invest. 1985;75(1):40-48. https://doi.org/10.1172/JCI111695.

Research Article

Phenylketonuria provides a human model for the study of the effect of phenylalanine on brain function. Although irreversible mental retardation is preventable through newborn diagnosis and dietary phenylalanine restriction, controversy exists regarding the effects of increased concentrations of phenylalanine in older patients. We have studied ten older, treated, phenylketonuric patients using a triple-blind, multiple trials, crossover design. Each patient was tested at the end of each of three 1-wk periods of high or low phenylalanine intakes. Tests included a repeatable battery of neuropsychological tests, analysis of plasma amino acids, and measurement of urine amino acids, phenyl organic acids, dopamine, and serotonin. In all 10 patients plasma phenylalanine rose (900-4,000 microM). In 9 of 10 patients there was an inverse relationship between plasma phenylalanine and urine dopamine excretion. When blood phenylalanine was elevated, these patients had prolonged performance times on neuropsychological tests of higher but not lower integrative function. Urinary serotonin fell during phenylalanine loading in six patients. The concentration of phenylacids in the urine was not proportional to the plasma phenylalanine at concentrations below 1.5 mM. In one patient, neither performance time nor dopamine excretion varied as blood phenylalanine rose or fell. We interpret these data as follows: blood phenylalanine above 1.3 mM impairs performance on neuropsychological tests of higher integrative function, this effect is reversible, and one mechanism may […]

Find the latest version:

https://jci.me/111695/pdf

Biochemical and Neuropsychological Effects of Elevated Plasma Phenylalanine in Patients with Treated Phenylketonuria

A Model for the Study of Phenylalanine and Brain Function in Man

Wilma Krause, Margaret Halminski, Linda McDonald, Philip Dembure, Rino Salvo, David Freides, and Louis Elsas
Division of Medical Genetics, Departments of Pediatrics and Psychology, Emory University, Atlanta, Georgia 30322

Abstract

Phenylketonuria provides a human model for the study of the effect of phenylalanine on brain function. Although irreversible mental retardation is preventable through newborn diagnosis and dietary phenylalanine restriction, controversy exists regarding the effects of increased concentrations of phenylalanine in older patients. We have studied ten older, treated, phenylketonuric patients using a triple-blind, multiple trials, crossover design. Each patient was tested at the end of each of three 1-wk periods of high or low phenylalanine intakes. Tests included a repeatable battery of neuropsychological tests, analysis of plasma amino acids, and measurement of urine amino acids, phenyl organic acids, dopamine, and serotonin. In all 10 patients, plasma phenylalanine rose (900–4,000 μM). In 9 of 10 patients there was an inverse relationship between plasma phenylalanine and urine dopamine excretion. When blood phenylalanine was elevated, these patients had prolonged performance times on neuropsychological tests of higher but not lower integrative function. Urinary serotonin fell during phenylalanine loading in six patients. The concentration of phenylacids in the urine was not proportional to the plasma phenylalanine at concentrations below 1.5 mM. In one patient, neither performance time nor dopamine excretion varied as blood phenylalanine rose or fell. We interpret these data as follows: blood phenylalanine above 1.3 mM impairs performance on neuropsychological tests of higher integrative function, this effect is reversible, and one mechanism may involve impaired biogenic amine synthesis.

Introduction

Nearly a half-century ago Følling (1) attributed a syndrome of mental retardation and aberrant behavior to an inherited metabolic error. Since then, phenylketonuria (PKU)[1] has been the prototype for investigations of the effect of phenylalanine on central nervous system function in man. It is clear that if plasma phenylalanine is normalized before age 3 wk through dietary restriction of phenylalanine, irreversible mental retardation is prevented (2). The mechanisms for producing this permanent structural damage remain unclear, but several hypotheses have developed. Decreased or abnormal myelin formation and/or impaired oligodendroglial migration during the first 6 mo of postpartum brain development are the most probable mechanisms (3–7).

Controversy persists regarding possible effects of elevated phenylalanine on brain function when development is nearly complete in older, treated patients with PKU. Whether or not elevated concentrations of phenylalanine disturb central nervous system function in these patients is unknown. Since ~1 in 16,000 Caucasian newborns (Georgia statistics) (8) is affected with PKU, and effective newborn screening has prevented permanent brain damage since 1970 in the newborn screenee, an answer to the question of whether high plasma phenylalanine affects mental function becomes more urgent for this accumulating population.

Silverman and Guthrie (unpublished observations) approached the question by administering one loading dose of phenylalanine to control subjects, heterozygotes, and homozygous affected patients with PKU and compared errors in response time among the three groups. Their results suggested a difference among the three groups which related directly to the concentrations of plasma phenylalanine achieved.

In 1980, Waisbren et al. (9) reviewed the available literature on psychological assessment of children after termination of phenylalanine-restricted diets. Results were mixed, some showing a drop in IQ and other achievement test scores and others showing no change. Numbers of patients, study design, and assessment tools varied greatly among the reports. The PKU Collaborative Study began a prospective study in 1967 (10). Results of achievement tests (Stanford Binet, Wechsler Intelligence Scale (WISC), Wide Range Achievement Tests [WRAT]) on 81 children, 38 of whom had continued the diet beyond 6 yr of age and 43 of whom had discontinued at 6 yr of age, were reported in 1982. Results at 8 yr of age showed slightly lower achievement in reading and spelling in the discontinuers. No significant difference in IQ between the groups was observed after this 2-yr interval (11). Brunner et al. (12) in a recent study (1983) reported a negative correlation between performance on neuropsychological tests and serum phenylalanine concentration on the day of testing in a group of early treated patients age 6–13 yr. Neither of these studies used the patient as his/her own control. Interindividual variation, differences in phenylalanine concentrations achieved and in techniques used by collaborating centers have hindered interpretation of results.

In in vitro systems, phenylalanine influences the synthesis of two biogenic amines, dopamine and serotonin, which are

This work was presented in part at the Combined Plenary Session American Society for Clinical Investigation/Society for Pediatric Research, Washington, DC, 1983.

Address reprint requests to Dr. Krause.

Received for publication 17 January 1984 and in revised form 10 July 1984.

1. *Abbreviations used in this paper:* PKU, phenylketonuria; WISC, Wechsler Intelligence Scale; WRAT, Wide Range Achievement Tests.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/85/01/0040/09 $1.00
Volume 75, January 1985, 40–48

critical compounds in neurotransmission (13). Both tyrosine-3-hydroxylase (E.C.C.1.14.16.2) and tryptophan-5-hydroxylase (E.C.C.1.14.16.4) are rate-limiting enzymes in the synthesis of dopamine and serotonin, respectively, and are competitively inhibited by phenylalanine at millimolar concentrations (14, 15). Another potential inhibitory effect of phenylalanine on biogenic amine synthesis is through impaired uptake of tyrosine and tryptophan across the blood-brain barrier. Phenylalanine, tyrosine, and tryptophan share the same transport system and compete for a common transport function at physiologic concentrations (16). Since transport of amino acids across the blood-brain barrier is the rate limiting step in the movement of amino acids from plasma to brain, and since their plasma concentration is near saturation of their transporter proteins, increased concentrations of plasma phenylalanine could limit the transport of tyrosine and tryptophan and thus their availability to the brain cell membrane for neuropeptide synthesis or conversion to biogenic amines (16–19).

The current study compares specific neuropsychological tests with changes in plasma phenylalanine and biogenic amine production in young adults and older children with PKU. Although the dopamine excreted in the urine is a reflection of multiple sources of dopamine synthesis (20), we chose to measure urine dopamine, since it reflects 24-h production of the amine, not an acute level, and because urine collection is a noninvasive method of obtaining biologic fluids. Assessment is made of competitive inhibition by phenylalanine of tyrosine and tryptophan transport by kidney tubule. We use a triple-blinded, crossover, clinical protocol to circumvent the influence of individual variation in this disorder (21, 22).

Methods

Study design. 10 patients with PKU, aged 6–24 yr, were admitted on a 21-d protocol to the Emory University Clinical Research Facility. Informed consent was obtained from adult patients or from the parents of patients <21 yr of age. Each patient served as his or her own control. Each patient was admitted on one of two double crossover protocols and five were studied in each protocol group. Either the patient entered on a low dietary phenylalanine which was increased the second week and decreased the third week (low-high-low) or in the reverse pattern (high-low-high). Patients equilibrated for 7 d after each change in dietary phenylalanine. Past plasma concentrations of phenylalanine on known intake and genotyping of parents were used to determine the amount of phenylalanine added to patient formulation for restriction and loading (23, 24). Patients whose entering concentration of plasma phenylalanine was high either because of poor control or because of diet discontinuation for several years were on the high-low-high protocol. Five other patients who had been in consistently good dietary control entered the study on the low-high-low protocol.

The study diet was based on Phenylfree or Lofenalac as a phenylalanine-free amino acid source. A specified amount of tasteless L-phenylalanine was added to the formula during the loading phases. The study was triple-blinded: neither the patients nor their parents could taste the difference in formula and were unaware of their experimental condition; the psychologist administering the neuropsychologic tests was uninformed of the patients' blood phenylalanine concentration; and the laboratory personnel performing amino acid, organic acid, and amine analyses did not know the condition under which samples were obtained.

Biochemical tests. Blood and urine samples were obtained on all patients at the beginning of the first week as a baseline and at the new equilibria achieved at the end of each 7-d interval.

Plasma and urine amino acids were analyzed by ion exchange chromatography on the Beckman model 119 CL using lithium buffers (Beckman Instruments Inc., Palo Alto, CA). Because tryptophan is somewhat labile in extraction from blood, recovery of "spiked" standards from whole blood and urine was quantitated to determine losses. In the physiological ranges measured from 50 to 200 μM, recovery was 82–90% efficient. Data are presented normalized to an internal standard without correction for these specific losses which are in the range for the internal standard, *S*-2-aminoethyl-L-cysteine.

Dopamine assays were performed using the single isotope radioenzymatic assay developed by Peuler and Johnson (25). This method used catechol-*o*-methyl transferase from rat liver to transfer a radioactive methyl group from *S*-adenosyl methionine to catecholamine, forming methyl catecholamine derivatives which were then characterized by radiochromatographic analysis. The assay was sensitive in urine to 120 pg/ml for dopamine.

Serotonin was determined by a radioimmunoassay developed by Peskar and Spector (26) using rabbit antibody prepared by coupling serotonin to bovine serum albumin. The antibody bound 50% of ^{3}H-serotonin in the absence of free serotonin. Less than 1 ng of free serotonin was detected by standard displacement methods.

Urine organic acids were analyzed by gas chromatography on a HP 5992 gas chromatograph/mass spectroscope and quantitated on a HP 5790 gas chromatograph. Organic acids were extracted with ethyl acetate and ether and derivatized with trimethylsilane and *bis*-(trimethylsilyl)trifluoracetamide (27). The level of sensitivity for phenylacids in urine was ~5 μg. Specific recovery of phenylacetic, phenyllactic, and phenylpyruvic acids were 68, 91, and 58%, respectively. All calculations are corrected for these losses by parallel external and internal standards used during extraction, derivatization, and quantitation.

Renal clearances were calculated for phenylalanine, tyrosine, and tryptophan, from timed 24-h urine collections and mid-point plasma collections. Both specimens were quantitated for concentrations of amino acids and creatinine. The glomerular filtration rate (GFR) was calculated from the creatinine clearance, as were the rates of a specific amino acid filtration, excretion, and reabsorption using the following formulation (28): $F_{AA} = GFR \times P_{AA}$, $E_{AA} = U_{AA} \times V$, $T_{AA} = F_{AA} - E_{AA}$, where U was urinary amino acid concentration in mg/ml, V (urine volume) in ml/min, and P_{AA} the plasma amino acid concentration in mg/ml. The F_{AA} (filtered aminoacid), E_{AA} (excreted aminoacid), and T_{AA} (reabsorbed aminoacid) were expressed in mg/min. Percent reabsorption was calculated as $T_{AA}/F_{AA} \times 100$.

Neuropsychological tests. Measurements of general intelligence and achievement were based on the Wechsler Intelligence Scales (The Wechsler Adult Intelligence Scale for adults and the WISC for children) and the WRAT. To determine the influence of phenylalanine concentrations on neuropsychological performance, a repeatable battery of tests was developed and administered as a baseline on admission to the study and at the end of each 1-wk treatment period. A confounding variable inherent in tests given multiple times is subject learning. Two procedures were incorporated in the study design to reduce the artifacts due to learning. For one group of tests (type 1, Table I), the subject was allowed to practice the task until the asymptote of the learning curve was reached. Any changes in performance after becoming maximally competent with the task then reflected experimental manipulation of the patient. This procedure would not eliminate learning artifacts from a second group of tests (type 2, Table I). Because of this limitation, equivalent forms of this latter group of tests were developed to be given at the end of each of the experimental conditions. Table I lists the test names and the neuropsychological variables they measured.

Interpretation of data. Data are arrayed for all subjects in tabular form (Tables II, III, IV, and V) to emphasize intraindividual differences because of the wide interindividual variability in age, sex, intellectual competence, and phenylalanine requirements. From these tables, individual differences in dopamine and choice reaction time and the direction of change between the two dietary conditions are calculated

Table I. Neuropsychological Tests Used and the Variables Measured by Them

Test name	Type*	Age‡	Variables
Tests of higher integrative function			
Choice reaction time			
Figures	1	6+	Visual-perceptual discrimination and associated latency
Letters	1	6+	
Rhyme	1	6+	
Trails B	2	8+	Complex visual-motor coordination Maintaining a set Following instructions
Digit span	2	8+	Short-term auditory memory Concentration
Benton visual retention	1	8+	Visual memory
Buschke-Morgan	2	8+	Attention Short-term auditory memory
Symbol digit	2	6+	Visual-motor speed Concentration
Tests of lower integrative function			
RAN	2	6+	Verbal visual integration
Trails A	2	6+	Visual-motor coordination
Grooved pegboard	1	5+	Visual-spatial-tactile coordination Visual-motor speed
Halstead finger tapping	1	5+	Fine motor skill

* 1, A test which can be repeated over several testing sessions. 2, A test which cannot be repeated and must be presented as equivalent forms over several testing sessions.

‡ +, The age listed in years plus greater ages.

and plotted against changes in plasma phenylalanine during the same intervals in Figs. 2 and 3.

Results

Patient profiles and study design. Age, sex, IQ, and achievement scores for all patients are listed in Fig. 1. Each patient was given a symbol which was used in subsequent graphs. IQ scores below 85 in A.S. and K.K. were explained by their late diagnoses and treatment at 5 and 18 mo of age, respectively. K.K. is the older brother of T.K. Both D.A. and W.J. were diagnosed and treated before 3 wk of age and both had IQ scores which were consistent with parental scores (D.A.'s parents' scores were 103 and 83; W.J.'s were 88 and 70).

Symbol	Initial	Age (y)	Sex	Full Scale IQ	Verbal/ Performance IQ	Wide Range Achievement Test		
						Reading	Spelling	Arithmetic
■	B.R.	24	M	93	93/94	98	86	91
○	D.A.	7 8/12	M	79	81/80	75	66	73
△	M.B.	6 10/12	M	94	97/92	98	103	108
□	M.K.	10 6/12	F	114	117/108	112	101	91
▲	T.K.	14 4/12	M	91	87/98	100	92	84
◇	T.W.	9 1/12	F	90	90/92	96	96	84
★	A.S.	23	F	83	87/81	109	106	77
●	K.K.	18 3/12	M	67	66/70	68	66	66
◆	W.J.	6 10/12	M	77	79/78	94	98	95
☆	M.F.	8 9/12	M	91	86/100	91	79	80

Figure 1. Identification of patients by age, sex, IQ, and achievement. The Wechsler Adult Intelligence Scale and the WISC-revised were used to determine IQ. Scores given for the WRAT are standard scores.

Biochemical results. Plasma and urine amino acid and urine organic acid concentrations are presented for all patients in Table II during each of the three experimental conditions. The level of dietary phenylalanine was calculated from actual intake in the Clinical Research Facility, Emory University. The time interval of 7 d required for stabilizing the plasma phenylalanine concentration on a constant diet was determined by sampling one subject daily. A new plateau of blood phenylalanine concentration was achieved on the sixth to seventh day after each diet change. The plasma phenylalanine reflected the diet changes, and the relationship between intake and plasma concentration demonstrated interindividual variation between ingested phenylalanine ranging from 36 to 130 mg/kg per d and plasma phenylalanine concentration ranging from 800 to 4,400 μM.

Urinary phenyl acids are not detected in the urine of normal subjects. Four of the five patients who were on the high-low-high protocol and had not recently been on restricted phenylalanine intake were excreting large amounts of phenylpyruvate and phenyllactate at the end of the first week of high phenylalanine intake. Excretion of both fell dramatically after 1 wk of restricted phenylalanine intake. However, excretion reached the original high levels at the end of the third week (high dietary phenylalanine). In the low-high-low group who had been on continuous dietary control before entry into this study, excretion of organic acids never reached the high levels of the other group despite comparable plasma phenylalanine levels. In general, <50 mg of phenylacids per gram creatinine were excreted until the plasma phenylalanine rose above 1,500 μM. Those with the highest plasma phenylalanine did not consistently excrete the greatest amount of derived organic acids.

The results of dopamine and serotonin excretion are arrayed in Table III. Results were normalized to creatinine excretion. Interindividual variation in dopamine excretion was great. In general, the patients who were on the low-high-low protocol and had been on consistent dietary management before the study achieved higher levels of dopamine excretion than did those patients in the high-low-high group who were not well controlled immediately before the study. This kind of separation was not seen for serotonin excretion. Changes in dopamine excretion varied inversely with changes in plasma phenylalanine in 9 of 10 patients. The inverse relationship of changes in plasma phenylalanine concentrations and urinary dopamine excretion are graphed in Fig. 2. Solid symbols represent patients on the high-low-high protocol. Open symbols represent patients on low-high-low protocols. Results from all patients cluster in quadrants I and III regardless of the protocol (high-low-high or low-high-low) where quadrants I and III circumscribe an inverse relationship between plasma phenylalanine and urinary dopamine concentrations. Symbols in quadrant I show an increase in urine dopamine with decrease in plasma phenylalanine; those in quadrant III show a decrease in urine dopamine with an increase in plasma phenylalanine. Serotonin excretion did not vary directly with changes in phenylalanine.

Studies of membrane transport. To explore the possibility that increased concentrations of phenylalanine might competitively inhibit tyrosine or tryptophan uptake by the only plasma membrane transport function available for study in

Table II. Effects of Dietary Manipulation of Phenylalanine (PHE) on Concentrations of PHE, Tyrosine (TYR), and Tryptophan (TRP) in Plasma and Urine and on Excretion of Three Organic Acids

Patient	Wk	Dietary PHE	Plasma PHE	Plasma TYR	Plasma TRP	Urine PHE	Urine TYR	Urine TRP	Urine organic acids (*mg/g creatinine*) Phenyl-pyruvate	Phenyl-acetate	Phenyl-lactate
		mg/kg/d	*μM*	*μM*	*μM*	*mg/g creatinine*	*mg/g creatinine*	*mg/g creatinine*			
B.R.	1	36	1,255	46	46	192	16	4.6	68	np	203
	2	4	252	65	66	99	8.5	2.4	28	np	36
	3	36	797	33	46	22	7.1	10.7	35	np	21
W.J.	1	105	1,790	33	24	155	6	2	639	150	950
	2	7	197	26	36	40	6	19	17	nd	37
	3	105	1,303	29	23	375	7	16	1,057	377	872
K.K.	1	82	2,317	37	34	256	5	6	597	87	1,233
	2	7.5	1,426	24	52	114	4	7	55	38	87
	3	81	3,296	49	58	295	6	10	1,126	103	2,143
T.K.	1	74	2,058	28	42	850	14	37	787	128	1,179
	2	8	753	35	63	88	6	3	59	63	56
	3	74	2,647	35	53	313	8	8	1,217	152	1,776
A.S.	1	69	4,405	33	36	496	14	20	877	79	689
	2	8	441	22	45	74	7	9	36	10	23
	3	69	3,900	26	35	178	6	15	894	94	515
D.A.	1	10	668	65	117	18	6	3	13	1	22
	2	100	3,260	85	94	361	18	11	179	71	329
	3	10	1,632	78	113	44	5	2	34	103	118
M.B.	1	17	304	46	65	38	20	8	nd	nd	15
	2	94	1,549	57	43	166	14	3	69	84	110
	3	16	634	51	63	76	6	7	34	5	71
M.F.	1	19	199	37	68	13	5	7	12	nd	12
	2	95	1,402	87	80	195	13	19	15	175	nd
	3	18	329	28	50	50	8	17	18	28	nd
M.K.	1	38	793	58	93	112	25	14	19	38	50
	2	130	2,460	62	43	367	22	11	791	130	751
	3	40	577	47	41	94	20	10	9	58	45
T.W.	1	17	352	45	40	48	9	26	nd	nd	nd
	2	100	2,290	32	33	509	15	32	464	273	450
	3	15	536	32	40	104	7	29	nd	nd	nd

np, not processed; nd, none detected. Data are single measurements. The space between patients A.S. and D.A. separates the patients on the high-low-high protocol above from those on the low-high-low protocol below.

children, we quantitated their renal tubular transport. Renal tubular reabsorption data were obtained on eight patients under these conditions of phenylalanine loading and are presented in Table IV. Phenylalanine did not inhibit tyrosine reabsorption by renal tubular epithelium at the levels of filtered phenylalanine reached in these patients. At the highest rate of filtered phenylalanine (45 mg/min/M^2 in patient A.S.), we observed no less than 99% reabsorption of tyrosine. Maximum renal uptake of tryptophan was also seen at these filtered loads of phenylalanine. These findings differ from earlier results reported by Lines and Waisman (29), who reported a generalized aminoaciduria in PKU patients and suggested the possibility of competitive inhibition of reabsorption by high filtered loads of phenylalanine. However, their data were not adjusted for surface area. Our data for renal tubular transport provide negative evidence for a significant effect of phenylalanine on tyrosine uptake in the proximal renal tubule at the same time that dopamine excretion is reduced. Whether or not the lack of effect of increased phenylalanine reabsorption of amino acids in the proximal renal tubule is an appropriate reflection of transport across the blood-brain barrier is not known. Evaluation of blood-brain barrier transport using in-

Table III. Effects of Dietary Manipulation of Phenylalanine (PHE) on the Excretion of Dopamine and Serotonin

Patient	Wk	Dietary PHE	Plasma PHE	Urine volume	Urine dopamine	Urine serotonin
		mg/kg/d	*μM*	*ml/24 h*	*μg/gm creatinine*	*μg/gm creatinine*
B.R.	1	36	1,255	1,700	6	114
	2	4	252	655	166	204
	3	36	797	940	88	127
W.J.	1	105	1,790	650	16	151
	2	7	197	900	26	1,280
	3	105	1,303	955	35	1,273
K.K.	1	82	2,317	800	48	343
	2	7.5	1,426	955	69	324
	3	81	3,296	1,498	36	1,014
T.K.	1	74	2,058	1,780	41	356
	2	8	735	1,940	94	726
	3	74	2,647	1,724	73	370
A.S.	1	69	4,405	2,670	15	749
	2	8	441	2,500	51	388
	3	69	3,900	2,910	20	166
D.A.	1	10	668	970	429	512
	2	100	3,260	602	48	259
	3	10	1,632	405	192	442
M.B.	1	17	304	338	124	408
	2	94	1,549	750	39	234
	3	16	634	342	158	577
M.F.	1	19	199	570	26	140
	2	95	1,402	475	116	877
	3	18	329	820	103	1,070
M.K.	1	38	793	735	294	914
	2	130	2,460	570	96	571
	3	40	577	928	194	596
T.W.	1	17	352	1,560	80	438
	2	100	2,290	1,340	35	626
	3	15	536	980	150	623

The space between patients A.S. and D.A. separates the patients on the high-low-high protocol above from those on the low-high-low protocol below.

vasive techniques is not ethical in healthy children. Nuclear imaging techniques may be useful in the future.

Neuropsychological tests. Part of the purpose of this study was to determine the kinds of tests most suitable for determining possible changes in performance in treated PKU children challenged with phenylalanine. We found that many of the standard tests were too difficult to be applicable across the age group we were assessing and data could not be obtained on all 10 subjects. More complete analysis of these issues will be presented in a separate paper. Results are presented here for those tests on which data were obtained for all subjects. Data were obtained from all 10 subjects on the Choice Reaction Time when figures were used for matching. They were also complete on the Pegboard Test, the Tapping Test, and on Trails "A". Table V summarizes the results of the Choice Reaction Time and the Grooved Pegboard Test. The latter is a test of visual-spatial-tactile coordination and motor speed, whereas the Computerized Choice Reaction Time is a test of visual-perceptual discrimination, and by comparison is a test of higher integrative function. The Grooved Pegboard Test results are typical of results of the tests of lower integrative function, i.e., no significant differences were seen between conditions. In three other tests of lower integrative function of which the Grooved Pegboard is representative, <3 of 10 showed changes consistent with changes in plasma phenylalanine. Those results are not reported here. In the Choice Reaction Time Test, 7 out of 10 subjects showed changes concomitant with changes in plasma phenylalanine, i.e., reaction time was prolonged with increased plasma phenylalanine. M.F., who did not demonstrate typical changes in his Choice Reaction Time, also did not have a decrease in urinary dopamine when plasma phenylalanine concentrations were elevated (compare Table V with Table III, week 1 to week 2). Differences in Choice Reaction Time were not as consistent in K.K. and A.S. as in the other subjects. It is pertinent to note that K.K. and A.S. were not treated effectively early in life, and were less competent by achievement testing than many of the other patients (see Fig. 1). A graphic display of changes in phenylalanine and changes in Choice Reaction Time among these 10 patients is shown in Fig. 3. A direct relationship was seen between changes in plasma phenylalanine concentration and reaction time. When plasma phenylalanine increased, the choice reaction time increased; that is, performance worsened. Conversely, when phenylalanine concentrations fell, choice reaction times were shorter, which indicated improved performance. Solid symbols again represent patients on high-low-high dietary protocol and open symbols those on low-high-low dietary protocol. Symbols in quadrant II represent changes of increased choice reaction time with changes reflecting increased plasma phenylalanine concentrations. Symbols in quadrant IV indicate decreased choice reaction time with decreased plasma phenylalanine (Fig. 3).

By comparing Figs. 2 and 3, one sees the inverse relationship between choice reaction time and dopamine excretion. As urinary dopamine fell, choice reaction time increased; that is, performance worsened.

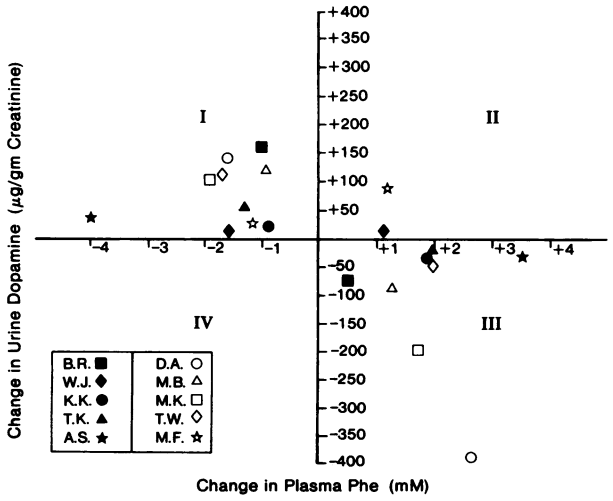

Figure 2. The relationship of changes in plasma phenylalanine to changes in urine dopamine during dietary manipulation of phenylalanine. Each patient is represented by a symbol that appears twice on the graph, indicating the difference in dopamine between the first and second week and the second and third week plotted against parallel changes in plasma phenylalanine.

Table IV. Absence of an Effect of Increased Filtered Phenylalanine on Renal Tubular Reabsorption of Tyrosine (Tyr) and Tryptophan (Trp)

Patient	Wk	Urine volume	^{F}Phe	^{F}Tyr	^{T}Tyr	Tyrosine transport	^{F}Trp	^{T}Trp	Tryptophan transport
		ml/24 h	*mg/min/M²*	*mg/min/M²*	*mg/min/M²*	*% Reabsorption*	*mg/min/M²*	*mg/min/M²*	*% Reabsorption*
B.R.	1	1,700	8.852	0.357	0.350	98	0.402	0.399	99
	2	800	1.945	0.547	0.542	99	0.627	0.626	99
	3	940	5.737	0.260	0.257	99	0.409	0.404	99
W.J.	1	650	10.03	0.201	0.199	99	0.166	0.165	99
	2	900	1.35	0.195	0.194	99	0.308	0.301	98
	3	955	6.94	0.134	0.132	99	0.148	0.145	98
T.K.	1	1,780	2.800	0.248	0.242	97	0.416	0.399	96
	2	1,940	4.311	0.360	0.357	99	0.723	0.722	99
	3	1,724	np*	np	np	np	np	np	np
A.S.	1	2,670	16.551	0.137	0.134	98	0.166	0.162	98
	2	2,500	3.146	0.121	0.120	99	0.272	0.269	99
	3	2,910	45.66	0.330	0.327	99	0.513	0.509	99
M.B.	1	338	1.558	0.259	0.254	98	0.413	0.411	99
	2	750	8.773	0.351	0.347	99	0.302	0.301	99
	3	342	np	np	np	np	np	np	np
M.F.	1	570	1.483	0.306	0.304	99	0.629	0.627	99
	2	475	6.120	0.418	0.415	99	0.434	0.430	99
	3	820	2.753	0.259	0.255	98	0.512	0.503	98
M.K.	1	735	6.468	0.519	0.508	98	0.939	0.934	99
	2	570	14.872	0.411	0.403	98	0.325	0.321	99
	3	928	4.552	0.426	0.418	98	0.401	0.397	99
T.W.	1	1,560	2.438	0.342	0.339	99	0.340	0.332	98
	2	1,340	18.641	0.285	0.278	97	0.331	0.316	95
	3	980	3.419	0.220	0.218	99	0.316	0.306	97

* np, not processed. The space between patients A.S. and M.B. separates the patients on the high-low-high protocol above from those on the low-high-low protocol below. Calculations for renal tubular reabsorption are detailed in the methods section. ^{F}Phe, ^{F}Tyr, ^{F}Trp, filtration rate of phenylalanine, tyrosine, and tryptophan, respectively. ^{T}Phe, ^{T}Tyr, ^{T}Trp, tubular reabsorption rate of phenylalanine, tyrosine, and tryptophan, respectively. mg/min/M², mg of indicated amino acid filtered or reabsorbed per minute normalized to meters squared of body surface area.

Discussion

Although mechanisms are unclear, the negative effect of increased blood phenylalanine on the developing human brain during infancy and early childhood is clear (1–7). Early dietary restriction of phenylalanine prevents irreversible brain damage in children detected and treated for phenylalanine hydroxylase deficiency (2). The studies reported here investigate whether elevated blood phenylalanine in the older child and young adult is associated with altered mental function, and if so, by what mechanism.

Early studies by Weil-Malherbe (30), Nadler and Hsia (31), and McKean (13) demonstrated decreased levels of catecholamines in blood, urine, and autopsied brains of untreated patients with phenylketonuria. McKean (13) also demonstrated improvements in visual evoked response in three severely retarded untreated patients when dietary phenylalanine was restricted or when catecholamine precursors were administered without restricting phenylalanine in the diet. He postulated that although concentrations of tyrosine (1.2×10^{-4} M/g of brain) in brain of hyperphenylalaninemic patients were well above the K_m reported for tyrosine hydroxylase in mammalian brain tissue (5×10^{-5} M) (32), phenylalanine itself might inhibit tyrosine hydroxylase activity directly. This hypothesis was supported by in vitro observations of Udenfriend (33), who found that phenylalanine was a competitive inhibitor of rat brain tyrosine hydroxylase with a $K_i = 1.7 \times 10^{-5}$ M. Since the concentrations of phenylalanine found by McKean (13) in his autopsy material averaged 8.4×10^{-4} M, such a mechanism of competitive inhibition was possible.

Phenylalanine may impair production of two psychoactive amines, namely dopamine and serotonin. Curtius et al. (34) described both decreased serotonin and dopamine synthesis in patients with high plasma phenylalanine concentrations caused by both phenylalanine hydroxylase deficiency and disorders in the tetrahydrobiopterin pathway. He also postulated competitive inhibition of both tyrosine and tryptophan hydroxylase by high phenylalanine at 1,500 and 600 μM concentrations,

Table V. Effects of Dietary Manipulation of Phenylalanine (PHE) on Choice Reaction Time and Grooved Pegboard Assembly

Patient	Wk	Plasma PHE	Choice reaction time	Pegboard $\bar{x}$ (s/10 pegs) Right	Left
		μM	*m/s*		
B.R.	1	1,255	811	57	73
	2	252	601	57	68
	3	797	654	51	66
W.J.	1	1,790	1,940	30	100
	2	197	1,407	50	33
	3	1,303	1,594	50	50
K.K.	1	2,317	1,085	34	31
	2	1,426	967	35	32
	3	3,296	901	29	31
T.K.	1	2,058	811	19	19
	2	735	621	19	21
	3	2,647	767	18	18
A.S.	1	4,405	669	26	29
	2	441	678	26	35
	3	3,900	769	25	28
D.A.	1	668	1,658	52	31
	2	3,260	2,113	56	29
	3	1,632	1,518	36	31
M.B.	1	304	1,215	36	36
	2	1,549	1,485	33	48
	3	634	1,221	40	39
M.F.	1	199	998	25	25
	2	1,402	980	20	20
	3	329	791	25	25
M.K.	1	793	910	20	24
	2	2,460	1,062	21	32
	3	577	744	19	23
T.W.	1	352	1,286	22	34
	2	2,290	1,297	32	33
	3	536	1,193	24	36

The space between patients A.S. and D.A. separates the patients on the high-low-high protocol above from those on the low-high-low protocol below. The choice reaction time represents a mean of 20 trials. The pegboard results represent a mean of four trials.

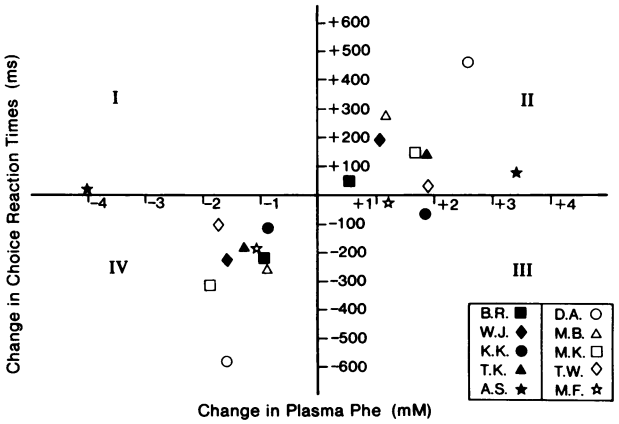

Figure 3. The relationship of changes in plasma phenylalanine to changes in choice reaction time during dietary manipulation of phenylalanine. Each patient is represented by a symbol which appears twice on the graph, indicating the difference in mean choice reaction time between the first and second week and the second and third week plotted against the parallel change in plasma phenylalanine.

respectively (34). Katz et al. (35) demonstrated the direct conversion of 20 μM phenylalanine to dihydroxyphenylalanine without the release of free tyrosine in rat brain striatal synaptosomal preparations. Phenylalanine was only 1/10 as good a substrate for this enzyme as tyrosine. However, he suggested that phenylalanine could be a substrate for tyrosine hydroxylase in the presence of saturating concentrations of tetrahydrobiopterin, and could be a competitive inhibitor as well.

Our results in vivo in treated PKU patients conform to the hypothesis that high phenylalanine inhibits dopamine synthesis, since 24-h urine dopamine excretion fell when plasma phenylalanine concentrations were maintained at an elevated concentration for days by dietary manipulation. A consistent relationship was not found between plasma phenylalanine and serotonin excretion in our study. Serotonin is stored in many tissues (36), and this inconsistency may be related to the high "background noise" of excretion of stored serotonin during a 24-h period.

The results from our experiments do support the hypothesis that brain function is altered by phenylalanine at the equilibrium concentrations achieved in the study. The battery of neuropsychological tests showed differences in a performance test which required higher integrative function rather than fine motor coordination. This was consistent over the whole group, regardless of the age or competence of the patients. Although many test batteries have been used in other surveys, the computerized reaction time has not been reported (9, 11, 12). We are currently attempting to determine whether patient competency, age, attention, or other factors influence the neuropsychological response to increased plasma phenylalanine, and deriving tests to maximize changes in accordance with patient competency.

Our data support a mechanism for prolonged performance through an inhibition by phenylalanine of biogenic amine synthesis. In our study when dopamine excretion fell, blood phenylalanine rose and performance times were prolonged. Data from two patients deserve special attention: the patient with the lowest IQ (K.K.) who was not diagnosed until 18 mo of age demonstrated expected biochemical changes in urinary dopamine excretion when phenylalanine concentrations were increased, but test scores on the Computerized Choice Reaction Time were unchanged. It is not surprising, in view of his overall low performance and achievement, that reaction time improved over the 3-wk period independent of the plasma phenylalanine, which suggested a gradual learning effect rather than relationship to biochemical status. It is also likely that intellectual competency of a patient will control the amount of change produced by altered phenylalanine. The lower the individual's competency, the less change might be expected. The one major outlier (M.F.) did not show consistent trends

in neuropsychological tests or in catecholamine excretion. Despite attaining a concentration of 1,402 μM plasma phenylalanine, he excreted barely measurable amounts of derived organic acids. We can speculate that he has other "protective" functions. Possible mechanisms include impaired transport of phenylalanine across the blood brain barrier or an increased rate of phenylalanine incorporation into new protein synthesis. He could also have some "protective" variation in tyrosine hydroxylase which prevents inhibition by phenylalanine. He emphasizes the individuality of patients with phenylketonuria and the "sensitivity" of brain function to phenylalanine loading.

The impairment in choice reaction time and decrease in dopamine excretion seen with increased plasma phenylalanine were reversible within the week periods studied. We are currently investigating a variety of repeatable neuropsychological and electrophysiological tests with which to assess performance in patients with varying competency, age, and achievement scores.

These data support the hypothesis that high concentrations of phenylalanine reversibly affect neuropsychological performance, probably through reduction in L-dihydroxyphenylalanine and dopamine production. The mechanisms may be through increased intracellular phenylalanine and competitive inhibition of brain tyrosine-3-hydroxylase. Whether intracellular concentrations of brain tyrosine are diminished is unknown. Although the concentrations of blood phenylalanine attained in our studies did not inhibit renal tubular reabsorption of tyrosine, it should be noted that the transport K_m of phenylalanine, tyrosine, and tryptophan in brain and kidney differ. Additionally, the blood-brain barrier is saturated at normal plasma concentrations, whereas the renal tubular epithelium is not (16–19). Since nearly 80% of all brain dopamine is found in the corpus striatum, decreases in dopamine synthesis could affect neuropsychological functions that involve both the nigrostriatal and corticostriatal pathways (37). This could explain the deterioration in response of our patients to a timed test, the Computerized Choice Reaction Time Test, which required integration of stimuli and a motor response. We have recently observed a change in the mean power frequency of electrical impulses detected by EEG in a different group of patients with phenylketonuria who were studied under similar clinical research protocols (38). This type of electrophysiological approach could assist in anatomical localization of changes in brain function.

Acknowledgments

The authors wish to express their appreciation to Dr. Bahjat Faraj and Mr. Vernon Camp of the Department of Radiology for performing the catecholamine assays. We also want to thank Mr. Robert Mapou for modifying the computerized choice reaction time tests.

References

1. Følling, A. 1934. Uber Ausscheidung von Phenylbrenztraubensaure in der harn als stoffwechselanomalie in verbindung mit imbezillitat. *Hoppe-Seyler's Z. Physiol. Chem.* 227:169–176.

2. Bickel, H., J. Gerrard, and E. M. Hickmans. 1954. The influence of phenylalanine intake on the chemistry and behavior of a phenylketonuric child. *Acta Paediatr.* 43:64–77.

3. Agrawal, H. C., A. H. Bone, and A. N. Davison. 1970. Effect of phenylalanine on protein synthesis in the developing rat brain. *Biochem. J.* 117:325–331.

4. Johnson, R. C., and S. N. Shah. 1980. Effects of alpha-methylphenyl-alanine plus phenylalanine treatment during development on myelin in rat brain. *Neurochem. Res.* 5:709–718.

5. Crome, L., and C. M. B. Pare. 1960. Phenylketonuria. A review and a report of the pathological findings in four cases. *J. Ment. Sci.* 106:862–883.

6. Agrawal, H. C., and A. N. Davison. 1973. Myelination and amino acid imbalance in the developing brain. *In* Biochemistry of the Developing Brain. W. A. Himwich, editor. Marcel Dekker, Inc., New York. 4:143–186.

7. Shah, S. N., N. A. Peterson, and C. M. McKean. 1972. Lipid composition of human cerebral white matter and myelin in PKU. *J. Neurochem.* 19:2369–2376.

8. Elsas, L., A. Brown, and P. Fernhoff. 1983. Newborn screening for metabolic disorders in the State of Georgia. *In* Neonatal Screening. H. Naruse and M. Irie, editors. Excerpta Medica, Princeton. 117–118.

9. Waisbren, S. E., R. R. Schnell, and H. L. Levy. 1980. Diet termination in children with PKU. A review of psychological assessments used to determine outcome. *J. Inherited Metab. Dis.* 3:149–153.

10. Williamson, M. L., J. C. Dobson, and R. Koch. 1977. Collaborative study of children treated for PKU: study design. *Pediatrics.* 60:815–821.

11. Koch, R., C. G. Azen, E. G. Friedman, and M. L. Williamson. 1982. Preliminary report on the effects of diet discontinuation in PKU. *J. Pediatr.* 100:870–875.

12. Brunner, R. L., M. K. Jordan, and H. K. Berry. 1983. Early treated phenylketonuria: neuropsychologic consequences. *J. Pediatr.* 102:831–835.

13. McKean, C. M. 1972. The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. *Brain Res.* 47:469–476.

14. Grahame-Smith, D. H., and L. Moloney. 1965. The subcellular localization, purification and properties of tryptophan 5-hydroxylase in brain. *Biochem. J.* 96:66P.

15. Ikeda, M., M. Levitt, and S. Udenfriend. 1967. Phenylalanine as substrate and inhibitor of tyrosine hydroxylase. *Arch. Biochem. Biophys.* 120:420–427.

16. Pardridge, W. 1977. Regulation of amino acid availability to the brain. *In* Nutrition and the Brain. I. R. J. Wurtman and J. J. Wurtman, editors. Raven Press, New York. 141–204.

17. Pardridge, W. M., and W. H. Oldendorf. 1975. Kinetic analysis of blood brain barrier transport of amino acids. *Biochim. Biophys. Acta.* 401:128–136.

18. Oldendorf, W. H. 1973. Saturation of blood brain barrier transport of amino acids in phenylketonuria. *Arch. Neurol.* 28:45–48.

19. Pratt, O. E. 1981. The needs of the brain for amino acids and how they are transported across the blood brain barrier. *In* Transport and Inherited Disease. W. R. Belton and C. Toothill, editors. MTP Press Ltd., Lancaster, England. 87–122.

20. Van Loon, G. R., and M. J. Sole. 1980. Plasma dopamine: source, regulation, and significance. *Metab. Clin. Exp.* 29:1119–1123.

21. McDonald, L., M. Halminski, and L. Elsas. 1983. Phenylalanine affects performance in phenylketonuria. *Clin. Res.* 30:891A.

22. Krause, W. L., L. McDonald, M. Halminski, and L. J. Elsas. 1983. Phenylalanine affects performance, dopamine excretion and renal tyrosine transport in phenylketonuria. *Pediatr. Res.* 17:213A.

23. Griffin, R. F., and L. J. Elsas. 1975. Classic phenylketonuria diagnosis through heterozygote detection. *J. Pediatr.* 86:572–577.

24. Acosta, P. B., P. M. Fernhoff, H. S. Warshaw, and L. J. Elsas. 1982. Zinc status and growth of children undergoing treatment for phenylketonuria. *J. Inherited Metab. Dis.* 5:107–110.

25. Peuler, J. D., and G. A. Johnson. 1977. Simultaneous single isotope radioenzymatic assay of plasma nerepinephrine, epinephrine and dopamine. *Life Sci.* 21:625–636.

26. Peskar, B., and S. Spector. 1973. Serotonin: radioimmunoassay. *Science (Wash. DC)* 179:1340–1341.

27. Goodman, S. I., and S. P. Markey. 1981. Appendix A: analytical methods for urine organic acids. *In* Laboratory and Research Methods in Biology and Medicine, Vol. 6. Diagnosis of Organic Acidemias by Gas Chromatography-Mass Spectrometry. Alan R. Liss, Inc. New York. 105–114.

28. Elsas, L. J., and L. E. Rosenberg. 1979. Renal glycosuria. *In* Strauss and Welt's Diseases of the Kidney. L. E. Earley and C. W. Gottschalk, editors. Little, Brown and Co., Boston. Third ed. II:1021–1028.

29. Lines, D. R., and H. A. Waisman. 1971. Urinary amino acid excretion in phenylketonuric, hyperphenylalaninemic, and normal patients. *J. Pediatr.* 78:474–480.

30. Weil-Malherbe, H. 1955. Blood adrenaline and intelligence. *J. Ment. Sci.* 101:733–745.

31. Nadler, H. L., and D. Y. Hsia. 1961. Epinephrine metabolism in PKU. *Proc. Soc. Exp. Biol. Med.* 107:721–723.

32. McGeer, E. G., P. L. McGeer, and J. A. Wada. 1971. Distribution of tyrosine hydroxylase in human and animal brain. *J. Neurochem.* 18:1642–1658.

33. Udenfriend, S. 1967. The primary enzymatic defect in PKU and how it may influence the central nervous system. *In* Phenylketonuria and Allied Diseases. J. A. Anderson and K. F. Swaiman, editors. U. S. Department Health, Education and Welfare, Washington, DC. 1–7.

34. Curtius, H-C. H., A. Niederwieser, M. Viscontini, W. Leimbacher, H. Wegman, B. Blehova, F. Rey, J. Schaub, and H. Schmidt. 1981. Serotonin and dopamine synthesis in phenylketonuria. *Adv. Exp. Med. Biol.* 133:277–291.

35. Katz, I., T. Lloyd, and S. Kaufman. 1976. Studies on phenylalanine and tyrosine hydroxylation by rat brain tyrosine hydroxylase. *Biochim. Biophys. Acta.* 445:567–578.

36. Essman, W. B. 1978. Serotonin distribution in tissues and fluids. *In* Serotonin in Health and Disease. I. W. B. Essman, editor. S. P. Medical and Scientific Books, a division of Spectrum Publications, Inc. New York and London. 15–180.

37. Coyle, J. T., and S. Snyder. 1981. Catecholamines. *In* Basic Neurochemistry. G. J. Siegel, R. W. Albers, B. W. Agranoff, and R. Katzman, editors. Little, Brown and Co., Boston. Third ed. 10:205–217.

38. Krause, W., A. Averbook, C. Epstein, and L. Elsas. 1983. Changes in EEG and L-DOPA in treated patients with phenylketonuria (PKU) during a phenylalanine challenge. *Clin. Res.* 31:898A.